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**ORIGINAL ARTICLE**

# Components of resistance to assess Black Sigatoka response in artificially inoculated *Musa* genotypes

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**ABSTRACT:** Components of resistance were evaluated in seven *Musa* genotypes artificially inoculated with mycelia fragment suspensions of *Mycosphaerella fijiensis* Morelet (strain CCIBP-Pf80). Incubation period, number and area of necrotic lesions, infection index, area under the disease progress curve, asexual latent period, and number of spermogonia were evaluated in the genotype leaves to dissect the infective cycle of the fungus under greenhouse conditions. Incubation period in the inoculated leaves began at 14-21 days post infection, and significant differences were detected in the response of the different genotypes. Necrotic lesions were only observed in Grande naine, Pisang Awak, and significantly less in Pisang lilin. Grande naine reached the highest percentage of leaflet area with necrotic tissue, followed by Pisang Awak and Pisang lilin. Grande naine and Pisang Awak reached the greatest areas under the disease progress curves, while the lowest values were calculated in FHIA-18 and FHIA-25. The asexual latent period in Grande naine and Pisang Awak was significantly shorter (approximately 14 days) than in Pisang lilin. Greater numbers of spermogonia were observed in Grande naine and Pisang Awak, followed by Pisang lilin. Conidia were only detected in Grande naine, Pisang Awak and, in a significantly less number, in Pisang lilin. The different response observed of *Musa* spp. genotypes to the causal agent of Black Sigatoka indicated that the components of resistance used allowed the quantitative assessment of their reaction to this fungus. These results could improve or facilitate the efficiency and precision of the early evaluation process in banana and plantain breeding programs.

**Key words:** early screening, inoculation, *Mycosphaerella fijiensis,* phytopathology, quantitative resistance.

## Componentes de la resistencia para cuantificar la respuesta a la Sigatoka Negra en genotipos de *Musa* inoculados artificialmente

**RESUMEN:** Se evaluaron los componentes de la resistencia en genotipos de *Musa* inoculados artificialmente utilizando suspensiones miceliales de *Mycosphaerella fijiensis* Morelet (cepa CCIBP-Pf80). El periodo de incubación, número de lesiones necróticas, área de lesiones necróticas, índice de infección, área bajo la curva del progreso de la enfermedad, periodo de latencia asexual y número de Espermogonios se evaluaron en siete genotipos para analizar el ciclo infectivo de *M. fijiensis* en condiciones de invernadero. El período de incubación en todos los genotipos se enmarcó entre 14-21 días posteriores a la inoculación y se observaron diferencias significativas en la respuesta de los diferentes genotipos de *Musa*. El mayor número de lesiones necróticas se observó en el genotipo Grande naine en comparación con el Pisang Awak, y este a su vez, respecto al Pisang lilin. Los mayores valores del área bajo la curva del progreso de la enfermedad se observaron en los genotipos Grande naine y Pisang Awak, mientras los menores se calcularon en FHIA-18 y FHIA-25. Grande naine y Pisang Awak tuvieron una reducción significativa del periodo de latencia asexual (aproximadamente 14 días) respecto al Pisang lilin. El mayor número de espermogonio se observó en Grande naine y Pisang Awak, seguido del Pisang lilin. Los conidios solo se detectaron en hojas necrosadas de Grande naine y Pisang Awak, las

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cuales fueron significativamente superiores al Pisang lilin. Las diferencias observadas en la respuesta de los genotipos de *Musa* spp. frente al agente causal de la Sigatoka Negra, indicaron que los componentes de la resistencia utilizados permitieron evaluar cuantitativamente la reacción de *Musa* spp. frente al agente causal de BLSD. Estos resultados podrían facilitar una mayor eficiencia y precisión de la evaluación temprana de genotipos de bananos y plátanos en programas de mejoramiento genético.

**Palabras clave:** fitopatología, inoculación, *Mycosphaerella fijiensis,* resistencia cuantitativa, selección temprana.

## INTRODUCTION

Black Leaf Streak Disease (BLSD), caused by the ascomycete fungus *Mycosphaerella fijiensis* Morelet, is the causal agent of the disease also known as Black Sigatoka (1). This fungal disease is the most damaging and economically important disease of banana and plantain (*Musa* spp.) worldwide (1). This leaf pathogen is an increasing threat in all areas where *Musa* spp. are grown. Several toxins are produced by *M. fijiensis* (2) that may destroy the photosynthetic capacity of banana leaves causing reduced yield and premature ripening of the fruit (3). Fungicides are used to control BLSD, but they are expensive and not fully effective (1). Therefore, resistant genotypes are valuable to breeders and farmers and precise evaluation procedures are required (4, 5).

