Epidemiology and Control of Citrus Greasy Spot on Valencia Orange in the Humid Tropics of Costa Rica

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ABSTRACT

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An epidemiological study of *Mycosphaerella citri*, the cause of greasy spot of citrus, was conducted for 2 years at Finca 6 and El Parque, Costa Rica. Ascospores were the primary source of inoculum; only a few conidia were trapped during the study. Ascospores were trapped first during late April or early May. The spore discharge pattern was associated closely with seasonal rainfall distribution; in both years, the number of ascospores trapped increased rapidly through May, peaked during early June, declined rapidly through July, and were negligible during the rest of the year. Most ascospores were trapped during a 6- to 8-week period. Three principal discharge patterns were observed. One pattern, associated with rainfall, was characterized by the release of large numbers of ascospores within 1 h of the beginning of rainfall. Ascospore release often continued for several hours after the rainfall ended. A second discharge pattern was associated with dew. Large numbers of spores were trapped during periods when dew was heaviest (0200 to 0700 h). A third pattern consisted of a combination of the first two patterns (i.e., discharge during evening rain followed by a second discharge associated with dew). No relationship was found between different combinations of rainfall data and either the first or peak ascospore catch. Similarly, there was no consistent relationship between weekly percentages of leaves at different stages of decomposition and ascospore catch that could be used to predict the period of peak ascospore discharge. Weekly total number of leaves on the orchard floor was a better predictor of total ascospore catch. Two sprays of a copper fungicide reduced greasy spot incidence, severity, and defoliation compared to an unsprayed control. Oil, sprayed twice as a standard grower treatment, did not reduce disease incidence or severity compared to the unsprayed control.

Greasy spot, caused by *Mycosphaerella citri* Whiteside (anamorph *Stenella* sp.), is one of the most important diseases of citrus leaves and fruit, often causing severe defoliation and yield losses up to 5 t/ha (1,5). Symptoms are characterized by the formation of raised, light-brown to black, oily lesions with diffuse margins, surrounded by a yellowish-green border of variable width that is most pronounced during the early stages of development. Infected fruit develop black specks on the surface between the oil glands.

M. citri overwinters in the leaves on the orchard floor and produces pseudothecia in densely packed groups on decomposing leaves. Little pseudothecial development occurs during the winter. Ascospores released from these pseudothecia are the main source of inoculum; conidia are believed to be of little epidemiological importance (9).

Ascospores germinate and produce hyphae that penetrate leaves and fruit rinds through stomata and cause death of guard cells (5). *M. citri* requires prolonged and repeated periods of relative humidity near 100% combined with warm temperatures (25 to 30°C) for infections via penetration of stomata by hyphae. Light is not a limiting factor for ascospore germination or hyphal growth. Infection usually occurs during periods of growth flush (7).

In Florida, the time of peak ascospore discharge ranges from April to July according to rainfall amount and frequency, use of microsprinkler irrigation, and grow-

ing region (2,4,9). However, during some years, significant discharges can occur outside the peak periods (2). Ascospore release usually follows flood irrigation during late June and July in Texas, but the majority of ascospores are released during rains during late August and early October (3). Dry weather prevents ascospore release and delays pseudothecial development. In contrast, wet weather causes more rapid leaf decay, hastens pseudothecial development, and increases the number of ascospores discharged. Light ascospore discharges have been recorded after dew (5, 6,9). There is no information on factors affecting ascospore maturity and release in humid, tropical environments such as in Costa Rica, where a distinct dry season is followed by a rainy one. Rainfall in the principal citrus-growing region in Costa Rica typically averages <100 mm/month from January to April and 100 to 500 mm/month from May to September (Table 1).

In Florida, greasy spot of both leaves and fruit is controlled with one (9) or two sprays (2) of copper fungicides applied in a mixture with mineral oil during or shortly after peak ascospore discharge (10). This treatment kills mycelial growth on the leaf surface and provides protection against future ascospore release (9). The actual role of oil in suppressing greasy spot is not well understood. Oil has no fungicidal or fungistatic effect, but it may play a role in increasing host resistance (7). When first registered, benomyl provided good control of greasy spot in leaves and fruit. However, resistance to benomyl was reported after 2 to 3 years of use in Florida and is no longer recommended (8).

