Reaction of Four *Musa* Genotypes at Three Temperatures to Isolates of *Mycosphaerella fijiensis* from Different Geographical Regions

Ronald A. Romero and Turner B. Sutton, Department of Plant Pathology, North Carolina State University, Raleigh 27695-7616

ABSTRACT

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Two tetraploid banana hybrids, FHIA1 and FHIA2, with resistance to black Sigatoka, and two highly susceptible, naturally occurring triploids, Grand Naine and False Horn, were evaluated at three temperatures for their resistance to isolates of *Mycosphaerella fijiensis* from five geographical regions. The youngest open leaf of young plants was inoculated, and plants were incubated at 22, 26, and 30°C in growth chambers. Duration of the incubation period and disease severity were used to evaluate the reactions of the genotypes. The incubation period was the shortest at 26°C. Disease severity was greatest at 26°C on Grand Naine and False Horn, but there was no clear temperature effect for the FHIA genotypes. The incubation period was longer on both FHIA genotypes than on Grand Naine and False Horn. With few exceptions, isolates with the shortest incubation periods caused greater disease severity than those with longer incubation periods. The level of resistance between the two FHIA genotypes was similar, and both expressed high resistance across temperatures and isolates of *M. fijiensis*, indicating that no physiological races of the pathogen were detected. There were differences in durations of the incubation periods and disease severities associated with the geographical origin of the isolates. Isolates that originated in Honduras, Colombia, and Costa Rica produced more disease on Grand Naine and False Horn than did isolates from Cameroon and Asia. However, no differences associated with the geographical origin of the isolates were observed for both FHIA genotypes. Also, there were no differences in disease severities within isolates that originated from Honduras, Colombia, and Costa Rica.

Black Sigatoka, caused by *Mycosphaerella fijiensis* Morelet, is the most destructive disease of bananas and plantains worldwide (18). For many years, resistance to black Sigatoka has been the objective of breeding programs. Female sterility and undesirable agronomic characteristics of the diploids bearing the resistance genes for *M. fijiensis* have been major constraints to the rapid development of resistant hybrids (13). However, after many years of research at the Banana Breeding Program of the Honduran Foundation for Agricultural Research (FHIA, formerly the United Brands Banana Breeding Program), improved diploids with excellent agronomic traits and resistance to black Sigatoka and other important diseases were obtained (14). These diploids were crossed with seed-fertile

triploids, such as the Highgate dwarf mutant (AAA Gros Michel), Dwarf Prata (AAB Pome), and French plantain (AAB Plantain), to obtain tetraploid hybrid progenies (13,14) that were then selected for their resistance to black Sigatoka and agronomic value.

Two dessert banana hybrids with resistance to black Sigatoka were released recently from FHIA for testing under field conditions. Recent reports on their resistance to black Sigatoka in different environments showed that these hybrids can be grown without the use of fungicides (8). Differences in the latent period and in disease severity among locations were observed and have been attributed mostly to differences in environmental conditions among locations and to differences in the amount of inoculum (12). Thus, this study was undertaken to compare the aggressiveness of isolates of *M. fijiensis* from different geographical regions at three temperatures and to examine possible *Musa* genotype-isolate and environment interactions in the expression of resistance to black Sigatoka.

MATERIALS AND METHODS

***Musa* genotypes.** Four genotypes of *Musa* spp. were used. Two genotypes were synthetic hybrids with resistance to black Sigatoka: FHIA1 (SH3481) and FHIA2

(SH3486). Both are tetraploids, with the genomes AAAB and AAAA, respectively. The A and B genomes are contributed by the diploid species *M. acuminata* and *M. balbisiana*, respectively. FHIA1 was derived from a cross between SH 3142 (AA), the male parent, × Prata Ana (AAB), a subacid banana. FHIA2 was derived from a cross of SH 3393 (AA) × Cavendish (AAA). The resistance to black Sigatoka in the diploid male parent SH 3142 was conferred by the naturally occurring diploid Pisang Jari Buaya (13), whereas the source of resistance of SH 3393 comes both from Pisang Jari Buaya and Pisang Lilin (P. Rowe, *personal communication*). Other *Musa* genotypes tested included the triploid (AAB) plantain cultivar False Horn, a very popular cooking type banana that is highly susceptible to black Sigatoka, and Grand Naine, another triploid banana (AAA) widely grown for the export market and also highly susceptible to black Sigatoka.