Measurements of *Musa* spp. germplasm resistance to *M. fijiensis* are often carried out in field trials under conditions of natural infestation, where ascospores and conidia are the main infective structures (6). Field evaluation are particularly time-consuming and costly because the plants have to be evaluated for several vegetative cycles and in different phenological stages, commonly affected by environment fluctuations (7).

Furthermore, tissue-culture-derived banana plants have been artificially inoculated with *M. fijiensis* and the disease development symptoms have been characterized in greenhouse and environmental growth chamber assays with stringent lighting and humidity controls (8), but without quantification of *Musa* response. However, the evaluation of the efficiency and durability of partial resistance in several genotypes of *Musa* have been performed by measuring some variables (size of lesion, number of perithecia and disease severity) in the life cycle of *M. fijiensis* at field and in detached leaf assay, but no greenhouse determination was done (9).

Nevertheless, the fungus cannot complete the entire infection cycle *in vitro* plantlets and detached leaf assay, due to the slow symptom development of *M. fijiensis* during the infection process. *M. fijiensis* symptoms can take up to two months or longer under optimal growth conditions, and normally senescent and not specific symptoms may be observed in that period. Although the response to BLSD has been evaluated by different qualitative ways, components of resistance in greenhouse conditions, with the mycelia fragment inoculation procedure, have never been used for this purpose.

As with other pathogen causing diseases, knowledge of the infective cycle under controlled condition is desirable to propose new quantitative variables and improve the effectiveness in evaluation of *Musa* breeding programs. The aim of the present study was to evaluate components of resistance to assess Black Sigatoka response in artificially inoculated *Musa* genotypes with mycelia fragment suspensions of *M. fijiensis*.

## MATERIAL AND METHODS

### Plant material and inoculation

Plants of seven *Musa* genotypes with different levels of resistance to BLSD, were obtained by tissue culture via organogenesis and acclimatized during 12 weeks. Calcutta 4 (AA) and Yangambi km 5 (AAA) were selected as resistant, Pisang lilin (AA), FHIA-18 (AAAB) and FHIA-25 (AAA) as partial resistant, while Grande naine (AAA) and Pisang Awak (ABB) as susceptible. Plants were planted in 0.5 L-plastic pots filled with humus, compost and zeolite mixture in a 5:3:2 $^{v}/_{v}$ ratio.

All inoculation experiments were performed with a single-ascospore *M. fijiensis* isolate (strain CCIBP-Pf80) obtained from naturally infected banana leaves at stage 6 (10) from the susceptible cultivar Grande naine (AAA) showing typical symptoms of BLSD. This strain is preserved in the culture collection of *M. fijiensis* at the Applied Microbiology Laboratory of the Instituto de Biotecnología de las Plantas, Universidad Central «Marta Abreu» de Las Villas Carretera a Camajuaní km 5.5. Santa Clara, Villa Clara, Cuba. Ten plants per genotype were used for the artificial inoculation assay. Other five plants were not inoculated and left as control. Mycelia

suspension was prepared following the protocol described by Leiva-Mora (11). Mycelial suspensions were filtered through sieves with a mesh of 40 µm and adjusted to $10^5$ mycelia fragments.$ml^{-1}$. Finally, gelatin at 1% (w/v) was added to improve adhesion of infective structures to the abaxial leaf surface.

The first three open leaves of each plant were inoculated on the abaxial leaf surface using a camel brush. Inoculated leaves were marked on the adaxial side with a black permanent marker. The plants were allowed to dry for two hours and humidity was maintained over 90% during the first three days by spraying water continuously. Afterwards, the humidity was saturated only during the night.

**Components of resistance**

The following components of resistance were evaluated separately in the third, second and first open leaves of the inoculated plants:

*Incubation period (IP)*: Defined as the time elapsed between inoculation and symptom appearance in the inoculated plants of each genotype. IP was assessed daily by visual inspection of symptom development starting at 7 days post-inoculation (DPI).