In Costa Rica, control of greasy spot has been accomplished with three to four sprays

Table 1. Monthly rainfall totals (millimeters) and 6-year mean for Finca 6 and Tico Fruit-Procisa, from 1990 to 1995, Los Lirios, Costa Rica

Month	1990	1991	1992	1993	1994	1995	Mean
January	96	20	106	146	63	92	87.2
February	84	101	47	23	43	65	60.5
March	20	21	28	77	36	32	35.7
April	14	36	46	44	38	82	43.3
May	182	350	95	329	346	102	234.0
June	210	255	269	619	295	395	340.5
July	420	449	252	431	489	418	409.8
August	350	383	387	306	332	383	356.8
September	230	391	423	482	338	266	355.0
October	98	276	337	133	168	234	207.7
November	15	257	82	130	344	280	184.6
December	48	321	333	151	535	303	281.8
Total	1,767	2,860	2,405	2,871	3,027	2,652	...

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of 3 to 4 kg of Cu a.i./ha in a mixture with mineral oil (1%) during the rainy season; one application is made each time a growth flush occurs. However, these applications have resulted in high copper levels in leaves. More recently, two to three oil applications have replaced copper in the control program, but these applications have not been efficacious. A better understanding of the conditions that favor ascospore production, maturity, and release could lead to improved disease management strategies.

This paper reports the results of an epidemiological study of citrus greasy spot in the San Carlos region of Costa Rica and a fungicide timing experiment for greasy spot control. The objectives of the study were to determine the seasonal ascospore discharge patterns of *M. citri* in relation to weather variables and to establish a relationship between leaf litter decomposition, rainfall or dew, and seasonal discharge pattern that could be used as a guide to more effective timing of fungicide applications.

MATERIALS AND METHODS

Seasonal ascospore discharge pattern.

Airborne dispersal of ascospores of *M. citri* was studied from 6 May to 14 December in 1994 and from 29 April to 20 October in 1995 at Finca 6, Los Lirios, and Finca El Parque, El Parque, both located within the San Carlos region of Costa Rica. Finca 6 was a 6-year-old orchard operated by Tico Fruit and was sprayed with copper and/or oil each year for greasy spot control. El Parque, a 7-year-old private commercial farm, received no fungicide spray. Ascospores were trapped at each location with a Burkard volumetric spore trap (Burkard Scientific Sales, Ltd., Rickmansworth, Hertfordshire, England). Traps were adjusted to sample 10 liters of air per min ($0.6 \text{ m}^3/\text{h}$); the trap orifice was approximately 50 cm above the orchard floor. Melinex tapes were cut into 48-mm sections, mounted on glass slides, stained with cotton blue in lactophenol, and examined at $400\times$. Daily ascospore counts were determined by making one longitudinal pass through the center of the slide and adjusting this count for the area of the tape counted. Preliminary studies indicated that one pass through the center of the tape provided an estimate of ascospore catch statistically similar to that of two passes. Hourly discharge patterns during dew and rain events were determined by making a transverse pass through the center of the tape for each hourly exposure at $400\times$ and adjusting the counts for the area sampled. In 1994, ascospore counts were made for each day from 6 May to 14 December; in 1995, daily counts were made only for the period from 30 April to 30 July. From 30 July to 20 October, counts were made during selected rainfall and dew periods each week to verify that only low numbers of ascospores were trapped.

The days with rainfall and rainfall amount from 1 January or 1 April each year to the week of peak ascospore discharge were tabulated and examined to determine whether they could be used to predict the time of first ascospore catch or peak ascospore catch.

