

Comparison of Development of *Mycosphaerella fijiensis* and *Mycosphaerella musicola* on Banana and Plantain in the Various Ecological Zones in Cameroon

A. Mouliom-Pefoura, Research Plant Pathologist, Centre de Recherches Régionales sur Bananiers et Plantains (CRBP), Box 832 Douala, Cameroon; A. Lassoudière, Agronomist, Cirad-Flhor, box 5035 - 34032 Montpellier, France; and J. Foko and D. A. Fontem, Plant Pathologists, University of Dschang, Box 110 Dschang, Cameroon

ABSTRACT

Mouliom-Pefoura, A., Lassoudière, A., Foko, J., and Fontem, D. A. 1996. Comparison of development of *Mycosphaerella fijiensis* and *Mycosphaerella musicola* on banana and plantain in the various ecological zones in Cameroon. *Plant Dis.* 80:950-954.

Mycosphaerella fijiensis (black leaf streak disease) has replaced *M. musicola* (yellow Sigatoka disease) as the primary pathogen of banana and plantain in many of the low (0 to 500 m) and mid (500 to 1,000 m) elevations, but does not yet exist in the highland (>1,300 m) banana- and plantain-producing areas of Cameroon. Host-pathogen relationships of the two diseases were evaluated in the three ecological zones of Cameroon during 1993 and 1994 by experimental inoculations. In the low altitude zone (80 m), the first phases of parasitic infection (conidial germination, growth of germ tubes, and incubation period) were identical for both *M. fijiensis* and *M. musicola*. However, the duration of lesion expansion at low altitudes was shorter for *M. fijiensis* than for *M. musicola*, which may help explain the disappearance of the latter pathogen in the lowland areas. In the mid-altitude (900 m) and high-altitude (1,350 m) zones, conidia of *M. musicola* developed faster than those of *M. fijiensis*. The incubation period was also shorter for yellow Sigatoka disease (17 to 20 days) than for black leaf streak disease (22 to 24 days); however, black leaf streak disease could develop in these zones. These results should be taken into consideration in production strategies of bananas and plantains, especially when transferring planting materials between different ecological zones.

Bananas and plantains constitute an important staple food crop for millions of people in the tropics. In Cameroon, they are cultivated extensively in the southern part, where diseases constitute an important constraint on increased productivity. The most important disease of these crops in Cameroon is *Cercospora* leaf spot *sensu lato*.

Two *Cercospora* diseases are usually distinguished: yellow Sigatoka caused by *Mycosphaerella musicola* J. L. Mulder in J. L. Mulder & Stover (6), and black leaf streak (black Sigatoka) incited by *M. fijiensis* Morelet. Losses, in Cameroon and elsewhere, attributable to these diseases have been estimated at 50 to 100% (2,4, 15,20,22).

M. fijiensis was first reported in 1963 (18) while *M. musicola* was first reported in 1902 (25). The distribution of both pathogens appears to be determined by altitude (14). *M. fijiensis* is more virulent than *M. musicola* and tends to replace the latter in lowland areas. The process of this replacement is quite similar in many producing zones and arises after a period of coexistence of the two pathogens (1,7,11,

12,23). *M. fijiensis* also infects all plant cultivars that are resistant to *M. musicola*. The distribution of *M. musicola* now appears to be limited to highland regions (8, 13,16). The occurrence of black Sigatoka appears to be limited to areas below 1,200 m (14). Between 700 and 1,200 m, there is an equilibrium between the populations of both pathogens. The absence of black Sigatoka in highland regions makes these areas potential production areas of banana and plantain in Cameroon.

This work compares the development of the two pathogens under natural conditions in order to explain the disappearance of yellow Sigatoka disease in lowland areas and to evaluate the risks of spread of black leaf streak disease in highland regions.

MATERIALS AND METHODS

Host plants. The material used for inoculation was cv. Grande Naine (subgroup Cavendish AAA) plantlets from tissue culture, obtained according to the technique used by Escalant (5).

Inoculum production. Isolations were made from diseased leaves using techniques previously described (17). The inoculum was maintained by monthly transfers on potato dextrose agar (PDA). Two isolates were used in this study: *M. fijiensis* obtained from Njombé (80 m), and *M. musicola* obtained from Dschang (1,350 m). *M. fijiensis* is not found in Dschang while *M. musicola* is not found in Njombé. The cultural characteristics of each isolate were

similar. Despite of the importance of the ascospores in the epidemiology of these diseases (19), conidia were used as inoculum because they are easier to work with than ascospores.

