fruit from plots receiving K fertilization. The K_1 rate used on Sunniland soil was the most satisfactory from the external grapefruit appearance standpoint since K_2 fruit often were rated during the experiment as being more sheepnosed, rougher textured, and greener than K_1 fruit (data not presented).

The data obtained from the K rate experiment over the 20-year period indicate that tree condition was affected at the K_0 rate by moderate to extreme K deficiency, while deterioration in tree condition at the K_2 rate probably was caused by the massive applications of K. Further evidence that tree condition in the experimental area was on the decline is substantiated by comparing the grapefruit yields obtained for all treatments during the last 5 years of the experiment with yields obtained in earlier years. In general, yield from all plots, regardless of treatments, were considerably lower in the last 5 years. Observations made in later years of the experiment indicate that symptoms of "Rio Grande" gummosis were both more prevalent and more severe in both K_1 and K_2 plots than in the K_0 plots on Sunniland soil. This would indicate that incidence of gummosis was related to the application of the potassium salts. More specific soil and leaf analyses data collected from this experiment which may relate to this problem will be presented in a later paper.

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THE POSSIBILITIES OF USING GROUND SPRAYS TO CONTROL CITRUS GREASY SPOT

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Abstract. **Descriptions are given of the pilot methods used to determine whether fungicides can reduce the ascospore inoculum supply of the greasy spot fungus (Mycosphaerella citri) when sprayed onto fallen perithecial-bearing grapefruit leaves. Samples of leaves were taken from sprayed and unsprayed areas of the grove floor and placed in a wind tunnel-spore trapping device to determine their remaining inoculum potential. Of the 4 materials (Tribasic copper sulfate, Difolatan, Bravo, and Benlate) tested, only Benlate substantially reduced the inoculum supply from the leaf litter, but only for 4 to 6 weeks after ground spraying.**

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Introduction

Spore inoculum of the citrus greasy spot fungus, *Mycosphaerella citri* Whiteside, originates mostly from fruiting bodies (perithecia) produced on decomposing fallen citrus leaves (5). The potential supply of spores (ascospores) from this substrate gradually increases during the spring and early summer and reaches a peak

in the early part of the rainy season, at a time of year when the climatic conditions are becoming more favorable for spore release and host infection.

As the rainy season proceeds, the supply of ascospore inoculum decreases and generally reaches very low levels by August or September. This is because the leaf litter eventually becomes too decomposed to support further fungal development and because there is generally little leaf drop after May to replenish the inoculum supply. Thus, greasy spot severity can be related partly to the number of infected citrus leaves present on the grove floor before the summer rainy season commences.

Theoretically, disking under the leaf litter in May or early June should assist greasy spot control. In practice, however, disking of citrus groves has to be very shallow to avoid excessive root damage, and only partial leaf burial is generally achieved. Furthermore, an area of the grove floor close to the trunk generally has to be left completely undisturbed. Consequently, large amounts of leaf litter may still remain on the surface even in groves that have been disked. Consideration has therefore been given to the possibilities of eradicating the main source of greasy spot fungal inoculum by applying chemicals to the leaf litter. This would involve the same princip'e of disease control as that previously used to eradicate primary inoculum of the apple scab fungus by spraying the orchard floor in early spring with dinitro-o-cresol and related compounds (3), or with benomyl, as more recently proposed (4).

Ultimately, the practical value of a ground spray for supplementing or replacing other methods of greasy spot control would have to be measured in terms of reduced disease severity. However, such data could be difficult to obtain, because greasy spot ascospores are wind-borne and the tests would therefore have to be made on large replicated plots with wide buffer zones between the treated and untreated sampling areas. Pilot methods were therefore devised to determine whether a material possessed sufficient activity against the phase of the fungal life cycle contained in the fallen leaves to justify large scale testing as a ground spray.

Results are given here of the ascospore inoculum potential from decomposing fallen leaves at various times after spraying such leaves on the grove floor with Tribasic copper sulfate (53% copper), Difolatan (cis-N-[(1,1,2,2-tetrachloroethyl)thio]-4-cyclohexene-1,2 dicarboximide), Bravo (chlorothalonil), or Benlate (benomyl). These materials were selected for initial study because they are all effective against greasy spot when sprayed onto the tree canopy (7).

Materials and Methods

The procedure used for determining whether a fungicide could reduce the supply of ascospores from treated fallen leaves consisted of placing a dried sample of test leaves in a special spore trapping device, spraying the sample with water to release any mature ascospores into the air, and then collecting these spores on vaseline-coated microscope slides.

