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Original article

Impact of the extension of black leaf streak disease on banana susceptibility to post-harvest diseases

Cécile Annie EWANÉ[1,5,8], Marc CHILLET[3,6], Florence CASTELAN[3], Yves BROSTAUX[4], Ludivine LASSOIS[1], Josué ESSOH NGANDO[5], Olivier HUBERT[7], Yolande CHILIN-CHARLES[7], Philippe LEPOIVRE[1], Luc de LAPEYRE de BELLAIRE[2,5]*

[1] Univ. Liege, Gembloux Agro-Bio Tech, Plant Pathol. Unit, Passage des Déportés 2, B-5030 Gembloux, Belgium

[2] CIRAD, Persyst, Res. Unit, Banana, plantain and pineapple cropping systems, TA B-26 / PS4, Boulevard de la Lironde, F-34398 Montpellier, France, luc.de_lapeyre_de_bellaire@cirad.fr

[3] Dep. Aliment. Nutr. Exp., Fac. Cienc. Farm., Univ. Sao Paulo, Av. Lineu Prestes, 580, 05508-900 Sao Paulo, Brasil

[4] Univ. Liege, Gembloux Agro-Bio Tech, Appl. Stat. Comput. Sci. Math. Unit, Av. Fac. 8, B-5030 Gembloux, Belgium

[5] CARBAP, Afr. Cent. Res. Bananas and Plantains, Phytopathol. Unit, P.O. Box 832, Douala, Cameroon

[6] CIRAD, UMR Qualisud, F-34398 Montpellier cedex, France

[7] CIRAD, Stn. Neufchâteau, 97130 Capesterre Belle-Eau, Guadeloupe

[8] Present address: Univ. Yaoundé I, Fac. Sci., Dep. Biochem., P.O. Box 812, Yaoundé, Cameroon

* *Correspondence and reprints*

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***RESUMEN ESPAÑOL**, p. 365*

Impact of the extension of black leaf streak disease on banana susceptibility to post-harvest diseases.

Abstract – Introduction. The susceptibility of banana fruit to crown rot and anthracnose, the two main banana post-harvest diseases, is influenced by many pre-harvest abiotic factors. *Mycosphaerella* leaf spot diseases (MLSD) of bananas are biotic pre-harvest factors, which have an influence on fruit physiology. The fruit's susceptibility to post-harvest diseases may also be influenced by foliar diseases caused by *M. fijiensis*, responsible for black leaf streak disease (BLSD), and *M. musicola*, which causes sigatoka disease (SD). The aim of our study was to determine the influence of these biotic pre-harvest factors on banana fruit's susceptibility to crown rot and anthracnose. **Materials and methods**. A disease severity gradient was established in two experimental fields (Cameroon for BLSD and Guadeloupe for SD) where, at the flowering stage, six different levels of MLSD severity were selected. Fruit susceptibility was determined through necrotic surface assessments after artificial inoculation by *Colletotrichum musae* on the 3rd hand of harvested bunches. **Results and discussion**. BLSD significantly influenced banana sensitivity to crown rot ($P < 0.001$) but only had a slight effect on the development of anthracnose ($P = 0.041$). SD had no effect ($P > 0.05$) on banana susceptibility to either post-harvest disease. These results are discussed with emphasis on the influence of variations in the source-sink ratio on fruit physiology. The influence of BLSD on crown rot disease suggests the need to take into account the management of these foliar diseases for an alternative control method of post-harvest diseases through integrated pest management programs.

Cameroon / Guadeloupe / *Musa* / fruits / *Mycosphaerella fijiensis* / *Mycosphaerella musicola* / black sigatoka / black leaf streak disease / anthracnoses / crown rots

Impact de l'extension de la maladie des raies noires sur la sensibilité des bananes aux maladies post-récolte.

Résumé – Introduction. La sensibilité des bananes aux maladies post-récolte (pourriture de couronnes et anthracnose) est influencée par plusieurs facteurs abiotiques pré-récolte. Les maladies foliaires de bananes sont des facteurs biotiques pré-récolte causés par *Mycosphaerella fijiensis* pour la maladie des raies noires (MRN) et *M. musicola*, pour la maladie de Sigatoka (MS). Ces maladies foliaires pourraient avoir une influence sur la physiologie du fruit. L'objectif de cette étude a été de déterminer l'influence de ces facteurs biotiques pré-récolte sur la sensibilité du fruit à la pourriture de couronnes et à l'anthracnose. **Matériel et méthodes**. Un gradient de sévérité de la maladie a été établi dans deux parcelles expérimentales (Cameroun pour la MRN et Guadeloupe pour MS). À la floraison, six niveaux différents de sévérité de ces maladies foliaires ont été sélectionnés. La sensibilité du fruit a été déterminée en évaluant la surface de couronnes nécrosée après inoculation artificielle des fruits de la 3e main du régime par *Colletotrichum musae*. **Résultats et discussion**. La MRN a influencé significativement la sensibilité des bananes à la pourriture de couronnes ($P < 0{,}001$), mais a eu seulement un léger effet sur le développement de l'anthracnose ($P = 0{,}041$). La MS n'a eu aucun effet ($P > 0{,}05$) sur la sensibilité des bananes à ces deux maladies post-récolte. Ces résultats ont été discutés avec un accent particulier sur l'influence des variations du ratio source-puits sur la physiologie du fruit. L'influence de la MRN sur la pourriture des couronnes suggère la nécessité de la prise en compte de la gestion des maladies foliaires dans les méthodes alternatives de contrôle des maladies post-récolte au travers des programmes de gestion intégrés.