Mycelial fragments were obtained from cultures growing on PDA medium. Mycelium (2.5 g) was ground in 10 ml of water with a mortar and pestle. One milliliter of this solution was spread on sterilized cellophane placed on a modified V8 medium (100 ml of V8, 0.2 g of CaCO₃, 20 g of agar per liter, pH 6.0) in a 9-cm-diameter petri dish (17). The cultures were incubated under continuous light at 20°C for 10 days, and then flooded with sterile distilled water containing 1% gelatin at 3 ml per petri dish.

Inoculation. A conidial suspension for each isolate was quantified to a concentration of 10⁵ conidia/ml with a hemacytometer. Inoculations were made on the youngest leaf of each plant. The inoculated leaf was protected with a polyethylene bag before it unfolded to prevent natural contamination. The polyethylene bag was removed at inoculation and kept off during incubation. The leaf was gently rubbed with cotton soaked in water to remove the wax layer of the leaf before inoculation and conidial suspension was deposited on one side of the leaf with a light brush. Plantlets inoculated in the dry season were watered every 3 to 4 h by misting in Njombé or by manual rainfall in Melong and Dschang in order to ensure good conditions for conidial germination and germ tube growth (10). Inoculated plantlets were arranged in a randomized complete block design with three replicates (four plantlets per replicates per treatment). Plantlets were incubated in Njombé, Melong (900 m), and Dschang.

Conidial germination and growth, incubation period, and life cycle duration. One foliar piece per plantlet was removed with a 10-mm-diameter punch 72 h after inoculation. The pieces were stained in 0.1% cotton blue-lactophenol medium for 20 min, mounted on a slide and observed with a compound microscope (eyepiece 10, objective 40, Leitz Wetzlar, Wetzlar, Germany). Conidial germination and germ tube length were determined. About 300 conidia were observed for conidial germination and 60 germ tubes were measured for each site. A conidium was considered to be germinated when the length of its germ tube was at least greater than its diameter.

Corresponding author: A. Mouliom-Pefoura.

Accepted for publication 16 March 1996.

Publication no. D-1996-0624-04R
© 1996 The American Phytopathological Society

The incubation period of the disease was determined as the difference between the date of appearance of stage 1 symptoms and the date of inoculation. Stage 1 symptoms consist of the first external manifestation of the disease, i.e., a small depigmentation mark, whitish or yellow. Duration of lesion development was studied at Njombé, where the two diseases are well established, in order to explain the disappearance of yellow Sigatoka in this zone. The same study was not carried out in Melong or in Dschang; there was no strong reason to carry it out in Melong, where the two diseases yet exist and develop in mixture, and such study would have exposed the Dschang zone (where only yellow Sigatoka is present) to the risks of the spread of *M. fijiensis* spores after the necrotic phase. So the infectious cycle (life cycle), which is the total duration of the incubation period and that of lesion expansion, was studied only in Njombé; it was determined as the difference between the date of appearance of the necrotic stage and that of inoculation, while the duration of lesion development was determined as the difference between the date of appearance of the necrotic stage and that of the stage 1 symptoms of the disease.

Data analyses. All data were analyzed statistically with version C of MSTAT statistical package (Michigan State University, East Lansing) and means were separated by means of the Student-Newman-Keuls test.

RESULTS AND DISCUSSION

Development of both pathogens. At Njombé (80 m elevation). In 1993 and 1994, percent germination, germ tube length, and incubation period were similar for the two species (Table 1). Mean percent germination was 90 to 95% and mean germ tube length was 72 to 73 μm . The incubation period was between 16 and 17 days for the two treatments (Table 1). Mean daily temperatures recorded during the incubation period ranged between 21 and 30°C (1993) and 22 and 33°C (1994) (Fig. 1A, B).

Differences were observed in the rate of lesion development for the two isolates in 1994. Necrotic symptoms incited by *M. fijiensis* appeared earlier (32 days) than those caused by *M. musicola* (42 days). These necrotic symptoms corresponded to spore production by the isolates.

At Melong (900 m elevation). In 1993, percent germination but not germ tube length, was significantly greater for *M. musicola* than for *M. fijiensis* (Table 2). Minimum daily temperatures recorded during the incubation period varied between 11 and 20°C while the maximum temperatures ranged from 21 to 29°C (Fig. 2A) and were lower than those observed in Njombé.

