Effects of Leaf Wetness Duration and Temperature on Development of Black Sigatoka Disease on Banana Infected by *Mycosphaerella fijiensis* var. *difformis*

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ABSTRACT

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Severity of Black Sigatoka disease on banana leaves infected by either conidia or ascospores from two isolates of *Mycosphaerella fijiensis* var. *difformis* was influenced by temperature and leaf wetness duration. A film of water on the leaf surface was required for ascospore infection. Conidial infection was observed at a leaf wetness of 0–18 h. Initial foliar symptoms of Black Sigatoka disease required an incubation period of 14 days on leaves subjected to 18 h of leaf wetness after inoculation. Symptom expression was delayed 7–14 days at lower levels of leaf wetness. Disease severity increased with increasing leaf wetness duration. The optimal temperature range for disease development was 25–28 C. Older leaves were more conducive to disease development. The arcsin of disease severity as a linear combination of temperature, leaf wetness, and leaf age best described the infection response ($R^2 = 0.84$–0.91). Differences in area under the disease progress curve were found between the isolates.

Banana leaf spot or Black Sigatoka, caused by *Mycosphaerella fijiensis* Morelet var. *difformis* Mulder and Stover (9,14), is a destructive disease of banana (*Musa acuminata* Colla) in the tropics (4,6,17). Between 1977 and 1980, the Black Sigatoka pathogen spread to southern Mexico and throughout Central America (14,17). In Central America, Black Sigatoka is the most damaging and costly disease; it accounts for 27% of production costs (16).

At present, little information on the epidemiology of *M. f. difformis* has been published. Most of our knowledge is from

field observations of the disease and pathogen under natural infection (2,15). Studies conducted in controlled environments would make it possible to isolate the effect of specific factors and remove the confounding stresses imposed under field conditions.

Ascospores are considered the major source of inoculum of *M. f. difformis* responsible for Black Sigatoka infections in banana plantations (14). Stover (14,15) suggested that temperatures above 21 C and a film of water favor rapid development of leaf spotting; the optimum temperature range for disease development was 26–28 C. A previous study (5) indicated that ascospores required free water or a nearly saturated environment (relative humidity [RH] of 98–100%) for germination and germ tube growth. Conidia, in contrast, germinated in a wider range of RH (92–100%). The conditions in Honduras, Central America, during the dry season (February–May) are not conducive to ascospore infection, and ascospores are not generally trapped with Kramer-Collins spore traps (Electric Manufacturing Co., Manhattan, KS) during this period. But, disease symptoms do appear on new leaves. If one considers the lower RH requirements for germination, conidial infection could be responsible for disease development during the drier part of the growing season. The effect of environmental factors on disease development from conidial infection has not been studied. In this study, we investigate the effects of selected environmental factors on infection of banana plants by conidia and ascospores of *M. f. difformis* under controlled conditions. Determination of optimal conditions for infection should help in the understanding of the development of field epidemics, assist in the formulation of mathematical models, and contribute to the improvement of forecasting and disease management methods.

MATERIALS AND METHODS

Plant material. Banana plantlets of the susceptible Grand Nain (Musa, AAA) cultivar, obtained by tissue culture (Oglesby Plant Lab Inc., FL), were transplanted into 10-cm-diameter plastic pots containing a steam-pasteurized mixture of soil/peat/perlite (1:1:1, v/v/v). Clonal host material was used to remove the effect of genetic variability. The plants were grown for 3 wk in a growth chamber (M12-3.5K, EGC, Chagrin Falls, OH) at 26 C, with a RH of >90%, and a 12-h photoperiod. During this period, the plants were watered with Hoagland's solution. The plants were then transferred to greenhouse benches, where they were grown under 47% shade for 2–3 wk. After this acclimatization period, the shade was removed, and the plants were maintained in the greenhouse until inoculation. The plants were fertilized every 2 wk with Peter's 20-20-20 (N-P-K) fertilizer.