Weather stations. Hourly records of temperature, leaf wetness, rainfall, and relative humidity were collected at each location during the same period that spore traps were operated in both years. In 1994, temperature, leaf wetness, and rainfall data were recorded with a thermistor, a leaf wetness sensor, and a tipping bucket rain gauge attached to a CR21 data logger (Campbell Scientific, Logan, UT). Additional records of temperature, relative humidity, and leaf wetness were collected with a hygrothermograph (Belfort Instruments Co., Baltimore) and a DeWit leaf wetness meter (Instrumentenfabriek I.F.G. DeWit, Rhoden, the Netherlands). The hygrothermographs and the data logger were placed inside a slatted, white wooden box covered with a tin roof, located 30 cm above ground and placed approximately 2 m from the spore trap. The DeWit leaf wetness

Fig. 1. Weekly pattern of ascospore catch of *Mycosphaerella citri* from 7 May to 14 December 1994 at El Parque and Finca 6, Costa Rica. Blank spaces represent weeks when no spores were caught.

Fig. 2. Weekly pattern of ascospore catch of *Mycosphaerella citri* from 30 April to 2 July 1995 at El Parque and Finca 6, Costa Rica. Spore counts for 28 May to 4 June at El Parque were estimated from a partial spore trap tape.

meter was located approximately 1.5 m above ground and was placed in the drip line of the tree on the west side. In 1995, the same weather variables were recorded, except a CR10 data logger was used, and temperature and relative humidity were measured with a UVL235 sensor (Campbell Scientific). Daily rainfall data for 1994 to 1995 from Finca 6 (Tico Fruit) and 1994 from CitroRico, located at approximately 2 km from Finca El Parque, were used as supplemental data when the tipping-bucket rain gauge malfunctioned. Data from the DeWit leaf wetness meter were used in all studies requiring leaf wetness data. A rainy day was defined as a day with ≥ 0.252 mm of rain.

Measurement of disease incidence and severity. Disease intensity, measured as incidence, severity, and number of leaf scars and leaves still attached to the shoots (a measure of defoliation), was determined on 14 December 1994 and 24 February 1996 on 16 trees in each orchard selected arbi-

trarily within a ~1-ha area surrounding the spore trap. To measure disease severity, 10 terminals from May's growth flush, distributed equidistantly around the canopy, were tagged on each tree during June, and every leaf was rated according to a modified Horsfall-Barratt scale, consisting of six categories for percentage of area with greasy spot symptoms: 1 = 0 to 1%, 2 = 2 to 3%, 3 = 4 to 6%, 4 = 7 to 12%, 5 = 13 to 25%, and 6 = 25 to 50%. Disease severity >50% was not observed. Trees at Finca 6 received standard Cu + oil treatments; trees at El Parque were never sprayed.

Leaf-litter decomposition. Leaf-litter decomposition was monitored at both locations from 2 June to 6 July in 1994, and from 18 May to 27 June in 1995. A 21 × 21-cm square frame was placed 1 m from the base of each of four trees surrounding the spore trap at each location, and leaves found inside the frame were examined weekly. The frame was placed on the west

side of the tree in the same location on each sample date; any leaves removed for examination were replaced. Leaves were separated into four decomposition stages as follows: 0 = leaves recently fallen from trees, showing a greenish color with no visible signs of decomposition; 1 = leaves brown with no signs of decomposition; 2 = leaves brown with 1 to 50% of the leaf area partially or completely decomposed; and 3 = leaves brown with more than 50% of the leaf area partially or completely decomposed. The relationship between leaf litter decomposition and ascospore catch was characterized by a general linear model procedure (SAS version 6.08, SAS Institute Inc., Cary, NC).

Timing of fungicide sprays. The fungicide spray timing experiment was conducted in 1995 at Finca 6, using 6-year-old Valencia orange trees spaced 8 × 4 m apart. Fungicide treatments were applied with a mist blower to single-tree plots separated

Fig. 3. Hourly pattern of ascospore catch of *Mycosphaerella citri* released by rain from 26 to 27 May 1995 at Finca 6, Costa Rica.