In 1994, differences in conidial germination and germ tube length were not sig-

nificant for the two treatments; however, values for *M. fijiensis* were slightly higher than those for *M. musicola* (Table 2). Daily temperatures recorded during the incubation at this site ranged between 19 and 29°C (Fig. 2B).

At Dschang (1,350 m elevation). In 1993 and 1994, *M. musicola* developed faster than *M. fijiensis*. Conidial germination and germ tube length were greater in *M. musicola* than in *M. fijiensis*. (Table 3). Values for conidial germination and germ tube

Table 1. Comparison of the different phases of infection by *Mycosphaerella fijiensis* (Mf) and by *M. musicola* (Mm) conidia after inoculation of Grande Naine (AAA) plantlets in Njombé (80 m elevation)

Date of inoculation	Variable	Isolate ^x		CV (%)
		Mf	Mm	
9 July 1993	Germination (%)	90.4 a ^y	92.5 a	1.9
	Germ tube (μm)	73.4 a	71.9 a	2.9
	Incubation (days) ^z	17.2 a	16.8 a	6.7
	Life cycle (days) ^z	40.1 a	44.5 a	7.7
13 April 1994	Germination (%)	96.4 a	94.8 a	3.8
	Germ tube (μm)	72.3 a	69.6 a	5.2
	Incubation (days)	15.8 a	18.3 a	8.9
	Life cycle (days)	32.5 b	42.4 a	4.1

^x Values are the means of three replicates with four plants per replicate; 300 spores were counted for percent germination and 60 germ tubes were measured 72 h after inoculation.

^y Means within a row followed by the same letter are not significantly different ($P = 0.05$) according to the Student-Newman-Keuls test.

^z Let D1, D2, and D3 be, respectively, the date of inoculations, of the appearance of stage 1 symptoms of the diseases, and of the appearance of the necrotic stage of the diseases: D2 to D1 is the incubation period, D3 to D2 the duration of lesion development, and D3 to D1 the duration of the life cycle of the disease.

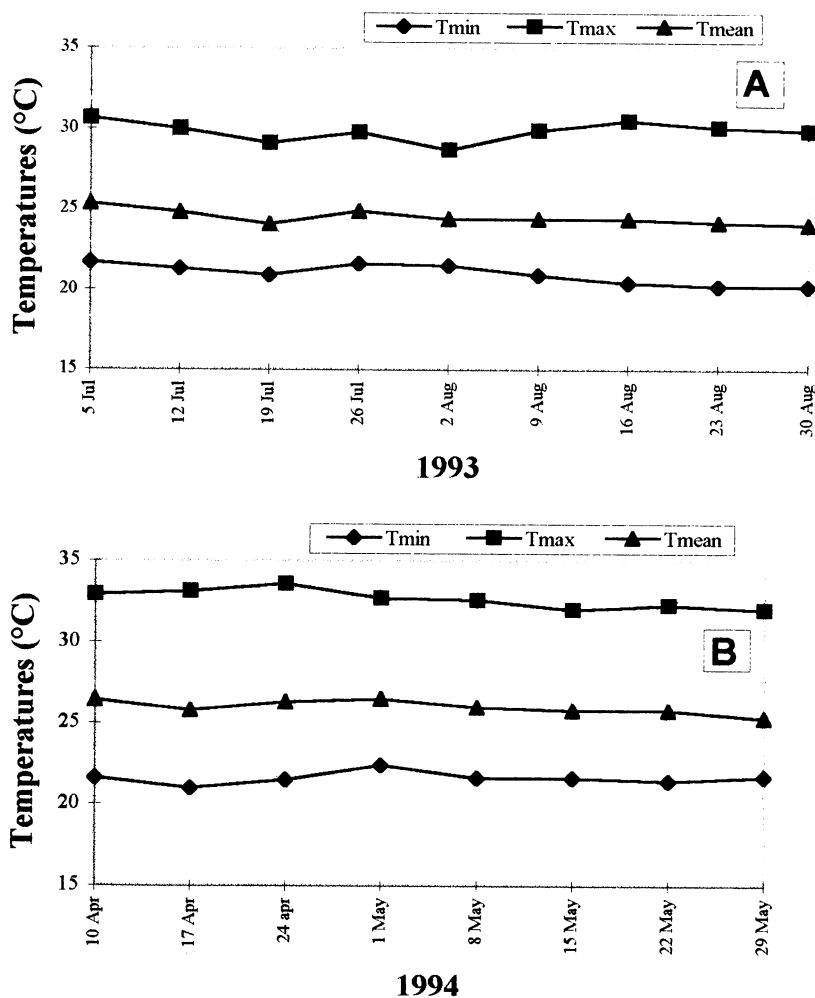


Fig. 1. Weekly means of the minimum (Tmin), maximum (Tmax), and mean (Tmean) temperatures recorded in Njombé (80 m elevation) during incubation periods of Sigatoka diseases after inoculation on banana and plantlets in (A) 1993 and (B) 1994.