The plants were propagated by tissue culture and were received in stage 4 of development (20). Plants were conditioned in a greenhouse by growing them in a mixture of Metromix 220 (Grace Sierra Horticultural Products, Miltipias, CA), sand, and soil (1:1:1, vol/vol/vol) in 5-cm-diameter pots. Plants were placed under a mist system that operated for 20 s every 3 min. After 6 weeks, plants were transplanted to 15-cm-diameter plastic pots and maintained in the greenhouse for 4 weeks. Before placing plants in growth chambers in the Phytotron (Southeastern Plant Environmental Laboratory, Raleigh, NC) they were sprayed with the insecticide nicotine sulfate and fertilized with Peters 20-20-20 (25 g liter⁻¹) by applying 50 ml of the solution around the base of each plant.

Isolates and inoculum production. Fifteen isolates of *M. fijiensis* were used. Three isolates were obtained from each of five different geographical regions: Asia (South Pacific Islands), Cameroon, Colombia, Costa Rica, and Honduras. Except for isolates from Asia and Cameroon, which were obtained from pure cultures, isolates were obtained by discharging ascospores from banana leaves bearing pseudothecia onto water agar and then transferring single ascospores to potato dextrose agar (PDA). All ascospore isolates were grown at 24°C for 20 days in the dark. The entire colony was removed from the PDA and vortexed for 10 s in 1 ml of sterile water in a test tube. One drop of the sus-

Corresponding author: R. Romero
E-mail: roromero@fractal.icr.co.cr

Current address of R. A. Romero: Corporación Bananera Nacional (CORBANA), San José, Costa Rica, Box 6504-1000.

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pension of mycelial fragments of each isolate was transferred to Mycophil-agar (MA) (6) using a Pasteur pipette and streaked over the surface of the agar. Culture dishes were incubated under continuous cool-fluorescent light for 2 weeks at 20°C for conidial production (6). Conidia were obtained by scraping the surface of the colonies on the MA medium and blending them for 10 s in 100 ml of distilled water. The suspension was passed through a double layer of cheesecloth, and the number of conidia was adjusted to 15×10^3 per ml. Tween 20 (0.05%) was added to each conidial suspension.

Inoculation. Fifteen plants of each *Musa* genotype, for a total of 60 plants, were placed in each of three chambers in the Phytotron set at 22, 26, and 30°C. Plants with five to six fully expanded leaves were inoculated with each of the isolates. Conidia were atomized onto the abaxial surface of the youngest expanded leaf to the point of runoff using an aerosol propellant atomizer (Crown Spra-Tool; Crown North American Professional Products, Woodstock, IL). Inoculations were performed inside a plastic chamber with one open side to avoid contamination of other plants. Leaves of inoculated plants were tagged, and plants were subjected to a photoperiod of 12 h day/night regime and a relative humidity above 92% for 5 con-

secutive days. The relative humidity was maintained by a mist system that operated for 30 s every 4 min. After the fifth day, the mist system operated with the same frequency, but only from 6 P.M. to 6 A.M. Four plants, each corresponding to a different genotype, were inoculated with each isolate of *M. fijiensis* and placed in each chamber. Plants were grouped according to isolate, and isolates within the chamber were arranged randomly. Three replications were conducted over time.