Fig. 4. Hourly pattern of ascospore catch of *Mycosphaerella citri* released by rain from 31 May to 1 June 1995 at Finca 6, Costa Rica.

from each other with buffer trees and replicated four times in a randomized complete-block design. The spray materials used were copper oxychloride (Cupravit 56 to 58% Cu, Bayer AG, Leverkusen, Germany) at 28 to 29 g of Cu a.i./liter and 1% spray oil (Agrol, 98% paraffinic oil, Chemol Trading Ltd. Co., Budapest). The treatments consisted of (i) a single copper spray applied at the time of first growth flush (1 June), (ii) two copper sprays (1 June and 9 August), and (iii) two oil sprays (30 August and 1 November [1% Agrol; Finca 6 standard treatment]). Ten shoots per tree were tagged after leaves from both first and second growth flushes were fully expanded. Shoots were distributed equidistantly around the canopy to reduce variability resulting from canopy location. Disease incidence, severity, and number of leaves attached to the shoots were quantified on 10 October 1995 and 16 January 1996, as described previously. Data were analyzed by a general linear model pro-

cedure (SAS version 6.08), and means were separated by a least significant difference test.

RESULTS

Ascospore dispersal pattern. During the two periods that ascospore dispersal was monitored, ascospores were trapped first during late April or early May. Peak ascospore discharge occurred at both Finca 6 and El Parque from 28 May to 4 June (Fig. 1) in 1994. In 1995, peak discharge occurred from 7 to 14 May at Finca 6 and from 4 to 11 June at El Parque (Fig. 2). Most of the spores were trapped during a 6- to 8-week period; few ascospores were trapped after early July. For example, in 1994 at Finca 6 more than 5,000 ascospores were trapped per m³ of air per week during the 4 weeks of peak discharge, but the concentration rarely exceeded 80 after mid-July. The greatest numbers of spores were trapped in the nonsprayed orchard at El Parque during both years; however, differences in weekly

numbers of spores trapped were not as great in 1995 as in 1994 (Figs. 1 and 2). Only a few conidia were trapped during the 2 years that the study was conducted.

Three principal discharge patterns were observed. One was associated with rainfall. Large numbers of ascospores were trapped, usually within 1 h of the beginning of rainfall, and discharge often continued for several hours after the rainfall stopped (Figs. 3 and 4). The concentration of ascospores generally increased as rainfall intensity decreased during a rain period. The second discharge pattern was associated with dew. The numbers of ascospores trapped were highest during the early morning (0200 to 0700 h), when the dew was the heaviest (Fig. 5). A third discharge pattern was triggered by a combination of rainfall and dew. Rainfall is frequent during the late afternoon in the San Carlos region of Costa Rica during the rainy season. These late afternoon rains triggered a discharge that occurred during and shortly after the rain-

Fig. 5. Hourly pattern of ascospore catch of *Mycosphaerella citri* released by dew from 21 to 22 May 1995 at Finca El Parque, Costa Rica.

Fig. 6. Hourly pattern of ascospore catch of *Mycosphaerella citri* released by rain and dew from 20 to 21 June 1995 at Finca El Parque, Costa Rica.

fall ended, followed by another discharge associated with dew formation the following morning (Fig. 6). We investigated the relative importance of discharges that occurred during rainfall and dew by tabulating the ascospore trap catches associated with rain, dew, and rain after dew for the principal discharge period each year (Table 2). Both rain and dew were very important in triggering ascospore discharges. In the three periods studied, rainfall alone resulted in the release of 3 to 30% of all ascospores caught during the period of maximum spore discharge, dew 19 to 66%, and combination of rain and dew 31 to 51% (Table 2).

Disease intensity. Generally, few greasy spot symptoms appeared on the May growth flush until August; both incidence and severity remained relatively low at this time. Incidence was <46%, and 1% of the leaf surface was affected (data not shown). During both 1994 and 1995, incidence and severity showed a large increase from August until the final assessment, reaching almost 100% incidence and a severity score of up to 3.2 (approximately 5% of the leaf surface affected) (Table 3). Defoliation, measured only during December 1994, reached an average of 16% for both locations.