*Number of necrotic lesions and area of necrotic lesions (NNL and ANL)*

*NNL* was counted in each inoculated leaves at 63 DPI. ANL was estimated in each inoculated leaves at 63 DPI by using the ellipse area formula ($A = {}^{a}/_{2} \cdot {}^{b}/_{2} \cdot \pi$), where *a* was the length and *b* the width of the necrotic spot, and $\pi$=3.14.

*Infection index (II)*: The percentage of leaflet area with necrotic tissue was estimated using a seven degree diagrammatic scale (12) modified (13) for BLSD resistance in *Musa* spp. The scale degrees were 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).

Disease severity assessments were calculated at 63 DPI by the expression:

$$\sum nb \div (N-1)T \times 100$$

where n= number of leaves at each level; b= value of severity according to the diagrammatic scale, N= 7, corresponding with the number of stages in the scale and T= total number of leaves evaluated per plant.

Three inoculated leaves on each plant were evaluated.

*Area under the disease progress curve* (AUDPC): AUDPC was calculated according to Shaner and Finney's (14) formula:

$$\sum_{i=1}^{n} [(Y_{i+n1} + Y_i)/2][(X_{i+1} - X_i]$$

where *Yi* =BLSD severity (per unit), *Xi* =time (days) at the *i*th observation and *n*=total number of observations, previously infection index was determined by the evaluating scale (15) (Table 1).

*Asexual latent period* (*ALP*): ALP referred to the days elapsed from inoculation to the occurrence of conidia sporulation. Detection of conidia was performed according to Aguirre (16), and the evaluations were done weekly beginning at 35 DPI until 63 DPI. ALP was considered completed when sporulation was observed on at least three lesions on each inoculated leaf.

*Number of spermogonia*

Discs (1 cm of diameter) from leaves with necrotic lesions (stage 4 and 5 in the evaluation scale (15) were decolorized in 10% ($^{w}/_{v}$) KOH for 24h. They were washed in deionized steril water three times. The discs were placed on slides with lactophenol (phenol 20 g; lactic acid 20 g; glycerol 40 g; water 20 mL) and mounted for their further observation under the microscope (Olympus) with 200x magnification. One hundred observations of each *Musa* genotype were done under the microscope with 200x magnification and the total number of spermogonia recorded.

**Statistical analysis**

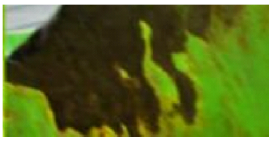
All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences Version 15.0) software (SPSS Inc., Chicago, IL, USA). The number of necrotic lesions, area of necrotic lesions, area under disease progress curve (AUDPC), number of spermogonia and conidia were compared according to Kruskall-Wallis non-parametric test. Infection index and asexual latent period were processed by One-way ANOVA and the means compared by *Duncan's* test.

## RESULTS AND DISCUSSION

*Incubation period (IP)*

The incubation period in all the inoculated leaves of the same genotypes began at 14-21 DPI, and significant differences were observed among the *Musa* genotype

**TABLE 1.** Descriptive scale to evaluate Black Sigatoka symptomatology in young micropropagated *Musa* genotypes artificially inoculated in greenhouse according with Alvarado *et al*. (15)./ *Escala descriptiva para evaluar la sintomatología de la Sigatoka Negra en plantas de Musa micropropagadas e inoculadas artificialmente en invernadero*

| Degree | Description of symptoms | Visual pattern |
|---|---|---|
| Stage 0 | Leaf symptoms mostly absent |  |
| Stage 1 | Reddish flecks on the abaxial leaf surface. No symptoms observed on the surface |  |
| Stage 2 | Regular or irregular reddish circular spots on the abaxial leaf surface No symptoms on the adaxial leaf surface |  |
| Stage 3 | Regular or diffuse light brown circular spots on the adaxial surface |  |
| Stage 4 | Black or brown circular spots on the adaxial leaf surface, with a halo or chlorosis in adjacent tissues. Areas of green tissues sometimes present |  |
| Stage 5 | Black spots with dry centre of grey color on the adaxial leaf surface. Leaf completely necrotic. |  |

response (Table 2; Figure). FHIA-18 and FHIA-25 had the longest incubation period, followed by Pisang lilin. Calcutta 4, Grande naine, Pisang Awak and Yangambi were the genotypes with the shortest incubation period.