Cameroun / Guadeloupe / *Musa* / fruits / *Mycosphaerella fijiensis* / *Mycosphaerella musicola* / cercosporiose / maladie des raies noires / anthracnose / pourriture de la couronne

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1. Introduction

Post-harvest diseases, especially anthracnose and crown rot, significantly affect the quality of exported bananas in major production zones. *Colletotrichum musae,* a banana-specific pathogen, is responsible for anthracnose lesions [1]. Fruits are mainly contaminated in the field following flowering, by conidia produced on the floral parts and on senescent leaves [2]. Conidia germinate rapidly and form melanized appressoria that remain quiescent until fruit approaches maturity. From there, they form penetration hyphae, which colonize the underlying tissues, leading to anthracnose lesions, especially in the case of quiescent anthracnose [3]. If the bananas are bruised, a rot can develop when the fruit is still green, and this later leads to larger lesions [1], technically called wound anthracnose.

A broad unspecific and opportunistic fungal complex causes crown rot. Within this complex, *Colletotrichum musae* is the most pathogenic species [4–6]. Rotting later evolves during shipping, storage, ripening and commercialization. This obviously has a negative impact on the commercial value of the banana fruit [7].

The incidence and severity of these post-harvest diseases are determined by pre-harvest factors, which contribute to determining the fruit's quality potential. As such, the quality potential of bananas depends on abiotic pre-harvest factors such as agronomic practices and soil-climate factors [8–10]. Concerning anthracnose, it has been demonstrated that pre-harvest factors contributing to the degree of fruit susceptibility at harvest and to the degree of contamination by *Colletotrichum musae* are key factors in the post-harvest development of this disease and of its control [11–13].

Moreover, Chillet *et al.* showed that the physiological age (based on temperature sums) is a critical factor which affects the susceptibility of bananas to wound anthracnose [12]. Recently, a similar relationship between the age of the fruit in degree days (dd) and its susceptibility to crown rot was reported [14]. Unfortunately, little is known about pre-harvest factors, which influence the post-harvest development of crown rot disease in bananas. Although pedo-climatic conditions and agro-technical factors are known to influence the development of this disease [15–17], only very few studies link such fluctuations to either fruit susceptibility [6, 10, 18] or to fruit contamination.

Among the pre-harvest factors that have an impact on the susceptibility of banana fruit to post-harvest diseases, the influence of biotic factors has been poorly studied to date. However, a study recently carried out in some localities in Cameroon has highlighted a potential effect of black leaf streak disease (BLSD) on banana susceptibility to crown rot [18]. BLSD and Sigatoka disease (SD) of bananas are foliar diseases caused, respectively, by the ascomycetous fungi *Mycosphaerella fijiensis* and *M. musicola*. Severe infections can lead to a substantial reduction of the leaf area. Moreover, these foliar diseases also have an indirect effect on the quality of the fruit, especially as bananas harvested from heavily infected plants cannot be exported because of early ripening. A relation has been particularly shown between the severity of these diseases and the greenlife (GL) of bananas harvested at a constant physiological age [19, 20]. These results clearly show that *Mycosphaerella* leaf spot diseases have a direct effect on banana physiology, although the mechanisms involved therein remain unknown.

Since these foliar diseases have an influence on fruit physiology, they could have a potential influence on banana susceptibility to post-harvest diseases. The purpose of our study was therefore to further investigate the influence of these foliar diseases on banana susceptibility to crown rot and anthracnose. This study was conducted in two different agro-ecological locations where each pathogen was exclusively present. Since *M. fijiensis* is an invasive pathogen that eliminates *M. musicola* progressively in all banana-growing countries where it is introduced [21], the effects of black leaf streak disease and Sigatoka disease were studied in Cameroon and Guadeloupe, respectively. *Mycosphaerella fijiensis* was effectively reported for the first time in Cameroon in 1980 and it totally replaced *M. musicola* [22]. The study was conducted 4 years before

M. fijiensis had been reported in Guadeloupe (2012) and *M. musicola* was prevalent at that time [21].

2. Materials and methods

2.1. Plant material

Banana fruits [*Musa acuminata* (AAA group, Cavendish subgroup) cv. Grande Naine] were harvested from plants grown in commercial banana farms. The banana plants were selected at the flowering stage (fingers in the horizontal position) and were covered with a plastic sleeve. Bunches were tied with a colored belt for them to be recognized at harvest (for each week of selection, a different color was chosen). The third hand of bunches was harvested and used for analysis.

2.2. Prediction of harvest time

In order to forecast the harvest dates, temperatures were recorded on the different plantations. An electronic probe (Tinytag Plus, Gemini Data Loggers Ltd., Chichester, UK) with regular statements (every 15 min) was installed in the experimental plots and the average daily temperature was calculated from all daily data as described by Ganry and Chillet [23]. The bunches were harvested at a constant physiological age [24], *i.e.*, when the sum of the mean daily temperature accumulated by the fruit at the 14 °C threshold, between flowering and harvest, reached 900 degree days (dd).

2.3. Experimental design

Our experiment was conducted in two commercial banana farms located in two different environments where climatic conditions are favorable for the development of leaf spot diseases:

– Cameroon: in Njombé (Mbomè), altitude 80 m, average temperature 27 °C, mean annual rainfall 3000 mm. Only *M. fijiensis* was present in this plantation.