Relationship of seasonal pattern of ascospore catch to measures of rainfall. No

consistent relationship was found between total number of rainy days or rainfall totals from 1 January and 1 April and the peak or first ascospore discharges (Table 4). The only association observed was that the accumulated rainfall total from 1 January to first ascospore catch ranged between 223 and 271 mm.

Relationship of ascospore catch to leaf decomposition. The percentage of leaves in stages 1 to 3 of decomposition and the total number of leaves in all stages was a good predictor of the weekly ascospore catch for Finca 6 in 1994 (Table 5 and Fig. 7). In 1995, the only significant relationship was found between the total amount of leaves in all growth stages and total ascospore catch per week (Table 5 and Fig. 8). Data from El Parque were not used, because it was learned that the owner of the orchard periodically raked the leaves from underneath some of the trees included in our study during the time we were collecting the data in both 1994 and 1995.

Timing of fungicide spray. Two copper sprays significantly reduced greasy spot incidence, severity, and defoliation (as indicated by the number of leaves per shoot) compared to the control. There was no significant difference between one copper spray and the control (Table 6). Disease

incidence, severity, and defoliation in the standard grower treatment with two oil sprays did not differ from the unsprayed control.

DISCUSSION

The ascospore discharge pattern observed in the San Carlos region of Costa Rica was closely associated with the seasonal distribution of rainfall. During both years, the number of ascospores trapped increased rapidly through May, reached a peak during early June, declined rapidly through July, and remained low throughout the rest of the year. Peak ascospore discharge occurred earlier in 1994 because of unseasonably wet weather during April when rainfall reached 82 mm, compared to an average of 36 mm, which hastened leaf decomposition and pseudothecial development.

The period of peak discharge observed was shorter than was observed in Florida, occurring over a 4- to 6-week period. Whiteside (5,9) reported that the peak discharge period lasted from 1.5 to almost 4 months. In Los Lirios, February, March, and April typically are drier months, with rainfall averaging 60 (February) and 36 mm (March to April); rainfall increases beginning during late April or early May (Table 1). Most leaf drop occurs during this dry period, because trees become moisture stressed,

Table 2. Number of days of rain, dew, rain plus dew, and percentage of total ascospores released by each event at Finca 6 and El Parque, Costa Rica, during the period of maximum ascospore discharge during 1994 and 1995

Year	Location	Period	Rain ^y		Dew ^z		Rain + Dew	
			Days	% Spores	Days	% Spores	Days	% Spores
1994	Finca 6	6 May–30 Jun.	17	30	20	19	16	51
1995	Finca 6	3 May–18 Jun.	9	13	16	36	22	51
	El Parque	3 May–18 Jun.	2	3	18	66	9	31

^y >0.254 mm/day.

^z Measured by the DeWit leaf wetness meter.

Table 3. Greasy spot intensity measured as incidence, severity, and leaves per shoot, at Finca 6 and El Parque, Costa Rica

Year	Location	Evaluation	Leaves/shoot ^x	Incidence ^y	Severity ^z
1994	Finca 6	14 Dec.	8.8	99.8	3.2
	El Parque	14 Dec.	7.1	95.2	1.9
1996	Finca 6	24 Feb.	6.2	100.0	2.0
	El Parque	24 Feb.	6.9	100.0	1.7

^x Based on 10 shoots on 16 trees in each orchard.

^y Percentage of leaves infected.

^z Severity scale based on percent leaf area with greasy spot symptoms: 1 = 0 to 1%, 2 = 2 to 3%, 3 = 4 to 6%, 4 = 7 to 12%, 5 = 13 to 25%, and 6 = 25 to 50%.

