Leaf Spot of Bananas Caused by Mycosphaerella musicola: Role of Conidia in Epidemiology

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ABSTRACT

Conidial production was greater on the upper than on the lower surface of banana leaf spots, and began when the lesion caused by *Mycosphaerella musicola* turned from pale brown to dark brown or black. Conidial sporulation ceased or was greatly diminished when the center of the spot began to turn gray, and did not occur on older spots with gray centers. An average of 5.3 crops of conidia were produced/spot on young plants. Sporulation occurred on successive nights in the absence of rain, provided dew was present, then ceased after a maximum of 12 crops of conidia had been produced. In areas of mass infection where mass necrosis occurred (leaf "burning"), conidial production was reduced about 50%. Conidia, when mature, readily floated off the sporodochia in morning dew or rain water. Conidia were responsible for maintaining various levels of infection in sprayed and unsprayed bananas during prolonged rain-free periods. In the absence of rainy weather favorable for ascospore production and dissemination, conidia were the major source of inoculum responsible for spotting. The amount and prevalence of this spotting was less than in rainy weather and was dependent on dew formation. Phytopathology 60:856-860.

In Honduras, few or no ascospores of *Mycosphaerella musicola* Leach are present during a 3-month dry period, but leaf spotting still persists. Thus, it is possible to determine the role of conidial inoculum in spotting prevalence in the absence of ascospores. The purpose of this paper is to describe the capacity for conidial production in individual leaf spots and the contribution these spores can make to leaf spot incidence.

MATERIALS AND METHODS.—Banana leaf spots on *Musa* AAA cultivar Valery were examined daily in situ with a dissecting microscope placed on an adjustable stool. Ten spots on three to five plants were selected once or twice weekly, and daily observations of number of sporodochia with conidia were made on each spot until sporulation stopped. The banana plants were in an unsprayed surface-irrigated area where mature growth was kept cut back, so only suckers 5 to 6 ft tall exposed to full sunlight were present. This facilitated observation of spots in situ on leaves numbered 6 to 8 counting down from the first open leaf. Young lesions that had recently changed from a pale brown to a dark brown or black color and with a first heavy crop of conidia were chosen. These spots were marked and numbered with a felt pen, and the conidia were washed off with a washing bottle after the dew had dried. These marked spots were then observed for amount of sporulation on successive days late in the morning after most of the dew had dried. Successive crops of conidia were removed with wash water after each observation. In addition, sporulation was observed only once on five spots on each of five randomly chosen plants to determine seasonal changes. Observations were made once or twice weekly over a period of several months encompassing wet and dry weather.

Spotting incidence was recorded bi-weekly in an adjacent unsprayed and sprayed mature plantation. Spray consisted of maneb in an oil-in-water emulsion applied by aircraft. The percentage of plants with spots and the average age of the youngest leaf with spots, as indicated by leaf number counting down from the first open leaf, were recorded on medium-sized plants that had not flowered. The younger the leaf on which spotting first appears, the more favorable are conditions for infection and disease development. Also, average age of youngest leaf spotted can be correlated with the prevalence and intensity of spotting in the area (9). For example, spotting is much more severe when the average age of the youngest leaf spotted is number 8 down from the first open leaf than when it is number 11. This system (9) of measuring spotting prevalence and intensity was used to determine the role conidia played in maintaining spotting at a time when ascospores were few or absent because weather was unfavorable for their production. These data were supplemented with streak counts made every 1 to 2 weeks on leaves 2 to 4 in a nearby commercially-sprayed plantation.

Maximum and minimum temperatures were recorded 16 ft above the soil in a standard U.S. meteorological box. A Hirst spore trap was also in operation on the platform to monitor presence or absence of ascospores of *M. musicola*. Hours of leaf wetness were recorded at the same height on a DeWit instrument having an accuracy of plus or minus 5%.