– Guadeloupe: in Capesterre-Belle-Eau (Montbelley), altitude 180 m, average temperature 25 °C; mean annual rainfall 2800 mm. Only *M. musicola* was present here.

In each plot, a gradient of Leaf Spot Disease (LSD) was obtained through differential chemical control of the foliar diseases, one part of the experimental design being untreated. Moreover, mechanical deleafing of diseased leaves was not done on the plots. Six groups (treatments) of banana plants were selected at different flowering dates (horizontal finger stage) according to their level of BLSD or SD severity: A: severity between 0% and 5%; B: severity between 6% and 15%; C: severity between 16% and 25%; D: severity between 26% and 35%; E: severity between 36% and 45%; F: severity > 46%.

When the gradient of leaf spot disease (LSD) was obtained in both locations (Cameroon and Guadeloupe, June 2008), banana trees were selected according to the level of severity of *Mycosphaerella* leaf spot disease (MLSD) at the flowering stage. In Cameroon, ten banana plants were selected per week for three consecutive weeks for each disease severity index (DSI) group and harvested for three consecutive weeks as well after a flowering-to-harvest-period (FHP) of about 77 days. In Guadeloupe, since the banana plot was smaller, such a design could not be used and the banana plants were selected for two labeling periods (2 weeks), and banana trees were placed into each DSI group according to their availability; they were also harvested for two harvest periods after an average FHP of 82 days (*table I*).

2.4. Assessment of leaf spot diseases

During the flowering-to-harvest-period, different effects of leaf spot diseases were assessed on banana plants and on fruit morphology.

2.4.1. Disease severity

Disease severity was evaluated to characterize the intensity of necrotic surface: disease severity was observed on all selected banana plants from flowering to harvest.

Table I.
Number of banana trees selected at flowering (NBT_F) and number of banana trees harvested (NBT_H) for the different replicates of the experiments conducted in Cameroon for black leaf streak disease and in Guadeloupe for sigatoka disease. Bunches were harvested at a constant physiological age of 900 dd. The duration (days) of the flowering-to-harvest-period (FHP) is also indicated for each replicate. The NBT_H and the number of banana fruit inoculated for analysis are different because of early ripening of fruit before harvest.

Disease severity index group	Replicate	Black leaf streak disease				Sigatoka disease			
		Flowering date	NBT_F	Harvest date (FHP)	NBT_H	Flowering date	NBT_F	Harvest date (FHP)	NBT_H
A	1	11 June2008	10	27 August 2008 (77 days)	10	From 11 to 30 June 2008	8	From 1 to 18 September 2008 (80-82 days)	7
B	1		10		10		10		8
C	1		10		10		15		13
D	1		10		8		14		9
E	1		10		9		7		7
F	1		7		5				
A	2	19 June 2008	10	3 September 2008 (76 days)	10	From 16 September to 3 October 2008	9	From 11 to 23 December 2008 (81-86 days)	4
B	2		10		10		10		10
C	2		10		10		13		10
D	2		10		10		12		5
E	2		10		10		9		8
F	2		10		10		–		–
A	3	25 June 2008	10	10 September 2008 (77 days)	10		–		–
B	3		10		10		–		–
C	3		10		10		–		–
D	3		10		9		–		–
E	3		10		10		–		–
F	3		10		10		–		–
Total	3		177		171		107		81

The severity index (SI), which is an estimation of the percentage of necrotic leaf area per plant, was then calculated according to the method described by Gauhl *et al.* [25]. Following this method, the severity index of each leaf is scored according to the following scale: 0: no necrotic lesions; 1: less than 1% necrotic lesions; 2: 1–5% necrotic lesions; 3: 6–15% necrotic lesions; 4: 16–33% necrotic lesions; 5: 34–50% necrotic lesions; 6: more than 50% necrotic lesions. The SI is calculated as {$SI = [(\sum scores / 6) \times NTL] \times 100$}, where $\sum$scores is the sum of all infestation indices of the banana plant and NTL is the number of leaves of the banana plant. The black leaf streak disease and the sigatoka disease severities (BLSDS, SDS) at flowering ($_F$) and at harvest ($_H$) were respectively named: $BLSDS_F$; $BLSDS_H$; SDS_F and SDS_H. A higher degree of the disease severity index at flowering (group F, SI > 45%) could not be achieved in Guadeloupe for Sigatoka disease (*table I*).

2.4.2. Number of functional leaves

The number of functional leaves was evaluated to characterize the photosynthetic potential of the banana tree: the number of functional leaves at flowering (NFL_F) and at harvest (NFL_H). In our study, NFL was determined with the assumption that functional

leaves should have less than 30% of necrotic surface, *i.e.*, an infestation index of the leaf < 5.

2.4.3. Youngest leaf spotted

The youngest leaf spotted was also assessed. This indicator is commonly used to characterize the efficiency of BLSD control methods [21]: the youngest leaf spotted first at flowering (YLS_F) and later at harvest (YLS_H). The youngest leaf spotted (YLS) was assessed according to the method of Stover and Dickson [26], which involves the monitoring of the youngest leaf (from the top of the plant) bearing at least 10 necrotic lesions.