Table 2. Comparison of the first phases of infection by *Mycosphaerella fijiensis* (Mf) and by *M. musicola* (Mm) conidia after inoculation of Grande Naine (AAA) plantlets in Melong (900 m elevation)

Date of inoculation	Variable	Isolate ^x		CV (%)
		Mf	Mm	
10 August 1993	Germination (%)	35.1 b ^y	44.2 a	8.0
	Germ tube (µm)	23.0 a	32.1 a	15.7
	Incubation (days) ^z	23.0 a	18.6 a	8.4
20 April 1994	Germination (%)	69.1 a	65.3 a	6.9
	Germ tube (µm)	51.9 a	44.8 a	3.7
	Incubation (days)	20.5 a	25.3 a	10.4

^x Values are the means of three replicates with four plants per replicate; 300 spores were counted for percent germination and 60 germ tubes were measured 72 h after inoculation.

^y Means within a row followed by the same letter are not significantly different ($P = 0.05$) according to the Student-Newman-Keuls test.

^z Let D1 and D2 be, respectively, the date of inoculation and that of the appearance of stage 1 symptoms of the diseases; D2 to D1 is the incubation period.

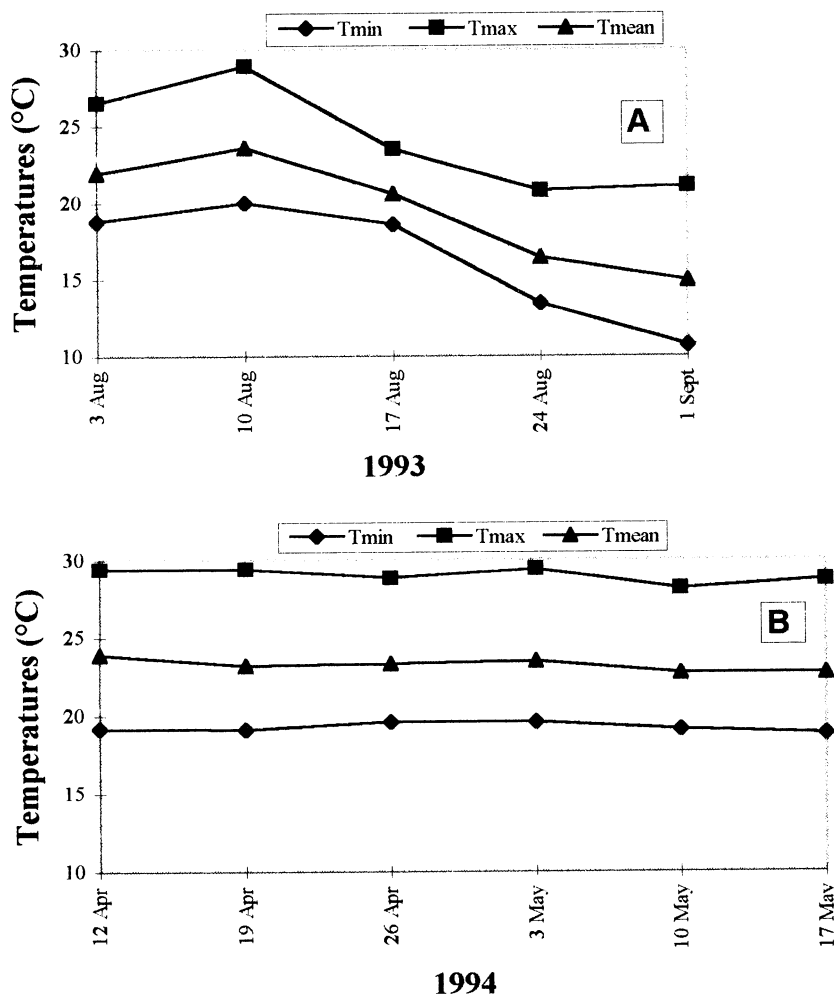


Fig. 2. Weekly means of the minimum (Tmin), maximum (Tmax), and mean (Tmean) temperatures recorded in Melong (900 m elevation) during incubation periods of Sigatoka diseases after inoculation on banana and plantlets in (A) 1993 and (B) 1994.