RESULTS.—*Conidial production from individual spots.*—Conidia were produced more frequently and in larger numbers on the upper than on the lower surface of spots. Conidia were more readily removed from the sporodochia in dew or guttation water on the lower than on the upper surface. Mites were frequently numerous on the lower leaf surface, and moved over the leaf spot removing conidia from the sporodochia. For these reasons, studies were conducted on the upper surface of the spots.

Sporulation began in quantity when the brown lesion began to turn into a dark brown or black spot with clearly defined borders (Fig. 1-A). Conidial sporulation was greatly diminished when the center of the spot began to turn light gray (Fig. 1-B). Sporulation usually did not occur on mature spots with a completely gray center (Fig. 1-C), and if conidia were present, they were few in number. For example, only 17% of the

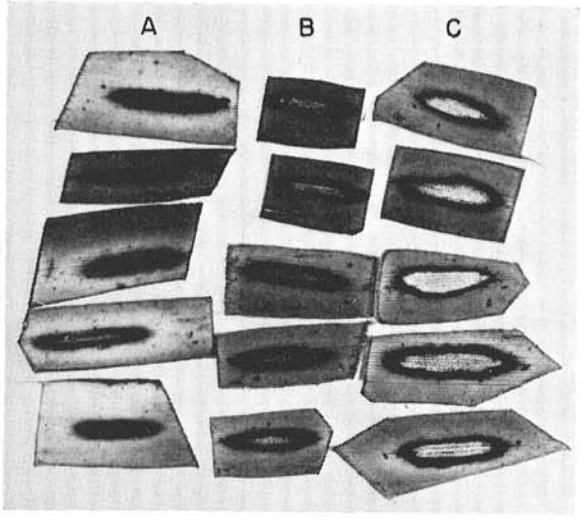

Fig. 1. Stages in maturation of banana leaf spots caused by *Mycosphaerella musicola*. **A)** Young spots that have recently turned from a brown lesion to a black spot with a well-defined border; **B)** centers of black spots have started to turn pale gray; **C)** mature black-bordered spots with center of spot pale gray. Conidia production is at a maximum in stage A, begins to decline and usually stops as the center turns grey (stage B) and sporulation seldom occurs in stage C. Spots pass from stage A to B in 3 to 5 days.

spots ceased sporulation in stage A (Fig. 1), 82% at stage B, when the centers began to turn light gray, and only 1% at stage C—mature spots with pale grey centers. Thus, when the black spots begin to turn grey, the period of maximum conidial production has passed.

An average of 5.3 crops of conidia were produced/spot, and a maximum of 8-12 crops were obtained on some spots (Table 1). In areas where leaf "burning" or rapid necrosis of tissue in between spots occurred as a result of mass infections, an average of only 3-4 crops of conidia was produced. Sporulation occurred

TABLE 1. Conidia cropping history of individual banana leaf spots (*Mycosphaerella musicola*)

Date observations began	No. crops	No. crops
1967	*range*	*avg*
9/29	3-12	6.9
10/24	2-8	4.8
11/20	4-8	6.3
11/27	2-4	2.7
12/8	2-4	3.2
12/11	3-5	4.4
12/18	2-7	4.8
1968		
1/3	4-6	4.9
1/9	4-6	5.6
1/19	3-5	4.3
2/12	3-9	5.8
2/22	4-8	5.9
3/4	4-7	5.8
3/18	4-7	5.3
4/3	4-7	5.6
4/15	7-10	7.9
4/23	3-7	6.1
5/10	2-8	5.2
5/20	4-5	4.5
Average		5.3

on successive nights in the absence of rain, provided dew was present. Not all sporodochia, however, produced spores on successive nights. Conidia that did not mature before the dew dried could mature the following night and be removed in dew water. Sporulation did not occur during nights when breezes, often from the south, or heavy cloud cover prevented dew formation. Southerly breezes were associated with hot, dry weather. Conidia, when mature, readily floated off the sporodochia in moving dew or rain water. For this reason, it is likely that the number of crops of conidia produced is underestimated.