2.4.4. Fruit diameter

Fruit diameter (grade) was evaluated in order to characterize the effect of leaf spot diseases on fruit growth (fruit morphology and fruit filling rate). The diameter (mm) of a fruit was measured in the third hand (median position in the internal row) of each bunch harvested.

2.4.5. Percentage of banana trees with at least one ripe fruit at harvest

The percentage of banana trees with at least one ripe fruit at harvest was also measured in both locations (Cameroon and Guadeloupe) for each disease severity index group, since the *Mycosphaerella* leaf spot disease induces early ripening of fruit.

2.5. Artificial inoculations for the evaluation of susceptibility to post-harvest diseases

Ripe fruits at harvest were not inoculated. Only the green ones were used for evaluation of fruit susceptibility to both post-harvest diseases starting from the same pathogen (*Colletotrichum musae*), which can induce anthracnose and crown rot development. Crown rot and anthracnose diseases were evaluated in both locations.

2.5.1. Fungal strains

Two *Colletotrichum musae* strains isolated in Cameroon and in Guadeloupe were used for artificial inoculations. In order to avoid fungal variations, all cultures were initiated 15 days before inoculation, starting from frozen cryotubes. Thereafter, the strain was transplanted into Mathur's medium ($MgSO_4.7H_2O$: 2.5 $g{\cdot}L^{-1}$; KH_2PO_4: 2.7 $g{\cdot}L^{-1}$; peptone: 1 $g{\cdot}L^{-1}$; yeast extract: 1 $g{\cdot}L^{-1}$; saccharine: 10 $g{\cdot}L^{-1}$; agar: 15 $g{\cdot}L^{-1}$) and incubated at 25 °C for 10 days. Conidia were recovered with sterile distilled water and later passed through a sterile sieve of 45 µm, and suspensions were calibrated with a Mallassez cell at a concentration of 10^4 and 10^6 conidia per mL for crown rot and anthracnose inoculations, respectively.

2.5.2. Evaluation of susceptibility to crown rot disease

A cluster of four banana fingers was cut at the median of each harvested hand. The cuttings were square, with regular and clear-cut sections in order to obtain similar crowns between the clusters. Once the latex ran out, crown tissues were dried with absorbent paper and sterilized by dipping them in 50° alcohol. Fruits were then laid out at room temperature for 2 hours to allow the crowns to dry. A droplet of 50 µL of the *C. musae* suspension (10^4 conidia$\cdot mL^{-1}$) was then deposited at the top of the crown. A sterilized square filter paper of 1 cm^2 was placed on the droplet in order to maintain the inoculum in place. Three hours after inoculation, clusters of the same treatment were packed in perforated plastic bags and in commercial boxes. They were stored under stable conditions at 13 °C for 13 days [18]. At the end of this storage period, an evaluation of the internal progression of the rot within the crown was made. The clusters were divided into two different parts and the transverse cutting of the crown allowed a visualization of the spread of the rot on the crown. The Internal Necrotic Surface (INS) expressed in mm^2 was calculated by assuming a rectangular shape to the internal necrosis. Its value was taken as a measure of fruit susceptibility to crown rot.

2.5.3. Evaluation of susceptibility to wound anthracnose

A median internal finger without defects was selected from each harvested hand. On each finger, two zones were located using a fine

marker. A droplet of 25 µL of the inoculum suspension (10^6 conidia·mL^{-1}) was deposited at the center of these zones. Fruits were laid at room temperature to allow the droplets to completely dry up. Once dried, a sterile humidified cotton swab was deposited on each inoculation point and wrapped with plastic film. Bananas of the same treatment were packed thereafter in perforated plastic bags placed in commercial boxes. The boxes were stored for 2 days at 25 °C to allow the development of appressoria [27]. Then, the inoculated areas were bruised by applying a standardized compression. In Guadeloupe, standardized compressions were carried out using a penetrometer with a rounded probe of 1 cm in diameter which exerted a deformation of 5 mm with a speed of 5 mm·s^{-1} for 4 s. The probe was controlled by a TA-XT2 texture analyzer (Rheo, Champlan, France) coupled with X-TRAD software. In Cameroon, this equipment was not available so wounds were inflicted manually with a rounded probe of 1 cm. Bananas were then packed and stored at 13 °C for 10 days in order to simulate industrial export conditions, as described by de Lapeyre de Bellaire *et al.* [27]. At the end of this storage period, fruits were laid at 20 °C and the length (l) and width (w) of the necrotic surface was measured at various dates. Evaluations were made (12, 14, 16 and 18) days after harvest (dah) in Cameroon and 20 dah in Guadeloupe. The Necrotic Surface (NS, mm^2) was then calculated by the ellipse area formula (length × width × $\pi/4$), and its value was taken as a measure of fruit susceptibility to wound anthracnose on these different dates.

2.6. Statistical analysis

For both locations, the effect of the different treatments on leaf spot severity (BLSDS, SDS), on the number of functional leaves (NFL) and on the youngest leaf spotted (YLS) at flowering and at harvest, on the grade of fruit, and on the internal necrotic surface (INS) and necrotic surface (NS) were analyzed by analysis of variance (ANOVA). The average values of $BLSDS_F$, $BLSDS_H$, SDS_F, SDS_H, NFL_F, NFL_H, YLS_F, YLS_H, grade, INS and NS (at different dates of evaluation calculated for both wounds) were subjected to a fixed two-way ANOVA performed with Minitab software v.15.1, with 'treatment' and 'date' as factors. Separations of means were based on Tukey's multiple range tests at a 5% probability level. $BLSDS_F$, $BLSDS_H$, SDS_F SDS_H, NFL_F, NFL_H, YLS_F, YLS_H, grade, INS and NS at different dates of evaluation were correlated via linear regression using Pearson correlation calculations and significance tests. The analysis was conducted using Minitab software v.15.1.