IP was found to be important because it showed significant differences among *Musa* artificially inoculated genotypes with mycelial suspension in greenhouse assays. It was the first time that incubation period has differentiated genotypes with differential response to Black Sigatoka in greenhouse using mycelial fragments. Similar results were obtained by Molina-Tirado and Castaño-Zapata (17), when they analyzed the incubation period to discriminate the reaction of three FHIA genotypes to Yellow and Black Sigatoka under natural infection. The incubation period was also used by Alvarez *et al*. (18) in the successful evaluation of

**TABLE 2.** Incubation period evaluated in genotypes of *Musa* artificially inoculated with mycelial fragments of *M. fijiensis* (strain, CCIBP-Pf80)./ *Periodo de incubación evaluado en genotipos de Musa inoculados artificialmente con fragmentos de micelio de M. fijiensis (cepa, CCIBP-Pf80)*

| Genotypes | Mean Rank | Mean |
|---|---|---|
| Grande naine | 50,00 c | 15,17 |
| Pisang Awak | 46,50 c | 14,78 |
| FHIA-18 | 99,00 a | 20,61 |
| FHIA-25 | 88,50 a | 19,44 |
| Pisang lilin | 64,00 b | 16,72 |
| Yangambi Km 5 | 39,50 cd | 14,00 |
| Calcutta 4 | 57,00 bc | 15,94 |

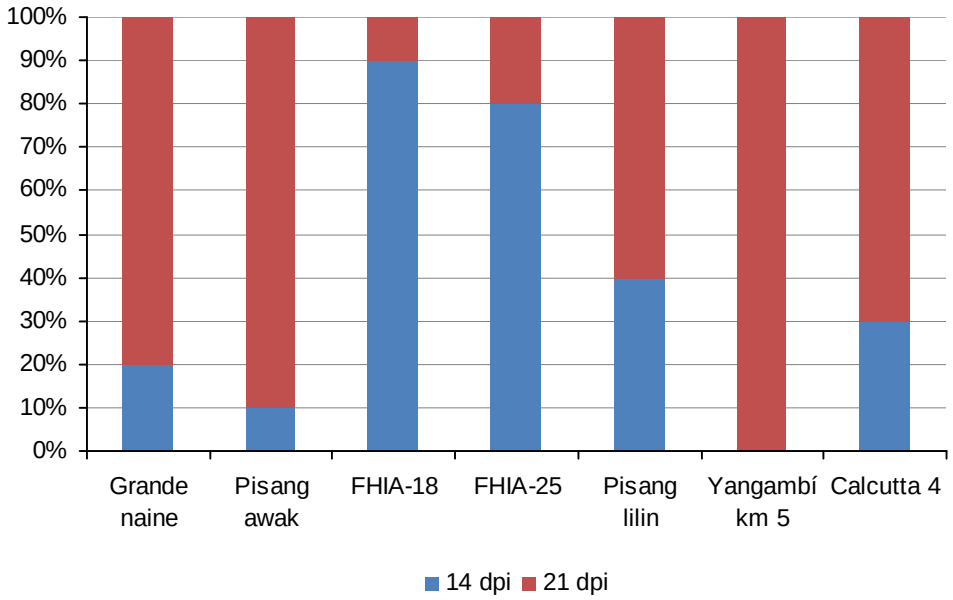



**FIGURE.** Distribution frequency of incubation period of artificially inoculated genotypes of *Musa* with mycelial suspension ($10^5$ cfu.ml$^{-1}$) of *M. fijiensis* (strain CCIBP-Pf80) greenhouse assay with different level of resistance to Black Sigatoka./ *Distribución de frecuencia (%) del Periodo de incubación evaluado en genotipos de Musa con diferentes niveles de resistencia a la Sigatoka Negra inoculados artificialmente en condiciones de invernadero.*

resistance to Black Sigatoka of plantain and banana genotypes under greenhouse conditions.

Similarly, Abadie *et al*. (9) demonstrated that incubation period also varied significantly in genotypes with different level of partial resistance in field and in detached leaf assays. Pondering the literature quoted, the incubation period can be consider useful for the early screening of *Musa* germplasm using mycelial suspensions as infective inoculums under controlled conditions; this variable is also very easy to evaluate, and it is not time consuming.