Inoculum production. Infected banana leaf tissue samples were collected from two locations in Honduras: Santa Barbara (SB), a northwestern region with no history of chemical control of banana diseases (wild-type); and La Fragua (FR), a commercial farm in the Sula Valley, where chemical disease control is employed. We obtained fungal cultures from ascospores discharged from infected tissue by using the technique described by Stover (12). Single germinating ascospores were transferred to 3.6% Mycophil agar (11445, Baltimore Biological Laboratory, Baltimore, MD) in petri dishes and incubated at 25 C. The resulting conidia-producing colonies were used for further subculturing. Mycophil agar in petri dishes was used for conidial production and was inoculated with a conidial suspension from each isolate (SB or FR). A conidial suspension was obtained by placing a 14- to 18-day-old fungal colony into a test tube containing 0.05% Tween 80 in distilled water and mixing on a vortex mixer for 1 min. Each dish was inoculated by uniformly spreading 0.25 ml of a conidial suspension, adjusted to 2×10^3 conidia per milliliter, on the agar surface with a sterile glass rod. All cultures were incubated at 20 C under 2.5 W m^{-2} of continuous, cool-white, fluorescent light.

Conidial suspensions for inoculation were prepared from the multiple-seeded Mycophil agar dishes. Cultures were 18–21 days old when used. Each dish was flooded with 5–10 ml of 0.05% Tween 80 in distilled water. The conidia were dislodged by gentle agitation with a camel's hair brush, and suspensions were adjusted to approximately 10×10^3 conidia per milliliter. The viability of the conidia was 90–96% in germination tests on water agar at 25 C.

Inoculation with conidia. Banana plants, bearing six expanded leaves, were used. Before inoculation, a target area of 7 × 12 cm was delineated with a water-resistant black marker on the right, lower surface of the leaf. The whole leaf, with the exception of the target area, was covered with a sheet of paper. A new paper cover was used for each leaf. We inoculated the four youngest open leaves by spraying 1 ml of the conidial suspension (10×10^3 conidia per milliliter) onto the target area with a Chromist atomizer (cat. no. 51901, Gelman Instrument Co., Ann Arbor, MI) from a distance of 20–25 cm. The conidial suspension was deposited as a uniform layer of minute droplets, and no runoff occurred. After plants dried for 30–45 min on the laboratory bench, they were subjected to the corresponding leaf wetness and temperature treatments. Leaf wetness treatments were done under continuous darkness.

Inoculation with ascospores. We inoculated banana plants, bearing four expanded leaves, by discharging ascospores from infected banana leaf tissue. The plants were suspended upside down at 90–120 cm from the ground and wired to a grill inside a large growth chamber. A two-piece cardboard platform around the pseudostem of each plant was also fixed to the grill about 20 cm above the leaves. Using the technique described by Stover (13) for ascospore discharge, we taped the lid of a petri dish containing wet infected tissue onto the cardboard platform just above and facing the target leaf. The two youngest open leaves were inoculated in this manner. After 30–45 min, the inoculum source was removed. Immediately afterwards, plants were subjected to the corresponding leaf wetness and temperature treatments.

Disease assessment. Disease severity was defined as the percentage of the target area covered by lesions of Black Sigatoka disease. Tagged leaves were monitored daily from inoculation to first symptom appearance. Thereafter, 10 weekly disease assessments were conducted. We scored disease severity separately on each leaf by using a diagrammatic scale developed from leaves showing various disease intensities. Sketches of actual Black Sigatoka lesions were drawn on transparent paper and then transferred to a 7- × 12-cm card of white paper. By cutting off the lesion surfaces, we computed the diseased and healthy areas with an electronic leaf area meter (model Li-3000, Li-Cor, Lincoln, NE). Thus, a set of cards diagrammatically representing 10 degrees of severity of Black Sigatoka disease (0.5, 1.0, 3.0, 5.0, 7.5, 10.0, 15.0, 20.0, 30.0, and 40.0% of the area covered) was used as standard reference. Symptom appearance and further disease development, number of lesions, and percentage of necrotic tissue were recorded. We computed area under the disease progress curve (AUDPC) by using the trapezoidal integration of disease severity over time for each treatment replication. Incubation period was defined as the time when 50% of the maximum lesion number became visible.