3. Results

3.1. Pre-harvest effects of leaf spot diseases on the banana plants and fruits

In both experimental locations, banana trees selected for the various groups (disease severity index) showed different significant disease levels, as shown for the severity index (SI), the number of functional leaves (NFL) and the youngest leaf spotted (YLS) (*tables II*, *III*). The gradient of the SI selected at flowering was maintained at harvest. Nevertheless, at the harvest stage, the differences among all the treatments for SI, NFL and YLS were not the same as in the flowering stage (*tables II, III*).

During the flowering-to-harvest-period, leaf spot diseases led to a significant reduction of the leaf area in the different disease severity index (DSI) groups. In all cases, the DSI increased dramatically, especially for black leaf streak disease (BLSD) in Cameroon (*table II*). The evolution of leaf spot disease was also confirmed by a significant decrease in the position of the youngest leaf spotted and by a significant decrease in the number of functional leaves (*tables II, III*). As such (especially for BLSD in Cameroon), banana trees selected at flowering with a high DSI (groups E and F) showed complete necrosis of leaves, leading to the absence of functional leaves on the banana plant at harvest (*table II*).

Morphological differences were observed among the fruits of the different

Table II.
Black leaf streak disease severity at banana flowering and harvest, number of functional leaves at flowering and harvest, youngest leaf spotted at flowering and harvest, fruit grade, crown rot, anthracnose mean values and standard deviations during the assessment period in Cameroon.

a) Banana tree

Disease severity index group	Black leaf streak disease (%)		Number of functional leaves		Youngest leaf spotted	
	at flowering	at harvest	at flowering	at harvest	at flowering	at harvest
A	3.1 ± 1.6 a	61.8 ± 16.4 a	12.6 ± 1.8 a	5.2 ± 2.2 a	8.2 ± 3.1 a	1.8 ± 1.0 a
B	11.5 ± 3.0 b	71.2 ± 14.6 b	12.8 ± 1.6 a	4.4 ± 2.1 a	6.5 ± 1.2 b	1.2 ± 0.5 b
C	21.0 ± 2.7 c	89.4 ± 11.8 c	13.3 ± 1.6 a	1.6 ± 2.0 b	5.8 ± 1.2 b	1.0 ± 0.0 b
D	31.6 ± 3.2 d	94.4 ± 11.8 c	11.6 ± 1.6 ab	0.7 ± 1.6 bc	5.5 ± 0.8 b	1.0 ± 0.0 b
E	40.1 ± 3.6 e	99.9 ± 0.6 d	8.8 ± 3.0 b	0.0 ± 0.0 c	4.3 ± 1.0 bc	1.0 ± 0.0 b
F	52.1 ± 5.6 f	100.0 ± 0.0 d	7.4 ± 1.3 b	0.0 ± 0.0 c	3.8 ± 0.6 c	1.0 ± 0.0 b
P obtained with ANOVA	0.000	0.000	0.000	0.000	0.000	0.000

b) Banana fruit

Disease severity index group	Fruit grade (mm)	Crown rot (internal necrotic surface) (mm^2)	Anthracnose (necrotic surface) (mm^2)			
			12	14	16	18
			Days after harvest			
A	37.5 ± 0.1 a	274.8 ± 52.3 a	69.1 ± 50.0 ab	229.8 ± 125.2 a	359.5 ± 176.7 a	518.4 ± 230.0 a
B	37.6 ± 0.1 a	256.9 ± 53.2 a	61.6 ± 41.3 a	182.6 ± 118.4 a	285.3 ± 170.1 a	458.1 ± 241.7 a
C	36.2 ± 0.2 a	303.2 ± 63.3 ab	81.9 ± 64.6 ab	222.5 ± 127.7 a	330.5 ± 163.9 a	516.1 ± 209.8 a
D	35.5 ± 0.2 ab	360.4 ± 53.2 bc	68.8 ± 59.6 ab	245.6 ± 173.4 a	370.3 ± 222.9 a	578.9 ± 289.9 a
E	34.1 ± 0.2 b	349.5 ± 76.5 b	78.2 ± 55.1 ab	217.6 ± 149.0 a	329.2 ± 187.5 a	515.6 ± 230.0 a
F	34.9 ± 0.2 ab	393.7 ± 60.2 c	105.6 ± 80.0 b	254.6 ± 142.0 a	368.5 ± 165.9 a	592.0 ± 259.5 a
P obtained with ANOVA	0.000	0.000	0.041	0.248	0.301	0.178

Means are the result of 10 replicates represented with standard deviation in the table.
For each column, the letters represent statistically different groups defined by the Tukey test (5%).

groups of the disease severity index (DSI) that were harvested at a constant physiological age of 900 dd in Cameroon (*table II*). Fruits with a lower DSI at flowering (groups A, B and C) had bigger grades than those with high DSI (groups D, E and F) (*table II*). Morphological differences among fruits of the different groups were not observed in Guadeloupe (*table III*).