In our experimental condition, it was used a reproductive protocol that described aspects related to the cultural, morphological, molecular, and aggressiveness characteristics of *M. fijiensis* strains. It also indicated how to use epidemiological variables and components of resistance to characterize the infective cycle of *M. fijiensis* in greenhouse. These reasons guaranteed incubation period to be useful for discriminating the response of *Musa* genotypes.

*Number of necrotic lesions and area of necrotic lesions (NNL and ANL)*

Mature necrotic lesions were observed only in Grande naine, Pisang Awak and Pisang lilin. No necrosis symptoms were observed in the rest of the genotypes until the final evaluation. Significantly greater were the numbers of necrotic lesions observed in Grande naine and Pisang Awak compared with those in Pisang lilin. The biggest areas of necrotic lesions were measured in Grande naine with significant differences with Pisang Awak and Pisang lilin. The size of necrotic lesions in Pisang Awak was also bigger compared with those in Pisang lilin (Table 3).

Differences regarding the formation of mature necrotic lesions were observed only in the genotypes Grande naine, Pisang Awak and Pisang lilin. This fact indicated that the number and area of necrotic lesions were good quantitative variables to differentiate inoculated *Musa* genotypes with a variable level of resistance to *M. fijiensis* in an early stage. This finding reinforced the results of Abadie *et al*. (9), where the resistant varieties Zebrina and Pisang ceylan had lesser and smaller necrotic lesions and more reduced necrotic areas than other resistant genotypes in the field and in detached leaf assays.

The use of the number and area of necrotic lesions in *M. fijiensis* to evaluate the epidemiology and ecology of Black Sigatoka on plantain and banana (*Musa* spp.) in Costa Rica under field condition was first reported by Gauhl (13). However, counting of total necrotic lesions on inoculated *Musa* leaves is a laborious, time

**TABLE 3.** Number and area of necrotic lesions in *Musa* genotypes artificially inoculated with mycelial fragments of *M. fijiensis* (strain, CCIBP-Pf80) under greenhouse conditions./ *Número y área de lesiones necróticas en genotipos de Musa, inoculados artificialmente en condiciones de invernadero con fragmentos de micelio de* ***M. fijiensis*** *(cepa, CCIBP-Pf80)*

| Genotypes | Number of necrotic lesions /leaf | | Area of necrotic lesions ($cm^2$) | |
|---|---|---|---|---|
| | Mean rank | Mean | Mean rank | Mean |
| Grande naine | 99,03 a | 85,35 | 313,34 a | 0,50 |
| Pisang Awak | 95,76 a | 77,88 | 281,68 b | 0,37 |
| Pisang lilin | 72,84 b | 2,56 | 231,48 c | 0,16 |

Means followed by different letter in each column indicate differences among the ranges according to Kruskal-Wallis/Mann-Whitney test ($p<0.05$) n=15

consuming and tedious task because of the great number of lesions and coalescences that may be observed. Nevertheless, the number and area of necrotic lesions may also contribute in studies related to *M. fijiensis* aggressiveness variability, the efficacy of fungicide protection, and the infective capacity of different inoculants.

The number of necrotic lesions was used successfully by Seifbarghi *et al*. (19) to determine the host range of Septoria species in inoculation experiments under controlled conditions of 27 genotypes of some cereals and wild grasses. Suffert *et al*. (20), by using the area of necrotic lesions, provided a good measure of *M. graminicola* fitness in estimating the quantitative resistance of wheat to *Mycosphaerella graminicola* blotch and characterizing the differences among isolates within a pathogen population.

*Infection index (II)*

The infection index was calculated only in Grande naine, Pisang Awak and Pisang lilin which showed mature necrotic lesions at 63 DPI. Statically significant differences were determined among the three genotypes, where Grande naine reached the highest percentage (87.03) of leaflet area with necrotic tissue, followed by Pisang Awak (61.28) and Pisang lilin (44.61).

Severity or Infection intensity caused by *Mycosphaerella* spp. has been used in the evaluation of *Musa* spp. response under natural infection conditions (21). However, other authors, instead of using diagrammatic scales, have calculated infection intensity according to the symptoms described by Meredith and Lawrence (22) and Fouré (10). Gauhl (13) determined the infection index by using the function proposed by Lehmann Danziger (21) and carried out an epidemiological and ecological study on Black Sigatoka disease in plantain and banana in Costa Rica where the response among the natural infected genotypes were statistically different. Orjeda (23) provides details on *Musa* spp. evaluation trials carried out in nine sites worldwide in which the infection index was successfully used to determine the response of the genotypes evaluated to Black Sigatoka.