length were lower in 1994 than those in the 1993 inoculations but the two experiments did not differ significantly. The incubation periods were significantly greater for *M. fijiensis* (23.6 days) than for *M. musicola* (15.2 days) in 1993, and were similar, with an average of 20 days, for the two treatments in 1994 (Table 3).

Mean temperatures recorded at this site were quite stable during the first 2 weeks

of incubation in the 1993 trial (Fig. 3A). Minimum temperatures varied between 15 and 18°C while the maximum was 25°C during both experiments.

Results confirm that conditions in lowland areas are favorable for the development of both pathogens. The mean daily temperatures decreased severely during the first inoculation but were almost constant during the second. These variations in tem-

perature influenced the development of both pathogens. The minimum and maximum temperatures for this region are around 22 and 30°C, respectively, while the mean is about 25°C, which is close to the optimum temperature for epiphyllous development of both species (3,13,21). The highest conidial germination and fastest germ tube development were recorded in this region and the incubation period was 15 to 17 days. These results agree with previous observations in this region for *M. fijiensis* (9). The life cycle of *M. fijiensis* was shorter than that of *M. musicola*. This disparity could be attributed to the shorter duration of lesion development observed for *M. fijiensis*. This could account for the dominance of black Sigatoka and the disappearance of yellow Sigatoka in lowland areas. Zadoks and Schein (24) reported that age distribution is an important factor in the development of pathogen populations and population growth depends on the reproductive fraction (age) of the population. Accordingly, a population with relatively more reproductive individuals would grow faster than that with young individuals.

In the mid-altitude zone (Melong), *M. fijiensis* had a weak epiphyllous development during the whole incubation period. The higher percent germination and the faster germ tube development observed for *M. musicola* than for *M. fijiensis* in this region could be attributed to the low temperatures recorded during this period, when mean temperatures varied from 15 to 24°C. In contrast, *M. fijiensis* developed slightly faster than *M. musicola* in the second trial (1994). This difference was similar to the results obtained under natural conditions for both pathogens (14). For instance, during this period of the year (March to April), both diseases are found in the area where this study took place, with a higher prevalence of black leaf streak than yellow Sigatoka disease, which generally disappears between early July and late November in the region.

The environment at the high-altitude area (Dschang) was characterized by great differences in minimal temperatures. During the first 2 weeks after inoculation, minimal temperatures were 18 and 15°C in the 1993 and 1994 inoculations, respectively. The incubation period was shorter for *M. musicola* (15 to 18 days) than for *M. fijiensis* (22 to 25 days) and this may account for the prevalence of yellow Sigatoka in the high-altitude regions.

However, the ability of *M. fijiensis* to develop at low temperatures in high-altitude regions should be taken into consideration when making recommendations for transfer of plant materials between different ecological zones.

ACKNOWLEDGMENTS

We thank the International Foundation for Science (IFS) for financial contribution of this work.

Table 3. Comparison of the first phases of infection by *Mycosphaerella fijiensis* (Mf) and by *M. musicola* (Mm) conidia after inoculation of Grande Naine (AAA) plantlets in Dschang (1,350 m elevation)

Date of inoculation	Variable	Isolate ^x		CV (%)
		Mf	Mm	
1 November 1993	Germination (%)	49.0 b ^y	92.5 a	12.0
	Germ tube (µm)	14.1 b	23.0 a	6.0
	Incubation (days) ^z	23.6 b	15.2 a	11.1
23 May 1994	Germination (%)	33.9 b	67.7 a	10.2
	Germ tube (µm)	9.1 b	16.8 a	9.9
	Incubation (days)	21.7 a	19.2 a	8.8

^x Values are the means of three replicates with four plants per replicate; 300 spores were counted for percent germination and 60 germ tubes were measured 72 h after inoculation.

^y Means within a row followed by the same letter are not significantly different ($P = 0.05$) according to the Student-Newman-Keuls test.

^z Let D1 and D2 be, respectively, the date of inoculation and that of the appearance of stage 1 symptoms of the diseases; D2 to D1 is the incubation period.