Disease assessment. The incubation period, defined as the time from inoculation to the appearance of 10 lesions at stage 1 (small necrotic streaks [5,16]) on a leaf, and disease severity were used to compare the reaction of *Musa* genotypes to the different isolates of *M. fijiensis* at each temperature. Disease severity was determined according to Stover's scale modified by Gauhl (5,16) in which 0 = no symptoms; 1 = presence of stages 1, 2, or 3, but not more than 10 stage 4 symptoms; 2 = more than 10 stage 4 but less than 5% of the leaf affected; 3 = from 6 to 15% of the leaf affected; 4 = from 16 to 33% of the leaf affected; 5 = from 34 to 50% of the leaf affected; and 6 = more than 50% of the leaf affected. Stages of symptoms are described according to Meredith and Lawrence (9) and Fouré (4). Disease severity was recorded 50 days after inoculation.

Experimental design and data analyses. The experiment was arranged as a split-split plot design with temperature as the main plot factor, isolate as the subplot factor, and genotype as the sub-subplot factor. Data were analyzed with an analysis of variance (PROC ANOVA, SAS Version 6.1, SAS Institute, Cary, NC) followed by protected LSDs. The importance of the geographic region (country) of origin of the isolates was examined also by analysis of variance and protected LSDs, partitioning isolate differences into region-of-origin differences and differences among isolates within region of origin.

RESULTS

Incubation period. Only the main factors of temperature, isolate, genotype, and region were significant for the duration of the incubation period. The incubation period was significantly shorter at 26°C (21.1 days) than at either 22°C (25.2 days) or 30°C (25.5 days) and was shorter on Grand Naine and False Horn than on either of the FHIA genotypes (Table 1). The incubation period was the shortest for isolates 275, 278, and 273 from Cameroon and longest for isolates 296 and 330 from Asia. There was no significant difference among the other regions of origin (Table 2). The duration of the incubation period associated with the 15 isolates ranged from 20.1 days to 29.4 days (Table 3). Except for isolates from Cameroon and Asia, which tended to have the shortest and longest incubation periods, respectively, there was no definite pattern in the duration of the incubation period associated with them.

Disease severity. The main factors, temperature, isolate, and country of origin, each interacted significantly with genotype. Disease was more severe on Grand Naine and False Horn than on FHIA1 or FHIA2 at each temperature (Table 4). Disease was most severe at 26°C on Grand Naine and False Horn. There was no difference among temperatures for FHIA2, and disease severity was similar at 26 and 30°C on FHIA1. All isolates produced more disease on Grand Naine and False Horn than on FHIA1 or FHIA2 (Table 5). There were no differences in disease severity among isolates on FHIA1 and only minor differences on FHIA2. Except for isolates 273 and 275, there were no differences in disease severity between Grand Naine and False Horn for any of the isolates. There were, however, differences among isolates within these two genotypes.

Table 1. Differences among genotypes in the duration of the incubation period of black Sigatoka, caused by *Mycosphaerella fijiensis*

Genotype	Incubation period (days) ^y
FHIA 1	26.2 a ^z
FHIA 2	25.1 b
Grand Naine	22.4 c
False Horn	22.1 c

^y Incubation period was defined as the time from inoculation to the appearance of 10 lesions of black Sigatoka, also known as stage 1 of symptoms (4,9).

^z Values followed by the same letter are not significantly different at $P = 0.05$ (LSD_{0.05} = 0.8). Values are means of 135 observations.

Table 2. Effect of geographical region of origin on the duration of the incubation period of *Mycosphaerella fijiensis*

Country or region	Incubation period (days) ^y
South Asia	27.4 a ^z
Costa Rica	24.5 b
Colombia	23.7 b
Honduras	23.6 b
Cameroon	20.6 c

^y Incubation period was defined as the time from inoculation to the appearance of 10 lesions of black Sigatoka also known as stage 1 of symptoms (4,9).

^z Values followed by the same letter are not significantly different at $P = 0.05$ (LSD_{0.05} = 2.5). Values are means of 108 observations.