Conidial production in relation to weather.—During rain-free periods in Honduras and during the dry sea-

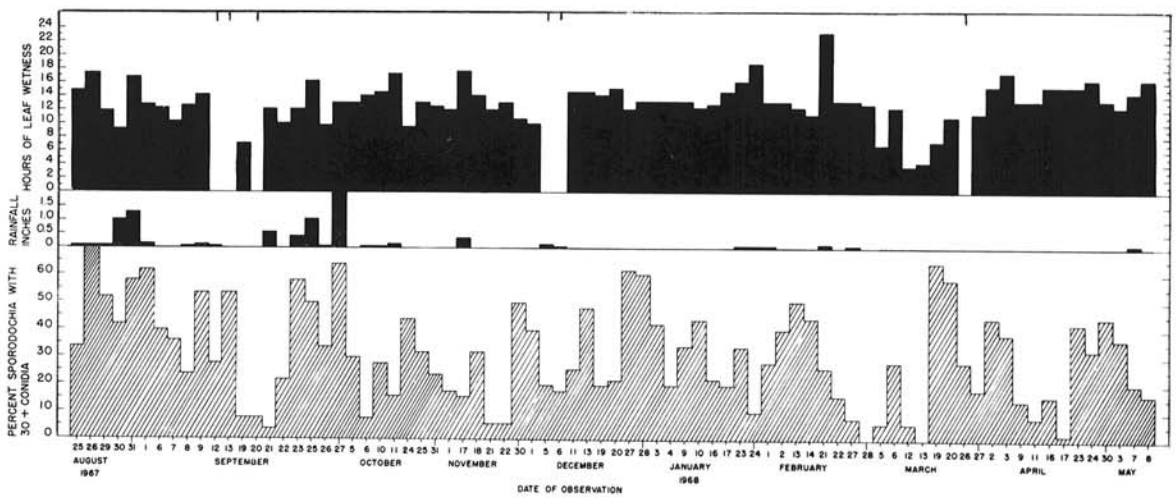

Fig. 2. Conidia production on sporodochia of *Mycosphaerella musicola* in relation to rainfall and hr of leaf wetness (mostly from dew). Short vertical lines at top indicate DeWit leaf wetness recorder not functioning.