Table 4. Cumulative rainfall (millimeters) and number of rainy days to first and peak ascospore catches at Finca 6 and El Parque, Costa Rica, during 1994 and 1995

Year	Location	First and peak catches	First catch				Peak catch			
			Rainy days		Rainfall		Rainy days		Rainfall	
			From 1 Jan.	From 1 Apr.	From 1 Jan.	From 1 Apr.	From 1 Jan.	From 1 Apr.	From 1 Jan.	From 1 Apr.
1994	Finca 6	30 Apr. and 4 Jun.	41	19	575	455	29	8	234	114
	El Parque	15 May and 4 Jun.	75	30	371	206	70	12	223	58
1995	Finca 6	23 Apr. and 14 May	24	10	339	150	25	7	271	82

Table 5. Regression analysis of leaf numbers in four stages of decomposition on spore catches at Finca 6, Los Lirios, Costa Rica, during 1994 and 1995

Year	Stage ^z	P > F	R ²
1994	0	0.060	0.62
	1	0.003	0.90
	2	0.002	0.92
	3	0.001	0.95
	Total leaves	0.006	0.88
1995	0	0.37	0.16
	1	0.15	0.37
	2	0.89	0.04
	3	0.70	0.03
	Total leaves	0.02	0.71

^z Stage 0 = leaves recently fallen from trees, showing a greenish color with no visual signs of decomposition. Stage 1 = leaves brown with no signs of decomposition. Stage 2 = leaves brown, 1 to 50% of the leaf area partially or completely decomposed. Stage 3 = leaves brown, >50% of the leaf area partially or completely decomposed.

Table 6. Fungicide control of greasy spot on leaves of Valencia orange at Finca 6, Los Lirios, Costa Rica, during 1995

Treatment ^v	Leaves/shoot ^w	Incidence ^x	Severity ^y
Control	6.88 b ^z	83.95 a	1.51 a
Oil (2 sprays)	6.59 b	81.06 ab	1.30 ab
Cu (1 spray)	7.40 ab	75.74 ab	0.91 ab
Cu (2 sprays)	8.17 a	60.75 b	0.83 b

^v Oil 1% in water, Cu 28 g a.i./liter.

^w Measured on 10 shoots in each of four repetitions.

^x Percentage of leaves infected.

^y Severity scale based on percent leaf area with greasy spot symptoms: 1 = 0 to 1%, 2 = 2 to 3%, 3 = 4 to 6%, 4 = 7 to 12%, 5 = 13 to 25%, and 6 = 25 to 50%.

^z Means within the same column followed by the same letter are not significantly different according to a LSD test ($P = 0.05$).

Fig. 7. Relationship between weekly percentage of leaves at different leaf decomposition stages and ascospore catches of *Mycosphaerella citri* at Finca 6, Costa Rica, in 1995. Stage 0 = leaves recently fallen from trees, showing a greenish color with no visual signs of decomposition. Stage 1 = leaves brown with no signs of decomposition. Stage 2 = leaves brown, 1 to 50% of the leaf area partially or completely decomposed. Stage 3 = leaves brown, >50% of the leaf area partially or completely decomposed.

and at the onset of the first growth flush, which usually occurs during May (Table 7). As rainfall increases, leaves on the orchard floor decompose rapidly and serve as a substrate for pseudothelial development and subsequent ascospore release. The rapid decomposition of the fallen leaves under very wet conditions in the San Carlos region and the lack of significant leaf drop throughout the remainder of the year are most likely responsible for the relatively short discharge period and low numbers of ascospores trapped from July through December. Although these ascospores provide inoculum for infection of subsequent growth flushes, these infections are not believed to be as important epidemiologically as those on May's growth flush, because in San Carlos most orange tree leaves are produced during the May flush (Table 7). Typically, >70% of the shoots initiate growth during the May flush and <30% do so in subsequent flushes.

In contrast to reports from Florida and Texas, we found that dew was as important as rainfall in triggering ascospore discharge. These discharges may be very important in the epidemiology of citrus greasy spot in the humid tropics, such as San Carlos. Heavy dew periods occur almost every day from May to January in San Carlos, even on days without rain. For example, in 1994 at Finca 6, during a 46-day period from 3 May to 18 June 1994, there was heavy dew on 12 days that was not accompanied by rainfall (data not shown), which resulted in 31% of the total ascospore catch. Many dew periods exceed 6 h at temperatures no lower than 22°C, which is sufficiently long for ascospores to germinate and initiate infection. Ascospores of *M. citri* can germinate after only 4 h at 30°C and 6 h at 25°C (7). In addition, ascospores can survive drying for periods of up to 142 h (7). Thus, ascospores discharged and deposited during one dew event could germinate or resume growth during a dew event the following night.