Table 3. Differences among isolates in the duration of the incubation period of *Mycosphaerella fijiensis*

Isolate	Country or region of origin	Incubation period (days) ^y
296	South Asia	29.4 a ^z
330	South Asia	28.7 ab
Col 14	Columbia	26.3 a-c
crgf	Costa Rica	26.1 a-c
h12	Honduras	25.2 b-d
crmj	Costa Rica	24.5 c-e
h13	Honduras	24.3 c-e
8837	South Asia	24.2 c-f
cr17	Costa Rica	22.8 c-g
Col 13	Columbia	22.4 d-g
Col 3	Columbia	22.3 d-g
h10	Honduras	21.3 g-f
275	Cameroon	20.0 g-f
278	Cameroon	20.6 gf
273	Cameroon	20.1 g

^y Incubation period was defined as the time from inoculation to the appearance of 10 lesions of black Sigatoka also known as stage 1 of symptoms (4,9).

^z Values followed by the same letter are not significantly different at $P = 0.05$ (LSD_{0.05} = 3.6). Values are means of 36 observations.

Table 4. Interaction of temperature and genotype on the severity^y of black Sigatoka

Temp. (°C)	FHIA1	FHIA2	Grand Naine	False Horn
22	1.02 b ^z	1.04 a	4.11 b	4.13 b
26	1.33 a	1.27 a	5.27 a	5.18 a
30	1.18 ab	1.27 a	3.41 c	3.42 c

^y See text for description of disease severity scale.

^z Values followed by the same letter in a column are not significantly different (LSD_{0.05} = 0.29). Values are means of 45 observations.

In general, disease severity was less for isolates from Cameroon and Asia than for those from the other regions.

There were no significant differences in disease severity associated with country of origin for both FHIA1 and FHIA2 genotypes (Table 6). Isolates from Asia and Cameroon produced less disease on Grand Naine and False Horn than did isolates from Costa Rica, Honduras, or Colombia (Table 6).

There were no significant differences in disease severity among isolates that originated from Colombia, Honduras, or Costa Rica (Table 7). One isolate from Cameroon (273) caused significantly more disease than did the other two from the same region. Similarly, one isolate from Asia (8837) caused more disease than did the other two isolates from the same location.

DISCUSSION

The results of these studies indicate that the resistance of FHIA1 and FHIA2 to *M. fijiensis* performs adequately in a range of temperatures and to different pathogen populations. This conclusion is also supported by results from field trials conducted worldwide in which both FHIA genotypes have shown high levels of resistance (12).

Our data indicate that no physiological races of *M. fijiensis* were detected in this study, since there was little or no effect of

temperature on the expression of resistance in the FHIA genotypes and no isolate-genotype interaction (19). Using segregation ratios for resistance with diploid bananas, Ortiz and Vuylsteke (11) showed that black Sigatoka is conditioned by one major recessive allele and two independent alleles with additive effects. They also suggested that in tetraploid banana hybrids, such as FHIA1 and FHIA2, favorable resistance alleles would have a dosage effect, thus conferring partial resistance to black Sigatoka. In our study, this partial resistance in both FHIA genotypes was expressed by slower disease progress and the absence of temperature-genotype and isolate-genotype interactions (3).

Although the exact mechanism of resistance of bananas to black Sigatoka is not known, low stomatal density and increased levels of epicuticular wax, phytoalexin production, the production of lignin or suberin, and resistance to phytotoxins produced by pathogen (15) have been suggested as possible mechanisms associated with partial resistance (11).

Our study indicates that artificial inoculations of 10-week-old tissue culture plants with conidia of *M. fijiensis* can be used to test the susceptibility of different genotypes. The reactions observed for the FHIA hybrids and the susceptible genotypes, Grand Naine and False Horn, were consistent with the results found in field experiments conducted in several locations (8).

The temperature most favorable for the incubation period and disease severity was 26°C, which agrees with previous studies showing that the optimum temperature for the development of *M. fijiensis* is around 26°C (6,10,17) for susceptible genotypes. Although the effect of temperature on the severity of black Sigatoka on the FHIA genotypes was not significant, indicating a stable expression of the resistance across different environments, 26°C should be used in studies for screening germplasm for susceptibility or resistance to *M. fijiensis* under controlled conditions. Differences in disease severity between the resistant and susceptible genotypes and variation in the aggressiveness of the isolates were expressed better at this temperature than at either 22 or 30°C.