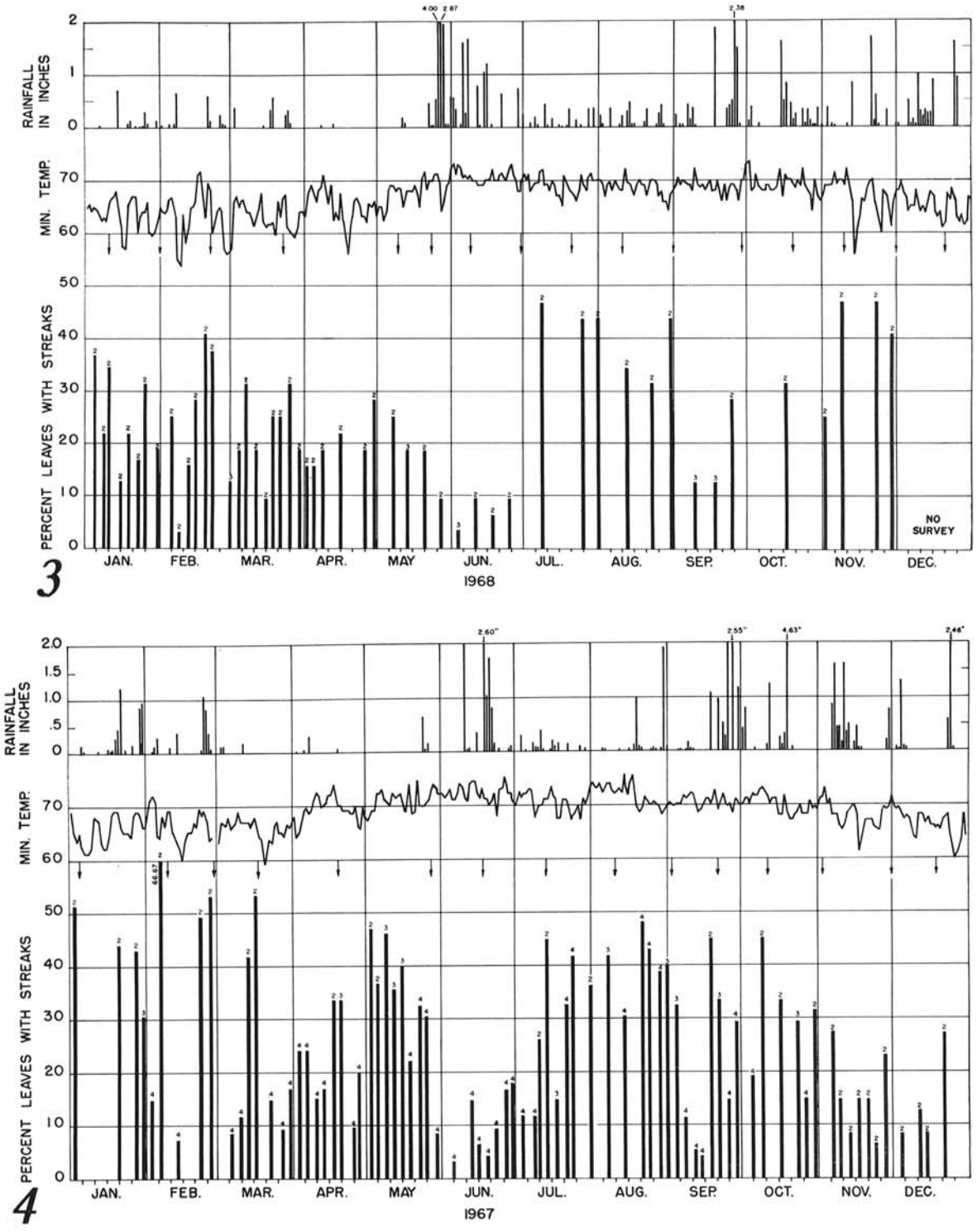

Fig. 3-4. Percentage of medium-sized nonflowered banana plants with streaks caused by *Mycosphaerella musicola* during 1967 (below) and 1968 (above) in relation to rainfall and temp. Streaks were counted twice weekly until April 1968, and weekly thereafter. Arrows indicate when spray was applied; numbers above bars refer to number of youngest leaf on which streaks first appear. Note that streaks appear throughout the year even in the absence of rain.

son of March to May, leaves are wet more than 10 hr nightly (8). Observations during prolonged rain-free periods in 1967-1968 (Fig. 2) showed that conidial production was frequently abundant, as a result of long hours of leaf wetness. Data from the DeWit leaf wetness recorder were verified by visual observation. Dew often began to form on leaves between 8:00 and 9:00 PM, and the heavy dew deposition did not completely disappear until after 10:00 AM the following day. This frequently resulted in 12 to 14 hr of leaf wetness (Fig. 2), and permitted production and maturation of some conidia even when minimum temperatures were below 20 C.

There was no day-to-day correlation between hr of leaf wetness, rainfall, temp, and number of sporodochia with conidia. Correlations of this nature are probably masked by factors such as spot maturity, vitality of the pathogen in spot tissue, and rapidity of necrosis, all of which could influence amount of sporulation. Also, conidia are often removed from sporodochia before counting by dew, rain, and guttation water, especially when there has been leaf movement.