In some cases, bunches harvested on heavily infested banana trees were fully ripe at harvest in both locations (*figure 1*). Fruits of the DSI groups A and B were not ripe at harvest as compared with fruits of other DSI groups (C, D, E and F). The highest percentage of ripe fruits was found in the DSI groups D and E. In addition, the percentage of ripe fruits increased with the level of leaf spot disease at flowering but for the higher disease level of black leaf streak disease in Cameroon (group F, severity at harvest > 46%). The percentage of *M. fijiensis* in ripe fruits at harvest was less significant as compared with *M. musicola*. The percentage of ripe fruit at harvest in Cameroon was (12, 15, 29 and 5)%, respectively, for the DSI groups C, D, E and F, while, in Guadeloupe, it was (23, 38 and 71)%, respectively, for the DSI groups C, D and E.

a) Disease severity index Group A, Sevetrity Index (SI) at flowering < 5% **b)** Disease severity index Group E, Sevetrity Index (SI) at flowering > 46%

Figure 1.
Effects of black leaf streak disease on early ripening of fruit before harvest: in cases of light infection, fruits are not ripe at harvest (a); in other cases with severe infection, fruits are ripe at the harvest stage (b).

3.2. Effects of leaf spot diseases on banana susceptibility to crown rot disease

In Cameroon, black leaf streak disease (BLSD) influenced the banana fruit susceptibility to crown rot disease. The internal necrotic surface (INS) continuously increased from the DSI groups A to F, and the groups had a significant effect on fruit susceptibility to crown rot ($P < 0.001$). As such, higher levels of BLSD severity corresponded to higher levels of fruit susceptibility to crown rot (INS) disease. Three statistically different groups were distinguished amongst the different DSI groups (*table II*).

Sigatoka disease in Guadeloupe did not influence ($P > 0.05$) fruit susceptibility to crown rot (*table III*). Because of the high amount of ripe banana fruits at harvest for the DSI group E (SI between 36% and 45%), this group was not taken into consideration for inoculation assessments.

3.3. Effects of leaf spot diseases on banana susceptibility to wound anthracnose

Black leaf streak disease (BLSD) in Cameroon influenced banana fruit susceptibility to wound anthracnose at the earliest dates of observation (12 dah). Among the various DSI groups, only group F had a significant effect on the necrotic surface (NS, $P = 0.001$) on the first date of evaluation (12 dah). Two statistically different groups were distin-

Table III.
Sigatoka disease severity at banana flowering and harvest, number of functional leaves at flowering and harvest, youngest leaf spotted at flowering and harvest, and anthracnose mean values and standard deviations during the assessment period in Guadeloupe.

Disease severity index group	Banana tree						Banana fruit		
	Sigatoka disease severity (%)		Number of functional leaves		Youngest leaf spotted		Fruit grade (mm)	Crown rot (internal necrotic surface) (mm^2)	Anthracnose (necrotic surface) (mm^2) 20 days after harvest
	at flowering	at harvest	at flowering	at harvest	at flowering	at harvest			
A	3.8 ± 1.9 a	33.5 ± 18.6 a	10.7 ± 1.4 a	8.0 ± 2.6 a	8.8 ± 2.2 a	1.5 ± 0.9 a	35.0 ± 2.8 a	92.7 ± 25.0 a	434.8 ± 221.6 a
B	10.2 ± 2.1 b	47.1 ± 28.5 a	10.7 ± 1.9 a	5.7 ± 3.3 a	7.1 ± 1.6 b	1.1 ± 0.3 ab	35.3 ± 1.1 a	63.5 ± 34.1 a	295.2 ± 173.1 a
C	20.0 ± 3.2 c	73.1 ± 20.1 b	10.3 ± 2.4 a	3.3 ± 3.1 b	5.1 ± 1.3 c	1.2 ± 0.4 ab	34.9 ± 2.1 a	81.0 ± 35.4 a	441.4 ± 273.1 a
D	30.3 ± 2.3 d	94.3 ± 7.2 c	9.4 ± 1.3 ab	0.2 ± 0.6 c	4.7 ± 0.8 c	1.0 ± 0.0 b	34.8 ± 2.0 a	100.8 ± 46.2 a	429.0 ± 207.7 a
E	39.5 ± 5.9 e	96.3 ± 9.9 c	8.5 ± 1.6 b	0.3 ± 1.1 c	4.1 ± 0.6 c	1.0 ± 0.0 b	33.6 ± 1.5 a	–	–
P obtained with ANOVA	0.000	0.000	0.001	0.000	0.000	0.032	0.575	0.133	0.139

Means are the result of 10 replicates represented with standard deviation in the table.
The letters a, b, c, d, e, f represent groups of statistically different fruits defined by the Tukey test (5%).

Table IV.
Analysis of correlation between black leaf streak disease severity (BLSDS) and some characteristics of banana trees (number of functional leaves, youngest leaf spotted) and fruit (fruit grade, crown rot and anthracnose) at flowering and harvest (degree of freedom = 169).