Experimental design. The experiment was repeated three times in a split-plot design with temperature as the whole plot and a factorial arrangement of isolates and leaf wetness as the subplot. The leaves were considered nested on plant. Twelve plants per isolate were inoculated each time the experiment was conducted. After being inoculated with conidia, the plants were subjected to 0, 9, or 18 h of leaf wetness (dew) in a dew chamber (E-540 DL, Percival Manufacturing Inc., Boone, IA) at 26 C. Immediately after being inoculated with ascospores, the plants were subjected to 0, 6, 12, or 18 h of leaf wetness in a dew chamber at 26 C. After the corresponding wet period, the plants were distributed among four growth chambers at 22, 25, 28, or 31 C. Because preliminary results showed no infection at an RH of 90%, the growth chambers were set at an RH of ≥94%. Temperature and relative humidity were monitored with a HMI 32 temperature-relative humidity sensor (Vaisala Corp., Finland). A combination of six incandescent and 16 fluorescent (Sylvania cool-white, Sylvania Lighting Equipment, Fall River, MA) lamps, with an intensity of 20 W m^{-2} at a distance of 60 cm from the lamps to the upper youngest leaves, provided a 12-h photoperiod.

Statistical analysis. Statistics were computed for final disease severity, number of lesions, and percentage of leaf necrosis. AUDPC was the random variable used for the overall comparison of the epidemics (1). We calculated preplanned orthogonal contrasts to evaluate whether younger and older leaves differ in their susceptibility to disease. We compared treatment combinations by using analysis of variance with the SAS general linear model (12).

We developed models describing the combined effect of temperature, leaf wetness duration, and leaf age on final disease severity by using the SAS stepwise procedure, with the maximum R^2 improvement option (12). The general model was

$$y = f(T,LW,A) \quad (1)$$

in which y is the arcsin transformed disease severity as a linear function of temperature (T), leaf wetness duration (LW), and leaf age (LA). Linear and quadratic terms of T and LW and their interactions were tested. The models were chosen based on the significance of the estimated parameters, normality of residuals, coefficient of determination, and Mallow's Cp values (7).

RESULTS

Symptom development. Under the conditions of this experiment, both kinds of propagules induced typical symptoms of Black Sigatoka disease. The first symptom noted was the appearance of brown streaks 1–2 mm long on the lower leaf surface. The streaks enlarged and darkened over time and became elongated or nearly rounded spots with dark brown to black centers. Advancing chlorotic and necrotic tissue was observed and recorded over time. Dead tissue of mature lesions became grayish-white. These symptoms were comparable to those observed under tropical conditions in the field.

Isolate effect. Both isolates, SB and FR, were pathogenic on young banana plants under the conditions of this study. In general, the disease severity induced by the wild-type isolate (SB) was higher than that induced by isolate FR. The wild-type isolate also caused more lesions and induced more necrosis independent of temperature and leaf wetness (Table 1). In general, higher AUDPC values were computed for the wild-type isolate (Table 2). Analysis of variance of final disease severity, AUDPC, and percentage of leaf necrosis data indicated highly significant ($P < 0.001$) effects of isolates, temperature, leaf wetness duration, and their interactions. Temperature had no significant effect on the number of lesions. The infection response was evaluated separately for each isolate, and means were compared at fixed levels of temperature and leaf wetness.

Leaf effect. Higher disease severity was observed as leaf age at inoculation increased (Table 3). Orthogonal contrasts indicated significant differences ($P < 0.006$) in final disease severity and AUDPC among leaves. Independent of isolates, younger leaves (1 and 2) developed significantly less disease ($P = 0.001$) than older ones (3 and 4). Leaf 4 (~32 days old at inoculation) developed significantly higher ($P = 0.0007$) disease severity than younger leaves. Orthogonal contrasts indicated no significant difference ($P = 0.52$) in number of lesions among leaves but highly significant differences ($P = .004$) in percentage of leaf necrosis among leaves. Older leaves (~24–32 days old at inoculation) developed more necrosis. Higher levels of leaf necrosis were observed earlier on leaf 4, followed by leaves 3, 2, and 1 (~24, 16, and 8 days old at inoculation, respectively).