Contribution of conidial inoculum to amount of spotting.—In Honduras, streaks appear on leaves numbered 2 to 4 throughout the year in fluctuating quantities regardless of spray applied (Fig. 3, 4). Even during the dry season months of March to May and in June, following the driest period in May, streaks appear. However, the percentage of leaves with streaks is lower, and they may appear on leaf number 4 instead of 2 or 3, indicating a longer incubation period as a result of unfavorable weather for infection and incubation. During these dry months, ascospores of *M. musicola* were rarely found on Hirst spore traps even when placed adjacent to spotted, unsprayed bananas. Furthermore, few perithecia are produced in the absence of rain (8). These streak data (Fig. 3, 4) and data in the previous section showing abundant conidial production during dry weather prove that conidia are the major source of inoculum during dry weather. Also, as a result of long hours of leaf wetness from dew on successive nights, this inoculum can be infective and may result in streaks.

The percentage of plants with spots and average age of youngest leaf spotted during the period when conidial production was observed are shown in Fig. 5 for the adjacent unsprayed and sprayed areas. During the dry, cool periods, the percentage of plants with spots declined in the sprayed area, and spotting appeared on older leaves in the unsprayed area. However, as a result of conidial production and sufficient leaf wetness from dew, spotting continued to develop on a majority of sprayed and unsprayed plants in the absence of rainfall and ascospores. In the sprayed area (Fig. 5), when the spray interval was increased to more than 40 days between March and May, spotting actually increased during the dry period in April-May. Almost 100% of the medium-sized plants had spots, and spots appeared on leaf No. 8. This shows that in the absence of spray, spotting can gradually build up in the dry season as a result of conidial inoculum.

DISCUSSION.—Studies reported here confirm previous research (2, 4, 5, 6) showing that dew is the essential factor for conidial production in the absence of rain. In Honduras, 10-12 hr of leaf wetness from dew is usually present during dry weather except for occa-