Source	BLSDS at flowering		BLSDS at harvest	
	r-value	*P*-value	*r*-value	*P*-value
BLSDS at harvest	0.74	< 0.001	–	–
Number of functional leaves				
at flowering	– 0.67	< 0.001	– 0.50	< 0.001
at harvest	– 0.73	< 0.001	– 0.94	< 0.001
Youngest leaf spotted				
at flowering	– 0.64	< 0.001	– 0.53	< 0.001
at harvest	– 0.40	< 0.001	– 0.54	< 0.001
Fruit grade at harvest	– 0.54	< 0.001	– 0.44	< 0.001
Crown rot (internal necrotic surface)	0.58	< 0.001	0.49	< 0.001
Anthracnose (necrotic surface)				
12 days after harvest	0.22	0.006	0.18	0.029
14 days after harvest	0.09	0.258	0.17	0.026
16 days after harvest	0.06	0.446	0.17	0.027
18 days after harvest	0.13	0.088	0.21	0.006

r is the Pearson correlation value and *P* its probability.

guished (*table II*). Higher levels of BLSD severity (group F) corresponded to a higher level of susceptibility to wound anthracnose, unlike with the other disease severity index (DSI) groups (A, B, C, D and E). However, for later dates of evaluation (14 dah, 16 dah and 18 dah), group F had higher values of necrotic surface (NS) but the differences among the groups were not significant ($P > 0.05$) (*table II*).

Sigatoka disease in Guadeloupe did not influence ($P > 0.05$) fruit susceptibility to wound anthracnose (NS, 20 dah) on the only date of observation (*table III*).

3.4. Relationships between leaf spot diseases and banana susceptibility to post-harvest diseases

For Sigatoka disease (SD), and particularly for black leaf streak disease (BLSD), disease parameters were well correlated with one another and better correlations were encountered for disease parameters observed at the same stage, *i.e.*, at flowering or at harvest (*tables IV, V*). The number of functional leaves (NFL) and the youngest leaf spotted (YLS) were inversely proportional to disease severity (*tables IV, V*). In addition, a significant correlation ($P < 0.001$) was found between leaf spot disease severity at flowering (SDS_F, $BLSDS_F$) and at harvest (SDS_H, $BLSDS_H$) (*tables IV, V*). A significant negative correlation ($P < 0.001$) was found between the grade of fruits and the $BLSDS_F$ and $BLSDS_H$ (*table IV*) but such a relation was not found for Sigatoka disease (*table V*).

BLSD severity at flowering ($BLSDS_F$) and at harvest ($BLSDS_H$) is therefore positively correlated with the development of crown rot and anthracnose on banana fruits. The relationship between the internal necrotic surface (INS) and BLSDS (at flowering and at harvest) is significant ($P < 0.001$) and linear (*table IV*). Thus, high levels of $BLSDS_F$ and $BLSDS_H$ are correlated with high levels of INS. Necrotic surface (NS) is slightly correlated ($P < 0.001$) with high levels of BLSDS. However, this effect is true only for the date of evaluation corresponding to 12 dah. No correlation ($P > 0.05$) was found

Table V.
Analysis of correlation between sigatoka disease severity (SDS) and some characteristics of banana trees (number of functional leaves, youngest leaf spotted) and fruit (fruit grade, crown rot and anthracnose) at flowering and harvest.

Source	Degree of freedom	SDS at flowering		SDS at harvest	
		r-value	*P*-value	*r*-value	*P*-value
SDS at harvest	79	0.75	< 0.001	–	–
Number of functional leaves					
at flowering	79	– 0.44	< 0.001	– 0.47	< 0.001
at harvest	79	– 0.72	< 0.001	– 0.90	< 0.001
Youngest leaf spotted					
at flowering	79	– 0.70	< 0.001	– 0.62	< 0.001
at harvest	79	– 0.26	0.019	– 0.37	0.001
Fruit grade	79	– 0.23	0.048	– 0.16	0.160
Crown rot (internal necrotic surface)	64	0.16	0.264	0.03	0.814
Anthracnose (necrotic surface) (20 days after harvest)	64	0.15	0.244	0.17	0.175

r is the Pearson correlation value and *P* its probability.

between the BLSDS and NS 14 dah, 16 dah and 18 dah (*table IV*).

Sigatoka disease severity at flowering (SDS_F) and at harvest (SDS_H) was not correlated ($P > 0.05$) with crown rot or wound anthracnose (NS) 20 dah (*table V*).

4. Discussion

The experimental designs put in place in Cameroon for black leaf streak disease (BLSD) and in Guadeloupe for Sigatoka disease (SD) generated disease gradients at flowering which were almost maintained until harvest, although the structures of these gradients were different at the harvest stage. The severity levels reached at harvest for the Disease Severity Index (DSI) groups A and B (disease severity < 15% at flowering) were higher for BLSD in Cameroon than for SD in Guadeloupe, thus suggesting differences in the kinetics of these diseases.

Leaf Spot Diseases had the expected impact on the banana trees through a significant reduction of the leaf surface. A significant reduction of the number of functional leaves at harvest was observed for highly infected plants, especially for the DSI groups D, E and F (severity at flowering > 26%), that had less than one functional leaf at harvest. This foliar reduction has been widely reported in previous studies [19, 20, 28, 29]. As expected, BLSD's impact on the leaf surface was more significant than SD's impact. Indeed, *M. fijiensis* is a more aggressive pathogen than *M. musicola* [30, 31].

Since the reduction in the photosynthetic area generates stresses during fruit filling, an impact on fruit morphology was also expected [10, 32]. Therefore, morphological differences in the diameter of fruits were observed because of heavy black leaf streak disease (BLSD) infestation among fruits of the different treatments harvested at the same physiological age. However, heavy Sigatoka disease (SD) infestation did not lead to a reduction in the diameter of fruit harvested at a constant physiological age, as previously reported [20]. This is probably because the level of stress induced by SD on fruit filling is lower than that induced by BLSD, which induces faster defoliation.