Because most of the ascospores are discharged during a relatively short period, the opportunity exists to control citrus greasy spot with one or two correctly timed, fungicide sprays. We found that a single spray of copper oxychloride applied in a mixture with oil provided good control of greasy spot at Finca 6 when applied on 2 June 1995, just after 74% of the shoots had flushed. An additional copper spray resulted in better disease control, because new shoots were not completely expanded at the time of the first spray. In contrast, we found that two applications of 1% oil in water did not provide satisfactory control of greasy spot. These oil sprays were timed on 30 August and 1 November to duplicate the greasy spot control program used by the growers. These results conflict with previous reports in which oil successfully controlled greasy spot on leaves regardless of the time of application (9).

Table 7. Date of main growth flush and flowering of Valencia oranges and cumulative rainfall from March to May during 1992 to 1995 at Finca 6, Los Lirios, Costa Rica

Year	Flush date	% Shoots flushing ^z	Flower date	Rainfall total (mm)		
				March	April	May
1992	May	83	May	28	46	95
1993	April	67	May	77	44	329
1994	May	94	May	36	38	346
1995	May	74	May	32	82	102

^z Percentage of shoots observed flushing on 22 to 25 April or May.

Fig. 8. Relationship between weekly percentage of leaves at different leaf decomposition stages and ascospore catches of *Mycosphaerella citri* at Finca 6, Costa Rica, in 1995. Stage 0 = leaves recently fallen from trees, showing a greenish color with no visual signs of decomposition. Stage 1 = leaves brown with no signs of decomposition. Stage 2 = leaves brown, 1 to 50% of the leaf area partially or completely decomposed. Stage 3 = leaves brown, >50% of the leaf area partially or completely decomposed.

We examined a number of variables to help predict either the onset or the peak period of ascospore release to help improve the timing of fungicide applications. None of the measures of rainfall was a good predictor. During the 2 years of this study, there appeared to be an association with the rainfall amount accumulated from 1 January and the time of peak catch; however, this is probably spurious, because there was no relationship with rainfall amount from 1 April, and there is little leaf fall during January and February. Unfortunately, we did not monitor dew, except during the peak seasonal discharge period. It is likely that dew influences both the maturation and discharge of ascospores. Dew and rainfall should be measured beginning during March, just prior to the major leaf drop period, to determine whether they can be used as predictors of ascospore maturity. We did not find a consistent relationship between the weekly percentage of leaves at the different stages of decomposition and the weekly numbers of ascospores caught at Finca 6 in either 1994 or 1995. We expected to find a significant relationship with leaves in stage 2 of decomposition because leaves in this stage have the more mature pseudothecia. However, there was a significant correlation between weekly numbers of leaves in all stages of decomposition and ascospores trapped.

We found that two sprays of copper fungicides were necessary to significantly reduce the incidence and severity of citrus greasy spot in the San Carlos region of Costa Rica. There was no difference in disease incidence and severity between one or two sprays. Although our fungicide trial needs to be repeated, our results suggest that citrus greasy spot can be controlled successfully in this region with a single copper spray in mixture with mineral oil applied as soon as May's growth flush has expanded fully. We believe that this is possible because of the relatively short peak discharge period characteristic of the region. The application should be made by the second half of June, during or just after peak discharge of ascospores in this region. In most years, the application would be made during the second or third week of June. This spray could be timed more precisely by monitoring the amount of leaves on the orchard floor from 1 April to determine inoculum availability and the stage of development of the susceptible tissue (May's growth flush) to ensure that the majority of new leaves have expanded fully at the time of application.

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