Likewise, Carlier *et al*. (24) confirmed the usefulness of the infection index to follow up *Mycosphaerella* spp leaf spot diseases throughout different phenological development stages (six months, flowering, harvest) in *Musa* germplasm. Krishnamoorthy *et al*. (25) differentiated the response of 11 banana hybrids and their respective parents after natural infection of *M. fijiensis* in field trials. Rocha *et al*. (26) used the infection index to develop a temporal analysis to assess the aerobiology of *Mycosphaerella musicola* spores and determine the evolution of the disease progression curve.

The infection index was successfully used by Portal *et al*. (27) to characterize *Mycosphaerella fijiensis* mutants transformed with a green fluorescent protein-carrying construct by using a restriction enzyme-mediated integration methodology. These authors observed that GFP-18 transformant showed increased aggressiveness in susceptible Grande naine and resistant Yangambi km5 plants once infection index was evaluated in a greenhouse.

Finally, the infection index depends on the level of resistance of the inoculated genotypes, and in the present study, it was calculated only in Grande naine, Pisang Awak and Pisang lilin, where necrotic tissues were observed.

*Area under disease-progress curve (AUDPC)*

The AUDPC values calculated from the data in all the evaluations until day 56 showed statistically significant differences among the inoculated genotypes respect to their quantitative disease resistance. The greatest AUDPC values were reached by Grande naine and Pisang Awak, while the lowest values were

calculated in FHIA-18 and FHIA-25. However, Pisang lilin, Yangambi Km 5 and Calcutta 4 revealed intermediate values.

This paper shows the first implementation of the AUDPC for the quantitative assessment of the disease resistance of *Musa* genotypes artificially inoculated with the causal agent of Black Sigatoka in greenhouse. Jeger *et al*. (28) used AUDPC to evaluate disease resistance in different crop cultivars, and they concluded that this variable was useful to measure the quantitative disease resistance in repeated assessments of the disease. Commonly, AUDPC may be practical for investigating the effectiveness of fungicide applications to control *Mycosphaerella* leaf pathogen diseases in field condition (29) and the evaluation of plant disease resistance (30).

Kablan *et al*. (30) showed that the AUDPCs calculated in banana plants grown with silicon were significantly lesser than those calculated for plants with no silicon. Thanks to the use of this quantitative variable, they integrated pest management against *M. fijiensis* by reducing the disease pressure on banana.

Otherwise, Cuéllar *et al*. (31) determined that the AUDPC and the apparent infection rate (r) were the only useful quantitative variables to differentiate resistance of plantain and banana genotypes and aggresiveness of Black Sigatoka strains during *Musa*-*M. fijiensis* interaction under controlled condition.

Significant differences were obtained among the inoculated *Musa* genotypes, and it may be the starting point for *Musa* breeders and epidemiologists to develop descriptive and more precise growth model for the early screening of BLSD that could be used in identifying resistant genotypes to *M. fijiensis*.

*Asexual latent period (ALP)*

Asexual latent period was detected only in Grande naine, Pisang Awak and Pisang lilin genotypes. Grande naine (41,22 days) and Pisang Awak (44,72 days) had statistically significant shorter (approximately 14 days) ALP than Pisang lilin (56,50 days).

The asexual latent period clearly differentiated the response of Grande naine, Pisang Awak and Pisang lilin from the rest of the inoculated genotypes. It was demonstrated experimentally for the first time that *M. fijiensis* conidia could be obtained with inoculation assays using mycelial fragments in greenhouse conditions. Indeed, some *Musa* spp. were able to complete the asexual cycle within the evaluated period (63 DPI), and there was correspondence between the asexual latent period and the level of resistance among the genotypes. Browne *et al*. (32) used the ALP as a component of partial disease resistance in wheat, detected in a detached leaf assay with the inoculation of *Microdochium majus*, and where cultivar responses were possible to be differentiated. Nevertheless, Dita *et al*. (33) evaluated the ALP and the spore production in four potato genotypes artificially inoculated with *Alternaria solani* Sorauer, but the separation of cultivars according to resistance levels could not be obtained. Similarly, no difference was found in the number of asexual spores observed on seven partially resistant and susceptible *Musa* cultivars in the field and ten cultivars under controlled conditions with detached leaf assays (9).