Leaf wetness and temperature effects. Infection of banana leaves by ascospores of *M. f. difformis* was not observed in the absence of leaf wetness. More variation in disease severity and number of lesions, between and within treatments, was observed at leaf wetness durations from 6 to 18 h. Thus, the data from ascospore infection was unusable for further analysis of main effects and is not included hereafter.

Infection of banana leaves by conidia of *M. f. difformis* occurred at leaf wetness durations of 0–18 h. Brown streaks (young lesions) were first observed 14 days after inoculation on plants subjected to 18 h of leaf wetness. Disease appearance was delayed 7 and 14 days on plants subjected to 9 and 0 h of leaf wetness, respectively. Disease symptoms developed at temperatures from 22 to 31 C. In general, the wild-type isolate (SB) induced more disease at 25 and 28 C than isolate FR. Disease severity was similar for both isolates at 22 C. At 31 C, isolate FR induced 2–2.5 times more disease than the wild-type isolate (SB). Disease severity increased at all temperatures when leaf wetness duration increased (Table 2). The optimum temperature was 28 C at the two lower levels of leaf wetness and 25 C at 18 h of leaf wetness. Higher AUDPC values were observed at 28 C and 25 C on leaves subjected to 0 or 9 h and 18 h of wetness, respectively. The highest AUDPC values were observed on the older leaves subjected to 18 h of wetness; these values were independent of isolates and temperature (Table 3).

More lesions and a higher percentage of leaf necrosis were observed at 22 and 25 C followed by 28 and 31 C, and at 25 and 28 C followed by 31 and 22 C, respectively, independent of isolates and leaf wetness. However, more lesions were observed at 25 C and 18 h of wetness and at 25 C and 9 h of wetness for isolates SB and FR, respectively. A higher percentage of leaf

TABLE 1. Effects of temperature and leaf wetness on mean (standard error) of final disease severity, number of lesions, and percentage of leaf necrosis resulting from conidial inoculations of two isolates of *Mycosphaerella fijiensis* var *difformis*

Symptom[a]	LW[b]	Temperature (C) 22	25	28	31
Disease severity (%)					
SB	0	1.68 (0.11)	7.40 (0.86)	11.54 (1.52)	1.88 (0.26)
	9	4.02 (0.46)	10.94 (1.08)	15.17 (1.62)	2.38 (0.26)
	18	4.88 (0.38)	30.00 (5.05)	24.67 (3.55)	3.02 (0.24)
FR	0	2.84 (0.26)	4.72 (0.65)	6.03 (0.53)	2.30 (0.11)
	9	3.12 (0.18)	11.80 (1.71)	12.96 (1.64)	3.80 (0.22)
	18	3.52 (0.30)	21.13 (1.84)	11.52 (0.66)	5.49 (0.57)
Number of lesions					
SB	0	48.50 (6.74)	25.17 (3.51)	50.00 (7.81)	25.83 (3.23)
	9	62.75 (5.21)	46.42 (4.45)	48.42 (7.76)	28.67 (4.50)
	18	72.67 (6.25)	81.67 (7.77)	62.08 (7.86)	32.58 (3.35)
FR	0	46.42 (4.27)	26.08 (1.82)	22.25 (1.82)	25.33 (1.86)
	9	52.67 (6.44)	66.83 (7.89)	58.58 (8.43)	28.42 (2.95)
	18	44.50 (5.81)	60.83 (7.92)	44.33 (7.74)	28.00 (2.18)
Percentage of leaf necrosis					
SB	0	17.92 (4.63)	46.83 (9.56)	42.75 (7.69)	29.08 (9.84)
	9	20.00 (4.69)	39.92 (8.56)	40.67 (9.87)	33.50 (8.53)
	18	15.00 (4.33)	66.33 (9.11)	49.58 (9.21)	38.67 (8.53)
FR	0	18.50 (4.89)	20.00 (4.32)	23.67 (7.09)	6.67 (4.14)
	9	18.25 (4.46)	25.50 (8.38)	36.50 (8.65)	23.58 (6.46)
	18	29.50 (6.51)	31.50 (9.36)	38.17 (8.42)	21.67 (6.11)

[a]SB = wild-type isolate from Santa Barbara, Honduras; and FR = isolate from La Fragua farm in the Sula Valley, Honduras.
[b]Leaf wetness duration in hours.