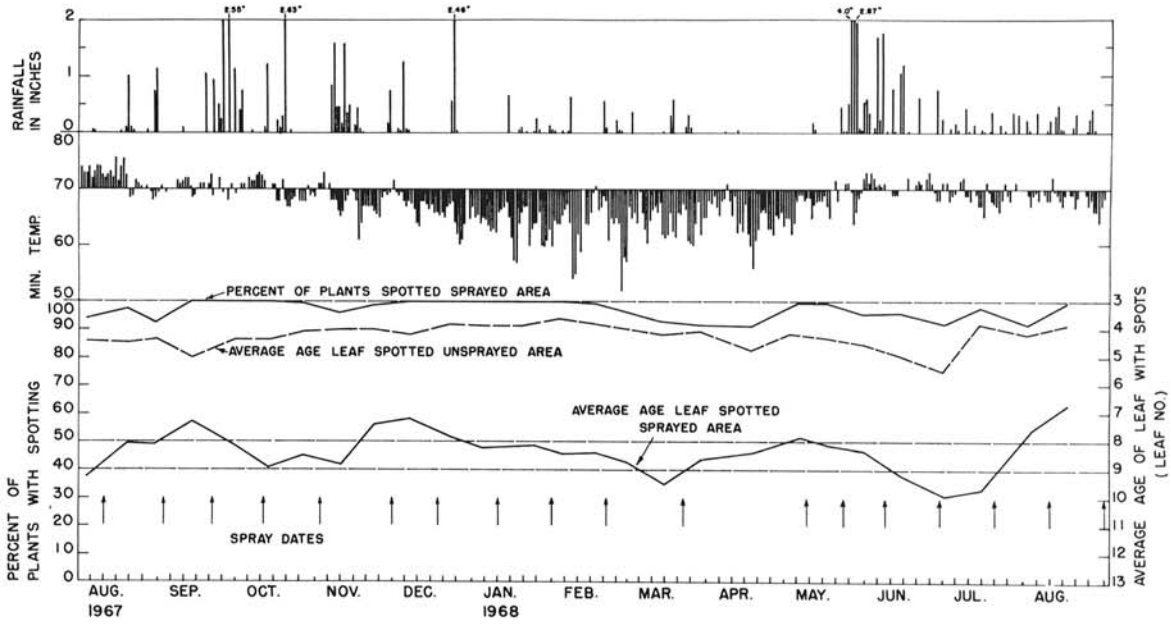

Fig. 5. Average age of youngest leaf with spots, and percentage of plants spotted in sprayed and unsprayed areas of Honduras in relation to rainfall and temp. In the unsprayed area, 100% of the medium-sized nonflowered plants were spotted throughout the year (not shown on graph). Note that various levels of spotting are present throughout dry weather, and spotting can actually increase in rain-free periods if spray cycles are omitted. This spotting resulted from conidial inoculum.

sional short periods of 1-3 days, when a southerly breeze is present or the sky is overcast at night. Thus, conidial inoculum is available continuously throughout the year and is the major source of infection during rain-free periods when conditions are unfavorable for ascospore production or dissemination. This was indicated in previous studies (10), where infections from conidia were identified on individual leaf blades by the basal orientation of spotting that appeared during or following dry weather. Ascopore infections result mainly in an apical and not a basal spotting pattern (1, 5). Nevertheless, as a result of minimum night temp below 20 C during March-April dry weather, fewer conidia are produced than during the warm wet months of June to October (7). Therefore, decreased conidial production during the dry season as well as the absence of ascospores contribute to the decline in spotting intensity. Nonetheless, sufficient spotting can build up from conidial inoculum to make spraying necessary.

Black spots are the major source of conidia, and once the spots turn gray, conidial production soon stops (3, 4). Thus, trash leaves are not an important source of conidial inoculum. Calpouzos (2) obtained a maximum of 13 spore crops/spot in Cuba. Kranz (4) found a maximum of 23 days for duration of sporulation in Guinea with an average of 7-18. In Honduras, an average of 5.3 and a maximum of 8-12 crops were obtained. Sporulation may be less on the younger plants observed than on more mature plants. Also, rapid necrosis of tissue adjacent to the spots reduced the sporulation period. In addition, cropping could have been underestimated because of prior removal of conidia in dew with leaf movement.

Observations with an incident light microscope showed that a sporodochium produced an average of 50 conidia/crop. Thus, 265 conidia/fructification were produced with an average of 5.3 crops. A perithecium produced an average of 160 ascospores, and there was no multiple cropping. Thus, a sporodochium produces more spores than a perithecium. Also, previous studies (8) showed that sporodochia are produced in much larger numbers than perithecia in Honduras at all times, and in other areas during most of the year. Therefore, conidia contribute many times more spores to the inoculum pool during the year than ascospores. Yet each spore form has a distinct but complementary role in pathogen survival and disease increase (Table 2). This comparison of the two spore forms shows that *M. musicola* is well adapted to a variety of tropical environments wherever rain or dew is present. Pleomorphism ensures not only survival but disease increase during both wet and dry seasons. When there is no rain but dew is present, the conidial form can increase spotting to a level necessitating control measures. When rain is frequent, both conidia and ascospores contribute to a rapidly increasing epidemic disease that is difficult to control. Windborne ascospores can rapidly move disease outward from primary foci of infection, whereas conidial infection is localized mostly on lower canopy leaves below or nearby spotted leaves.

TABLE 2. Contrasting features of *Mycosphaerella musicola* spores

Conidia	Ascospores
Produced daily when dew present	Produced periodically when rain present
Produced in absence of rain	Rain-dependent
Released by dew and rain	Released mainly by rain
Water-disseminated	Wind-disseminated
Infection over entire leaf but with basal tendency	Infection mostly apical
Survived 3-4 weeks on leaf surface[a]	Survived 8-14 weeks in the perithecium[a]
Lower temp optimum for germination and germ tube growth	Higher temp optimum
Can increase disease in "dry" season	Little or no "dry" season infection

[a] Survival times are from unpublished data.

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