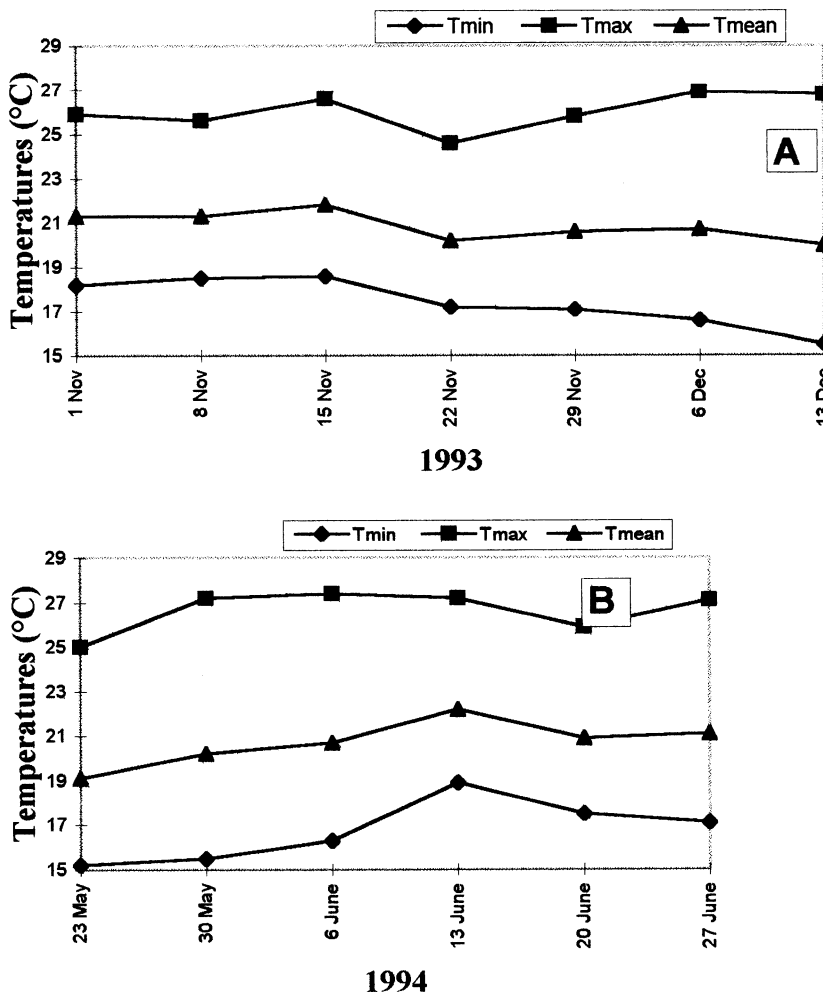


Fig. 3. Weekly means of the minimum (Tmin), maximum (Tmax), and mean (Tmean) temperatures recorded in Dschang (1,350 m elevation) during incubation periods of Sigatoka diseases after inoculation on banana and plantlets in (A) 1993 and (B) 1994.

LITERATURE CITED

- Avila Adame, C. 1991. Epidemiología de la Sigatoka negra (*Mycosphaerella fijiensis*) Del plátano (*Musa AAA*) bajo diferente nivel de control comercial en Tabasco, Mexico. Ph.D. thesis. Colegio de Postgraduados, Montecillos, Mexico.
- Bustamente, M. 1983. Impact du *Cercospora* noir sur la production du plantain au Honduras. Fruits 4:330-332.
- Calpouzios, L. 1955. Effect of temperature. Pages 40-50 in: Studies on Sigatoka Disease of Bananas and Its Fungus Pathogen. Atkins Garden and Research Laboratory, Cienfuegos, Cuba.
- Emebiri, L. C., and Obiefuna, J. C. 1992. Effects of leaf removal and intercropping on the incidence and severity of Black Sigatoka disease at the establishment phase of plantains (*Musa* spp. AAB). Agric. Ecosyst. Environ. 39:213-219.
- Escalant, J. V. 1987. Les bananiers diploides

en culture *in vitro* (*M. acuminata* et *M. balbisiana*). Etude du comportement et recherche de variabilité. Ph.D. thesis. Université des Sciences et Techniques du Languedoc, Montpellier, France.

- Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. American Phytopathological Society, St. Paul, MN.
- Fernandez, F. 1992. La Sigatoka negra: Origin, distribución e impacto económico y social. Pages 17-35 in: Preparandonos para una lucha eficaz contra la Sigatoka negra en el Ecuador. R. Velastegui and J. Chang, eds. Cabañilla, Quito, Ecuador.
- Fouré, E., and Lescot, T. 1988. Variabilité génétique des *Mycosphaerella* inféodés au genre *Musa*. Mise en évidence de la présence au Cameroun sur bananiers et plantains d'une cercosporiose (*Mycosphaerella musicola*) au comportement pathogène atypique. Fruits 42: 407-415.
- Fouré, E., and Mouliom-Pefoura, A. 1988. La cercosporiose noire des bananiers et plantains au Cameroun (*Mycosphaerella fijiensis* Morelet). Contribution à l'étude des premières phases de l'infection parasitaire. Mise au point de tests précoces d'inoculation sur plants issus de *in vitro* culture. Fruits 43:339-348.
- Goods, R. D., and Tsrch, M. 1963. Greenhouse studies on the *Cercospora* leaf of bananas. Trans. Br. Mycol. Soc. 46:321-330.
- Jones, D. R., and Mourichon, X. 1993. Black Leaf Streak/Black Sigatoka Disease. INIBAP, Montpellier, France.
- Mouliom-Pefoura, A. 1984. Distribution et incidence potentielle de la cercosporiose noire causée par *Mycosphaerella fijiensis* Morelet sur banane dessert et plantain dans le département du Moungo (Cameroun). Situation en Mai 1984. Rev. Sci. Tech. Ser. Agro. 1:9-13
- Mouliom-Pefoura, A. 1989. Contribution à l'étude d'une interaction atypique entre *Mycosphaerella musicola* (Maladie de Sigatoka) et les bananiers du sous-groupe plantain en zone d'altitude au Cameroun. M.S. dissert. Pierre et Marie Curie University, Paris.
- Mouliom-Pefoura, A. 1995. Les cercosporioses des bananiers et plantains (*Mycosphaerella musicola* Leach et *M. fijiensis* Morelet). Epidémiologie et Ecologie dans le contexte des zones de production du Cameroun. Ph.D. thesis. University of Dschang, Dschang, Cameroon.
- Mouliom-Pefoura, A., and Fouré, E. 1988. Efficacités comparées de différentes formulations de Triadiméol appliquées au sol sur *Mycosphaerella fijiensis* Morelet, agent de la maladie des raies noires des plantains au Cameroun. Evaluation des possibilités de lutte en milieu paysan. Fruits 43:201-210.
- Mouliom-Pefoura, A., and Mourichon, X. 1990. Développement de *Mycosphaerella musicola* (maladie de Sigatoka) et *M. fijiensis* (maladie des raies noires) sur les bananiers et plantains. Etude du cas particulier des productions d'altitude. Fruits 45:17-24.
- Mourichon, X., Peter, D., and Zapater, M. F. 1987. Inoculations expérimentales de *Mycosphaerella fijiensis* Morelet sur de jeunes plantules de bananiers issues de culture *in vitro*. Fruits 42:195-198.
- Rhodes, P. L. 1964. A new banana disease in Fiji. Commonw. Phytopathol. News 10:38-41.
- Stover, R. H. 1970. Leaf spot of bananas caused by *Mycosphaerella musicola*: Role of conidia in epidemiology. Phytopathology 60: 856-860.
- Stover, R. H. 1983. Effet du *Cercospora* noir sur les plantains en Amérique Centrale. Fruits 38:326-329.

21. Stover, R. H. 1983. The effect of temperature on ascospores germ tube growth of *Mycosphaerella musicola* and *M. fijiensis* var. *difformis*. *Fruits* 38:621-628.
22. Stover, R. H., and Simmonds, N. W. 1987. Bananas. 3rd ed. Longman Scientific and Technical, London.
23. Tapia Fernandez, A. C. 1993. Distribucion altitudinal de la Sigatoka amarilla (*Mycosphaerella musicola*) y la Sigatoka negra (*Mycosphaerella fijiensis*) en Costa Rica. Ph.D. thesis. University of Costa Rica, San José.
24. Zadoks, J. C., and Schein, R. D. 1979. *Epidemiology and Plant Disease Management*. Oxford University Press, London.
25. Zimmerman, A. 1902. Über einige tropischer Kul turpflanzen beobachtete Pilze. *Central f. Bakter* 2:219.