TABLE 2. Effects of temperature and leaf wetness on the mean (standard error) area under the disease progress curve (AUDPC) resulting from conidial inoculations of two isolates of *Mycosphaerella fijiensis* var *difformis*

Isolate[a]	LW[b]	AUDPC at temperature (C) 22	25	28	31
SB	0	0.36 (0.02)	1.16 (0.14)	2.38 (0.44)	0.35 (0.05)
	9	0.59 (0.07)	1.98 (0.15)	2.98 (0.39)	0.37 (0.04)
	18	0.70 (0.06)	6.91 (1.12)	4.96 (0.77)	0.42 (0.04)
FR	0	0.54 (0.06)	0.64 (0.10)	1.02 (0.12)	0.54 (0.04)
	9	0.59 (0.06)	2.04 (0.41)	2.51 (0.45)	0.67 (0.05)
	18	0.66 (0.07)	4.15 (0.49)	2.03 (0.15)	1.01 (0.10)

[a]SB = wild-type isolate from Santa Barbara, Honduras; and FR = isolate from La Fragua farm in the Sula Valley, Honduras.
[b]Leaf wetness duration in hours.

necrosis was induced at 25 C and 18 h of wetness and at 28 C and 18 h of wetness by isolates SB and FR, respectively.

No difference in AUDPC was found between the isolates at 22 C. The AUDPC for isolate SB at 25 and 28 C was significantly higher ($P = 0.001$) than that for isolate FR. However, a significantly higher AUDPC was computed for isolate FR at 31 C ($R^2 = 0.81$–0.94). On the other hand, no difference in AUDPC was found between the isolates at the intermediate level of leaf wetness (9 h). But, the AUDPC values for isolate SB were significantly lower ($P = 0.001$) and higher ($P = 0.003$) than those for isolate FR at 0 and 18 h of wetness, respectively ($R^2 = 0.72$–0.79).

AUDPC means were compared at fixed levels of leaf wetness and temperature by using the Waller-Duncan k-ratio t test with preassigned $P = 0.05$. Significant differences among temperatures were found at each level of leaf wetness, independent of isolates ($R^2 = 0.70$–0.86). Higher AUDPC values were observed at 28 C followed by 25, 22, and 31 C under 0 or 9 h of wetness for both isolates, and at 25 C followed by 28, 22, and 31 C for isolate SB and at 25 C followed by 28, 31, and 22 C for isolate FR under 18 h of wetness. However, no significant difference was found between 25 and 28 C for isolate SB at 9 h and at 25, 22, and 31 C at 0 h of wetness, respectively, or between 22 and 31 C for both isolates.

Significant differences ($P < 0.001$) in AUDPC were found among leaf wetness levels at each temperature ($R^2 = 0.73$–0.96). Independent of isolates, higher AUDPC values were observed at 18 h followed by 9 and 0 h of leaf wetness. No significant difference was found between the two lower levels of wetness (0 and 9 h) at 25, 28, and 31 C for isolate SB and at 22 C for isolate FR. No significant difference was found between 9 and 18 h of leaf wetness at 28 C for isolate FR.

No significant difference was found in the number of lesions at 22, 25, and 28 C, but a significant ($P = 0.01$) 50% reduction in the number of lesions was observed at 31 C, independent of isolates and leaf wetness duration. The number of lesions increased ($P = 0.002$) at higher levels of leaf wetness for both isolates. However, no significant difference was found between 9 and 18 h of leaf wetness for isolate FR. More lesions were observed at 18 and 9 h of leaf wetness for isolates SB and FR, respectively, independent of temperature (Table 1). A significant effect of leaf wetness duration was observed only at 22 and 25 C for isolate SB. The effect of leaf wetness on infection was only significant at 25 and 28 C for isolate FR.