Suffert *et al*. (20) identified ALP, development rate of sporulating area, maximal sporulating area, pycnidial density, and sporulation capacity traits as the most relevant variables to describe aggressiveness in *Mycosphaerella graminicola* (*Septoria tritici* blotch) population. They suggested that these variables could be used to estimate the quantitative resistance of wheat to this fungal pathogen.

Further studies should be led to improve the control of experimental conditions, the quantification methodology, and the inclusion of new *Musa* genotypes.

*Number of spermogonia*

The greatest number of spermogonia was observed in Grande naine and Pisang Awak, followed by Pisang lilin. Few spermogonia were detected in Calcutta 4 and Yangambi Km 5 and the lowest quantities were counted in FHIA-18 and FHIA-25 (Table 4). Spermogonia were only detected in Grande naine, Pisang Awak and, in a significant less number, in Pisang lilin (Table 4). The rest of the genotypes did not produced conidia.

In this work, the number of spermogonia was used as a component of resistance for the early evaluation of *Musa* genotypes artificially inoculated with Black Sigatoka in greenhouse. Significant statistically differences were obtained among the inoculated genotypes, and it was experimentally demonstrated that spermogonia of *M. fijiensis* could be obtained by inculating *Musa* spp. with mycelial fragments in greenhouse assays.

Pérez *et al*. (34) used the number of spermogonia and pseudothecia for the first time to compare Grande naine and FHIA-18 response to BLSD in two localities of Cuba, and they differentiated the resistance level of both genotypes. Nevertheless, this variable has not been used before under controlled conditions for the early discrimination of resistance in *Musa* spp.

In our experience, it was impossible to obtain pseudothecia of *M. fijiensis* because the inoculation assay was performed with only one isolate (CCIBP-

**TABLE 4.** Number of spermogonia and conidia/field per $mm^2$ in genotypes of *Musa* artificially inoculated with mycelial fragments of *M. fijiensis* (CCIBP-Pf80)./ *Número de espermogonios conidios/campo en genotipos de Musa, inoculados artificialmente con fragmentos miceliales de* ***M. fijiensis*** *(cepa, CCIBP-Pf80)*

| Genotypes | Number of spermogonia / 200x | | Number of conidia / 200x | |
|---|---|---|---|---|
| | Rank mean | Mean | Rank mea n | Mean |
| Grande naine | 581,98 a | 8,04 | 560,95 a | 1,76 |
| P. Awak | 565,58 a | 7,83 | 536,18 a | 1,61 |
| FHIA-18 | 203,38 d | 0,06 | 234,50 c | 0,00 |
| Yangambi km 5 | 216,16 c | 0,32 | 234,50 c | 0,00 |
| Calcutta 4 | 225,97 c | 0,39 | 234,50 c | 0,00 |
| P. lilin | 468,94 b | 4,94 | 418,37 b | 0,94 |
| FHIA-25 | 191,50 d | 0,05 | 234,50 c | 0,00 |

*Means followed by different letter in each column indicate differences among the ranges according to Kruskal-Wallis/Mann-Whitney test ($p<0.05$) n=15*

Pf80), and the heterothaly nature of *M. fijiensis* is well known. The sexual cycle of *M. fijensis* could be made possible under controlled conditions by inducing pseudothecia in plants artificially inoculated with isolates of different mating types in a greenhouse. The sexual latency period and the number of ascospores could then be calculated to be used as other components of resistance in *Musa* breeding programs.

## CONCLUSIONS

Results indicated that resistance to Black Sigatoka in artificially inoculated genotypes could be evaluated by components of resistance to assess the response of *Musa* spp. to *Mycosphaerella fijiensis* Morelet quantitatively, which would make more efficient and precise the early evaluation process to support banana and plantain breeding programs. Further genotypes with known resistance, partial resistance and susceptible phenotypes in field conditions must be confirmed in subsequent greenhouse tests. These results may be useful for screening BLSD *Musa* resistant breeding material, evaluation of aggressiveness of *M. fijiensis* isolates, studies related to molecular plant-pathogen interaction, and management schemes of BLSD. Additionally, these findings will supply information for guiding future studies on mechanisms involved in the BLSD resistance in *Musa* spp by dissecting the infection cycle of *M. fijiensis* under controlled condition.

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