On average, across temperatures and isolates of *M. fijiensis*, isolates showed longer incubation periods on both FHIA genotypes than on Grand Naine and False Horn. This further illustrates the potential for using incubation period as a variable in testing the reaction of *Musa* genotypes to *M. fijiensis*, as has been suggested previously (4,7). This variable has the advantage of being easy to measure and less time-consuming than measuring disease severity.

The observed differences in incubation period and disease severity associated with the geographical origin of the isolates indicates genetic variability in *M. fijiensis*. The apparent lack of genetic uniformity in this pathogen is in agreement with the recent findings from analyses for restriction fragment length polymorphisms (RFLPs) in ribosomal DNA (2) and nuclear DNA (1) that showed genetic differences among isolates of *M. fijiensis* and that the variant isolates are clustered according to geo-

Table 5. Interaction of 15 isolates of *Mycosphaerella fijiensis* with genotypes on the severity^y of black Sigatoka

Isolate	FHIA1	FHIA2	Grand Naine	False Horn
273	1.22	1.33 ab ^z	3.33 b	4.44 c
275	1.00	1.04 a	3.22 b	2.55 a
278	1.22	1.00 a	2.44 a	2.89 ab
296	1.00	1.11 ab	2.89 ab	3.33 b
330	1.00	1.00 a	2.44 a	2.78 a
8837	1.11	1.22 ab	5.33 e	5.22 de
Col 13	1.11	1.11 a	4.56 c	4.44 c
Col 14	1.11	1.00 a	4.00 c	4.22 c
Col 3	1.33	1.22 ab	5.44 e	4.89 d
Cr17	1.22	1.67 b	5.44 e	5.44 e
Crgf	1.11	1.00 a	4.89 d	4.33 c
Crmj	1.11	1.33 ab	4.83 d	4.56 c
h10	1.56	1.56 ab	5.56 e	5.67 c
h12	1.11	1.00 a	5.00 de	4.56 cd
h13	1.44	1.33 ab	4.55 cd	4.33 c

^y See text for description of disease severity scale.

^z Values followed by the same letter in a column are not significantly different from one another (LSD_{0.05} = 0.63). Values are means of nine observations.

Table 6. Interaction of geographic region of origin of isolates of *Mycosphaerella fijiensis* with genotype on the severity^y of black Sigatoka

Country or region	FHIA1	FHIA2	Grand Naine	False Horn
Cameroon	1.15 NS	1.11 NS	3.00 a ^z	3.30 a
Colombia	1.18	1.11	4.67 b	4.52 b
Honduras	1.37	1.27	5.04 b	4.85 b
Costa Rica	1.15	1.33	5.05 b	4.78 b
South Asia	1.04	1.11	3.55 a	3.77 a

^y See text for description of disease severity scale.

^z Values in a column followed by the same letter do not differ from one another (LSD_{0.05} = 0.58). NS = nonsignificant. Values are means of 27 observations.

Table 7. The variation in the severity^y of black Sigatoka associated with isolate within region effects

Country or region	Isolate	Disease severity
Cameroon	273	2.58 b ^z
	275	1.94 a
	278	1.88 a
Colombia	Col 13	2.81 a
	Col 14	2.58 a
	Col 3	3.22 a
Honduras	h10	3.58 a
	h12	2.91 a
	h13	2.91 a
Costa Rica	crl 7	3.44 a
	crgf	2.83 a
	crmj	2.96 a
South Asia	296	2.08 a
	330	1.81 a
	8837	3.22 b

^y See text for description of disease severity scale.

^z Isolates within a country followed by the same letter do not differ from one another (LSD_{0.05} = 0.69). Values are means of 36 observations.

graphical origin. The results of the present study suggest that a relationship exists between this genetic variation and differences in aggressiveness among the populations, but a much larger sample size is necessary to clearly establish such a relationship.

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