The percentage of leaf necrosis increased at higher levels of leaf wetness. However, no significant differences in percentage of leaf necrosis were found among the levels of leaf wetness for isolate SB, or between 9 and 18 h of leaf wetness for isolate FR, independent of temperature except for isolate FR at 22 C. No significant difference in leaf necrosis was found between 25 and 28 C or between 22 and 31 C for the isolate SB, independent of leaf wetness duration. Necrosis was significantly highest at 28 C for isolate FR.

The length of the incubation period, defined as the time until 50% of the maximum number of lesions became visible, was influenced by temperature and dew period for both isolates (Table 4). In general, extending the dew periods resulted in shortened incubation periods; this was independent of isolate. Shorter incubation periods were observed at 25 and 28 C as compared to 22 and 31 C. Isolate FR had shorter incubation periods at 31 C as compared to SB. The reverse situation was observed at 25 C.

Model development. The combined effect of temperature (T), leaf wetness duration (LW), and leaf age in days (A) on disease severity caused by conidial infection was best described for each of the isolates by the following equation:

$$y = b_0 + b_1T + b_2LW + b_3T^2 + b_4TLW + b_5T^2LW + b_6A \quad (2)$$

in which y = arcsin x, x = proportion of diseased area, and b_0–b_6 = the regression coefficients ($P < 0.001$). Disease severity followed a quadratic response function on temperature; higher values were observed at 25 and 28 C, followed by a drastic reduction at 22 and 31 C. Disease severity increased at all temperatures when leaf wetness duration increased. Both isolates showed similar response patterns, but each had different magnitudes (Table 5). The response of the isolate SB was more pronounced at 25–28 C when compared to the more flattened response of isolate FR.

TABLE 3. Effects of temperature and leaf wetness on the mean (standard error) area under the disease progress curve (AUDPC) from leaves resulting from conidial inoculations of two isolates of *Mycosphaerella fijiensis* var *difformis*

Isolate[a]	LW[b]	Leaf[c]	AUDPC at temperature (C)			
			22	25	28	31
SB	0	1	0.32 (0.03)	0.78 (0.16)	1.35 (1.26)	0.19 (0.02)
		2	0.35 (0.02)	1.00 (0.29)	1.77 (0.18)	0.22 (0.01)
		3	0.38 (0.06)	1.15 (0.17)	1.18 (0.10)	0.45 (0.02)
		4	0.40 (0.04)	1.71 (0.17)	3.21 (0.93)	0.55 (0.04)
	9	1	0.32 (0.02)	2.10 (0.24)	3.69 (1.41)	0.18 (0.01)
		2	0.50 (0.08)	1.70 (0.31)	2.36 (0.47)	0.32 (0.01)
		3	0.69 (0.08)	1.60 (0.06)	2.45 (0.38)	0.45 (0.02)
		4	0.86 (0.06)	2.52 (0.22)	3.42 (0.55)	0.53 (0.02)
	18	1	0.64 (0.17)	5.96 (1.93)	3.97 (1.44)	0.21 (0.03)
		2	0.63 (0.14)	6.42 (1.68)	4.19 (1.46)	0.38 (0.02)
		3	0.66 (0.13)	6.08 (1.36)	4.67 (1.94)	0.49 (0.03)
		4	0.84 (0.01)	9.19 (2.44)	6.99 (1.46)	0.58 (0.02)
FR	0	1	0.54 (0.09)	0.31 (0.02)	0.70 (0.15)	0.47 (0.03)
		2	0.34 (0.02)	0.41 (0.04)	1.34 (0.07)	0.41 (0.09)
		3	0.48 (0.03)	0.74 (0.16)	0.77 (0.19)	0.56 (0.06)
		4	0.80 (0.06)	1.10 (0.12)	1.27 (0.30)	0.70 (0.06)
	9	1	0.38 (0.03)	0.97 (0.09)	1.87 (0.79)	0.47 (0.03)
		2	0.52 (0.01)	2.26 (1.02)	3.20 (1.51)	0.66 (0.02)
		3	0.63 (0.09)	2.10 (0.75)	2.06 (0.45)	0.80 (0.10)
		4	0.86 (0.06)	2.83 (1.11)	2.89 (0.84)	0.77 (0.07)
	18	1	0.37 (0.03)	2.90 (0.42)	1.89 (0.30)	0.57 (0.05)
		2	0.60 (0.02)	3.69 (0.52)	1.72 (0.21)	0.87 (0.06)
		3	0.67 (0.02)	4.09 (0.53)	2.12 (0.31)	1.28 (0.10)
		4	1.02 (0.03)	5.93 (1.41)	2.39 (0.35)	1.34 (0.15)