Mycosphaerella leaf spot disease's effects have been previously reported to lead to a reduction in yield, as well as premature ripening of fruits in the field [20, 28, 29, 33]. As expected, in both locations (Cameroon and Guadeloupe), bananas harvested from

severely infected plants (DSI groups C, D, E and F) were at times already ripe at harvest.

Our results confirmed leaf spot disease's direct effects on fruit physiology [20]. Although BLSD's effect on fruit ripening has been widely documented, this is the first report of BLSD's effect on banana susceptibility to crown rot disease. This result confirms previous observations suggesting that some biotic factors had a potential effect on banana susceptibility to crown rot [18].

The presence of biotic stresses during fruit filling induced a reduction in sources (number of functional leaves) of the banana tree but not in sinks (fruits), leading to a reduction of the source-sink (So-Si) ratio. We can therefore hypothesize that such a decrease could account for a reduced susceptibility to crown rot, as previously shown [10]. Since the reduction of the source was more significant for black leaf streak disease (BLSD) than for Sigatoka disease (SD), this could account for the lack of increase in fruit susceptibility to crown rot which was observed with increasing SD severity. Also noteworthy is the fact that the values of internal necrotic surfaces (INS) measured in Guadeloupe were very low as compared with earlier studies [6], which could also explain the absence of SD's effect on the susceptibility of banana to crown rot.

In the case of banana anthracnose, there is a slight effect of black leaf streak disease (BLSD) on bananas harvested from heavily infested plants ($P = 0.041$) just for the first date of evaluation (12 dah). This effect is not found in the following dates of evaluation (14 dah, 16 dah, 18 dah). So, we can hypothesize that Sigatoka disease had no effect on fruit susceptibility to wound anthracnose, probably because the only evaluation date (20 dah) was rather too late. The lesser effect of BLSD on wound anthracnose could be explained by the fact that the modification of the So-Si ratio had less effect on fruit susceptibility to banana anthracnose as compared with crown rot. Effectively, in previous studies, the changes in the So-Si ratio were found to influence the banana fruit susceptibility to crown rot [10], but not to wound anthracnose [12].

Stresses generated by the effects of black leaf streak disease seem to trigger physiological changes in the banana fruit resulting from a disequilibrium between the availability of assimilates during the fruit growth. This disequilibrium could lead to a reduction of the formation of secondary metabolites such as phenolic compounds. Phenolic compounds have effectively been shown to be involved in the defence mechanisms of different banana tree tissues [34–38]. Moreover, products arising from the decomposition of banana dopamine seem to have a fungitoxic activity against *C. musae* [39, 40]. Since several phenolics are involved in the banana tree's defense mechanisms, they might also be involved in fluctuations observed in banana susceptibility to crown rot disease.

To sum up, our study highlighted a new effect of black leaf streak disease (BLSD) on banana fruit quality. However, the physiological mechanisms involved in the relationship between BLSD severity and post-harvest diseases still require research. The management of BLSD should also be considered in the elaboration of alternative control methods of post-harvest diseases, mainly through an integrated strategy.

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Impacto de la extensión de la enfermedad de las rayas negras en la sensibilidad de los plátanos a las enfermedades postcosecha.

Resumen – Introducción. La sensibilidad de los plátanos a las enfermedades postcosecha (podredumbre de la corona y antracnosis) se ve influida por numerosos factores abióticos precosecha. Las enfermedades foliares de los plátanos son factores bióticos precosecha causados por *Mycosphaerella fijiensis* en el caso de la enfermedad de las rayas negras (ERN) y por *M. musicola*, en el caso de la enfermedad de sigatoka (ES). Estas enfermedades foliares podrían influir en la fisiología del fruto. El presente estudio está encaminado a determinar la influencia de dichos factores bióticos precosecha en la sensibilidad del fruto a la podredumbre de la corona y la aracnosis. **Material y métodos**. Se estableció un gradiente de gravedad de la enfermedad en dos parcelas experimentales (Camerún para la ERN y Guadalupe para la MS). En el momento de la floración, se seleccionaron seis niveles distintos de dichas enfermedades foliares. La sensibilidad del fruto se determinó evaluando la superficie necrosada de la corona tras la inoculación artificial de los frutos de la tercera mano del racimo por *Colletotrichum musae*. **Resultados y discusión**. La ERN influyó significativamente en la sensibilidad de los plátanos a la podredumbre de la corona ($P < 0.001$), pero solo tuvo un ligero efecto en el desarrollo de antracnosis ($P = 0.041$). La MS no presentó ningún efecto ($P > 0.05$) en la sensibilidad de los plátanos a estas dos enfermedades postcosecha. Estos resultados se discutieron haciendo especial énfasis en la influencia de las variaciones del balance fuente-pozo en la fisiología de las frutas. La influencia de la ERN en la podredumbre de la corona sugiere la necesidad de tener en cuenta la gestión de las enfermedades foliares en los métodos alternativos de control de las enfermedades postcosecha a través de programas de gestión integrados.

Camerún / Guadalupe / *Musa* / frutas / *Mycosphaerella fijiensis* / *Mycosphaerella musicola* / sigatoka negra / enfermedades de estrías negras de las hojas / antracnosis / podredumbre de la corona