The trapping apparatus (Fig. 1) consisted of a wind tunnel, similar to that developed by Hirst and Stedman (2), and a spore impactor identical to that described by Brook (1). Air was drawn through the wind tunnel and impactor by a vacuum pump. Two such systems were always operated simultaneously from the same pump;

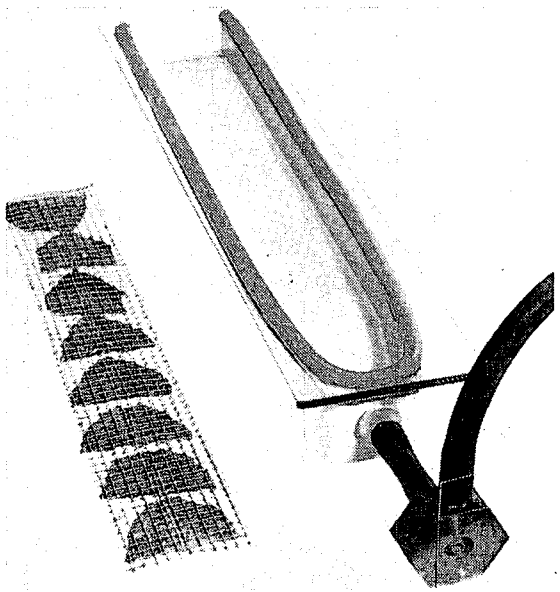

Fig. 1. Apparatus used for collecting ascospores discharged from fallen citrus leaves. Selected leaves or samples of leaf litter, sandwiched between hardware cloth, were placed in the tunnel, which had a removable plate glass lid resting on a seal of flexible tubing. Air was drawn by a vacuum pump through the upright tube on the impactor. Spores released after wetting a leaf sample were then impinged onto the vaseline-coated microscope slide. The type of carrier used for holding selected perithecial-bearing leaf halves is shown on the left of the tunnel portion of the spore trapping device.

one tunnel being used to hold a treated sample and the other tunnel to hold the corresponding untreated sample of fallen leaves.

Internal dimensions of the straight portion of the tunnel were: height 35 mm, length 400 mm, and width 75 mm. The tapered portion was 90 mm long and narrowed to a width of 45 mm. The length of the tube from the inside of the tunnel to the face of the microscope slide was 130 mm. The internal diameter of both the impactor intake and exit tubes was 6 mm. The intake tube was set back 0.6 mm from the surface of the impactor block and hence from the surface of the microscope slide. Air speed through each tunnel when empty was ca. 130 mm per sec.

The total number of ascospores caught from each sample was estimated after counting, at 400X magnification, the number of ascospores present on two 48 μ-wide transects running at right angles to each other through the center of the circular deposit on the microscope slide.

Two different procedures were used to determine the effectiveness of ground sprays for reducing the inoculum potential in fallen leaves. In both cases, however, the assessments were based on the number of ascospores deposited on the microscope slides after wetting a sample in the tunnel and running the vacuum pump for 30 min. This period was selected after determining that a majority of ascospores ready for release are discharged during the first 30 min after wetting.

In the first procedure, fallen 'Marsh' grapefruit leaves with numerous perithecia distributed over both the distal and basal halves of each leaf were cut in half across the midrib. The halves from 8 such leaves were placed flat between 2 pieces of 5 mm-mesh hardware cloth, the dimensions of which were slightly less than the interior of the straight portion of the spore trap tunnel. The other 8 leaf halves were placed in another such hardware cloth carrier to be used as an untreated check. The leaf halves were all placed in the carriers with the lower leaf surface facing the same direction. Subsequently, the carriers were always placed on the ground and in the spore trap with the lower leaf surface uppermost, thereby ensuring that this surface, which contains a majority of the perithecia, would always be exposed. The reason for cutting each leaf in half and placing one-half in a treated carrier and the other half in a corresponding untreated carrier was to reduce as much as possible the experimental variations caused by the different rates of decomposition and perithecial development that were observed between different leaves.

Six carriers, containing the half leaves, were placed flat on the ground in a citrus grove within a marked area, which was then sprayed, using a handsprayer, with a specified volume and concentration of fungicide suspension. The area was traversed several times to ensure greater uniformity of deposit. Another 6 carriers, containing the other leaf halves, were placed outside the sprayed area to serve as unsprayed checks. Following various periods of exposure in the citrus grove, the carriers were brought to the laboratory for the discharge and trapping of ascospores. In some tests, the carriers were returned to the citrus grove for a further period of exposure, before again testing their inoculum potential.

A material that showed promise when tested according to the above procedure was later subjected to a second test procedure that simulated grove conditions more closely. For this purpose, naturally deposited leaf litter under 50-year-old 'Marsh' grapefruit trees was sprayed at 300 psi from a single-nozzled handgun. Areas under nearby trees were not sprayed and served as checks. The samples to be treated for their inoculum potential were taken in the following manner to avoid disturbing the leaf litter as much as possible. A piece of hardware cloth, of the same width but only half the length of the carriers used in the first procedure, was placed firmly over the area to be sampled. Cuts were then made around the outside through the leaf litter with scissors. Another piece of hardware cloth of similar size was then slipped under the leaf litter to form a leaf litter-hardware cloth sandwich. This was held together with several paper clips for transportation to the laboratory. Before being placed in the spore trap, the samples were sun dried (if still wet) and the top layer of hardware cloth was removed, thereby allowing the leaves to spring back, at least partly, to their original curled orientation. Two carriers, containing subsamples of leaf litter from the same plot, were placed in each wind tunnel at the same time. The ground spraying treatments were replicated on 4 different plots.