[a] SB = wild-type isolate from Santa Barbara, Honduras; and FR = isolate from La Fragua farm in the Sula Valley, Honduras.
[b] Leaf wetness duration in hours.
[c] Leaf (leaf age): 1, 2, 3, and 4 = ~ 8, 16, 24, and 32 days old, respectively.

Ascospore infection was observed only in the presence of leaf wetness. Because of more variation in disease severity and number of lesions between and within leaf wetness duration or isolates, the data set from ascospore infection was not considered for model development.

DISCUSSION

The effects of high humidity and temperature on *M. musicola* have been reviewed (8) and, based on field observations, similar responses have been suggested for *M. f. difformis* (14). In this study, conidial infection by *M. f. difformis* and disease development strongly responded to isolates, leaf wetness duration, and temperature. Symptoms of Black Sigatoka disease were observed at all temperature and leaf wetness combinations. Ascospore infection was observed only on leaves subjected to leaf wetness after inoculation.

A film of water on the leaf surface was required for ascospore infection. The greater variation observed when the ascospore approach was used may be related to the nature of ascospore discharge. For instance, more variation in ascospore discharge potential occurs from piece to piece of infected tissue, and distribution of propagules is not controlled. Thus, a high number of leaves not receiving ascospores became missing values. Additionally, more genetic variation among ascospores may be involved, because the genetic makeup of the propagule is unknown.

A film of water on the leaf surface was not required for conidial infection provided the RH was high. Leaf wetness duration had a major effect on symptom appearance, which was delayed up to 14 days when no leaf wetness was present. The delay may be associated with a longer water absorption period required for conidial germination. The high osmotic pressure of the spore (18), which allows the conidium to absorb water from the air, could be related to germination and infection by conidia under such conditions. Conidia germinate at 20–35 C over a wide range (92–100%) of RH in less than 24 h (5). Because infection by conidia occurred even with no leaf wetness and when the RH was high, periods of high RH may be important for infection when duration of leaf wetness or a rain event is short or absent. Therefore, conidial infection may play an important role in the epidemiology of Black Sigatoka during the dry season, when humidity is the limiting factor for infection, and ascospores are not observed based on spore trapping results (L. H. Jacome, *unpublished data*). Conidial infection may represent a survival advantage to the pathogen. Maximum ascospore germination and growth on the leaf surface have been observed when a film of water is present (17). Thus, the greatest impact of ascospores as source of inoculum may be during the rainy season. Ascospore discharge and infection are less likely during the dry season.

The optimum range of temperatures for disease development was 25–28 C. Temperature did not cause any major effect on the number of lesions, except at 31 C. Thus, differences in disease severity, at a given temperature, among leaves and leaf wetness levels were primarily due to lesion expansion. Lesion size may be dependent on lesion number. Ricker et al (11) observed larger lesion size at lower inoculum densities of *Cercospora arachidicola*. Therefore, studies with different inoculum densities would elucidate the relationship between number of lesions and lesion size in the *Mycosphaerella-Musa* pathosystem.

The older leaves were more susceptible to disease. Because no significant difference in the number of lesions was found among the leaves, the susceptibility of a leaf to greater lesion expansion may be related to biochemical or structural changes in the tissue with age. The increased concentration of carbohydrates in the young and middle leaves of some banana cultivars has been associated with resistance to fungal diseases (10).