Results and Discussion

Because recent liberation of ascospores by

rain or heavy dew prior to testing would have reduced the actual catches in the spore trap, the results obtained from successive tests on the same sample did not accurately reflect the ascospore production trend with time. Valid comparison of data could therefore only be made between ascospore catches made from the corresponding treated and untreated samples tested on the same date.

In Table 1 is shown the effect of various fungicides on ascospore release from selected perithecial-bearing leaves that had been placed flat in hardware cloth carriers, and then treated and kept outdoors until testing time. The copper fungicide had no apparent effect on ascospore development and release. Difolatan 4F reduced ascospore numbers in only 1 of the 2 tests where it was applied. Bravo 6F reduced ascospore numbers only slightly. Only Benlate 50W consistently reduced the inoculum potential from the treated decomposing fallen leaves, but only during the first 4 weeks after treatment. After this time, the number of ascospores released from Benlate-treated leaves showed a proportionate increase when compared with the numbers released from untreated leaves. In the 1971 test, the number of ascospores released at 36 days after treatment with Benlate greatly exceeded the numbers released from unsprayed leaves, suggesting that this fungicide acted partly by delaying perithecial maturation.

Table 2 shows the number of ascospores trapped from samples of leaf litter collected from sprayed and unsprayed areas of the ground in a 'Marsh' grapefruit grove when Benlate was tested according to the second procedure. Because of below-average rainfall in June, leaf decomposition proceeded slowly at first and it was not until mid-August that the supply of inoculum was virtually exhausted. The ground spray of Benlate 50W, at 2 lb. in 400 gal/acre, substantially reduced potential ascospore release during the first 36 days after application. Under natural conditions, however, relatively few ascospores would have been released over this period because of the infrequency of rainfall. After mid-July, the number of ascospores trapped from treated litter, as compared with untreated litter, in-

Table 1. Catches of Mycosphaerella citri ascospores from fungicide-sprayed and unsprayed perithecial-bearing decomposing fallen 'Marsh' grapefruit leaves following various periods of exposure outdoors.

Date of spraying	Fungicide amount and volume of spray/acre	Days between spraying and trapping	Rainfall for this period (inches)	Ascospores trapped[z] from: Sprayed leaves	Ascospores trapped[z] from: Unsprayed leaves
June 11, 1971	Tribasic copper sulfate 3.0 lb/200 gal	16	2.88	16,228	16,935
June 25, 1971	Benlate 50W, 0.5 lb/100 gal	28	4.49	141	4,575
		36	7.52	9,850	2,472
		45	8.57	1,509	38
May 19, 1972	Difolatan 4F, 0.5 gal/200 gal	27	3.32	1,749	5,153
May 19, 1972	Benlate 50W, 1.0 lb/200 gal	27	3.32	7	1,649
		40	9.26	1,972	3,480
		55	9.60	750	34
May 15, 1973	Difolatan 4F, 2.0 gal/200 gal	20	3.52	9,170	10,700
May 15, 1973	Benlate 50W, 1.0 lb/200 gal	20	3.52	96	6,323
		36	4.00	364	8,645
		52	5.39	717	2,482
June 4, 1973	Bravo 6F, 1.33 gal/400 gal	21	1.08	21,625	31,394
June 4, 1973	Benlate 50W, 1.0 lb/400 gal	28	1.10	86	23,091

[z]Represents estimated average number of ascospores deposited on microscope slide from each carrier containing 8 leaf halves, after wetting the leaves and exposing them for 30 min in the wind tunnel.

Table 2. Number of ascospores of *Mycosphaerella citri* caught in a wind tunnel-spore trapping device from samples of leaf litter removed from Benlate-sprayed and unsprayed areas of ground in a 'Marsh' grapefruit grove.

Days between spraying and sampling[z]	Rainfall between spraying and sampling (inches)	Ascospores trapped[y] from samples of litter removed from:	
		Sprayed areas	Unsprayed areas
29	3.8	531	18,821
36	6.8	3,928	103,941
44	10.2	4,283	18,161
55	13.0	8,999	20,640
62	14.3	552	1,095

[z]Sprayed on June 6, 1973 with 2 lb Benlate 50W in 400 gal/acre.

[y]Estimated average number of ascospores trapped, after exposing a 300 cm^2 area of wetted leaf litter in the spore trap tunnel for 30 min.

creased considerably, but never reached the numbers released from unsprayed litter.

Further trials on a more extensive scale would be required to determine whether carefully timed ground sprays of benomyl could eliminate the need for canopy spraying for greasy spot control. The prospects for achieving greasy spot control by these means do not, however, appear very promising. For example, a single ground spray, applied in early June, might only be effective in years when the leaf litter decomposes rapidly and the fungal substrate becomes exhausted before the effect of the Benlate diminishes.

The idea of using ground sprays to control a disease such as greasy spot, by reducing the amount of inoculum released from infected leaf litter, certainly has a logical fascination. It should be emphasized, however, that greasy spot is not a difficult disease to control by present methods. In fact, 1 spray of an effective fungicide (7) applied to the canopy in late June or July generally gives adequate control of this disease, provided that good coverage is obtained (6). Therefore, ground spraying would not necessarily reduce the costs of greasy spot control.

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