Comparison of the isolates at each level of temperature and leaf wetness indicated that the wild-type isolate (SB) induced more disease under humid (9 and 18 h of wetness) and moderate (25 and 28 C) conditions. Isolate FR was less inhibited by the highest temperature or lowest wetness period than the wild-type isolate. The differences observed between the isolates suggest some degree of natural variability in the response of *M. f. difformis*-incited disease to environmental factors. A further indication of this variability is the length of incubation periods of the two isolates as influenced by the treatment combinations. A high number of isolates collected from different regions would be required to assess the full range of adaptability of this fungus to different environments.

The rate of Black Sigatoka development was modeled in Taiwan based on previous disease present, accumulated rainfall, and days of RH at ≥90% (2). Compared to the results of this study, rainfall presented conditions favorable for ascospore infection, whereas

TABLE 4. Length of incubation period (time until 50% of lesions become visible) of two *Mycosphaerella fijiensis* var *difformis* isolates on banana as influenced by several temperatures and dew period treatments during infection

Dew period (h)	Incubation period at temperature (C)							
	22		25		28		31	
	SB[a]	FR	SB	FR	SB	FR	SB	FR
0	63–70[b]	70–77	56–63	63–70	49–56	49–56	56–63	56–63
9	63–70	63–70	49–56	56–63	49–56	42–49	63–70	49–56
18	63–70	63–70	42–49	49–56	42–49	49–56	63–70	49–56

[a]SB = wild-type isolate from Santa Barbara, Honduras; and FR = isolate from La Fragua farm in the Sula Valley, Honduras.
[b]Time period in days. Values are the average of three runs.

TABLE 5. Estimated parameters ($P = 0.001$) from Equation 2 for the temperature (T in C), leaf wetness duration (LW in h), and leaf age (A in days) effects on final disease severity on banana leaves by *Mycosphaerella fijiensis* var. *difformis*

Isolate[b]	Parameter estimates[a]							R^2	Cp[c]
	b_0	b_1	b_2	b_3	b_4	b_5	b_6		
SB	−5.726	0.449	−0.348	−0.008	0.027	-5.375×10^{-4}	1.991×10^{-3}	0.913	8.1
	(0.832)[d]	(0.063)	(0.071)	(0.001)	(0.005)	(1.031×10^{-4})	(7.628×10^{-4})		
FR	−3.231	0.259	−0.257	−0.004	0.020	-3.814×10^{-4}	1.926×10^{-3}	0.857	7.8
	(0.727)	(0.055)	(0.062)	(0.001)	(0.004)	(9.015×10^{-5})	(6.665×10^{-4})		
CO	−4.479	0.354	−0.303	−0.006	0.024	-4.595×10^{-4}	1.959×10^{-3}	0.843	5.3
	(0.653)	(0.049)	(0.056)	(9.399×10^{-4})	(0.004)	(8.089×10^{-5})	(5.981×10^{-4})		

[a]Estimated parameters for Equation 2 corresponding to intercepts, T, LW, T^2, TLW, T^2LW, and A, respectively.
[b]SB = wild-type isolate from Santa Barbara, Honduras; FR = isolate from La Fragua farm in the Sula Valley, Honduras; CO = isolates combined.
[c]Mallow's Cp (7).
[d]Standard deviation.

RH of ≥90% presented favorable conditions for conidial infection. Because the model developed in Taiwan did not describe the influence of weather parameters on individual phases of the epidemic, the validity of the comparison is unknown. Our results indicate that a slightly higher RH is required for conidial infection in vivo, if leaf wetness is not present. Based on our response models, periods of RH (>92%) in which leaf wetness is not present, leaf wetness duration, and warm temperatures (25–28 C) can be important parameters in predicting the likelihood of Black Sigatoka disease outbreaks under the growing conditions in Honduras. However, the effect of temperature on disease development often becomes distinct only when optimum humidity conditions prevail at the same time (3). The importance of conidial infection on the disease epidemiology as suggested by results of this study needs to be confirmed under field conditions.

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