# Evidence of the effects of *Mycosphaerella* leaf spot diseases on fruit quality

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**Abstract**

*Mycosphaerella* leaf spot diseases Sigatoka disease (SD) and Black leaf streak disease (BLSD) are considered as the most severe diseases of bananas for the export market. Serious infestations can lead to a substantial reduction in the leaf area and thus to fruit yield losses. In addition, these diseases were found to have an impact on fruit quality, especially on ripening earliness, which causes some production losses for exported bananas. To assess the effects of these 2 diseases on the bananas quality (especially its physiological stage), plantation surveys and experiments were conducted in Guadeloupe (FWI) for SD and in Cameroon for BLSD. Bananas quality was characterised, on bananas harvested at a constant physiological stage evaluated in thermal time (temperature sums at 900 degrees day) from flowering to harvest, by its greenlife (duration of preclimacteric period) and by few physico-chemical (grade, weight). Diseases severity was measured either at flowering (for SD) or at harvest (for BLSD). Results of surveys revealed that bananas harvested on plants severely affected with SD or BLSD had similar diameter growth, but a shorter greenlife (GL) than bananas harvested from uninfected or lightly infected plants. A strong correlation was obtained between disease severity and fruit greenlife. All these results indicate clearly the direct effect of MLSD of banana greenlife rather than a trophic effect.The putative physiological mechanisms involved are also discussed.

**Keywords:** Banana, Sigatoka disease, Black leaf streak disease, Green life, Fruit Quality

## Introduction

Banana (*Musa acuminata,* triploid AAA, Cavendish cv Grande Naine) is a climacteric fruit (Burg & Burg, 1965) that is harvested at a green preclimacteric stage in commercial plantations prior to being exported. They must be marketed at a specific ripeness stage to ensure a sufficient shelflife. Fruit ripening can be artificially induced by exogenous ethylene treatment in commercial ripening sheds (Marriott, 1980). The time between harvest and natural fruit ripening initiation via the synthesis of endogenous ethylene (climacteric rise) is called the greenlife (GL) (Peacock & Blake, 1970). It should reach around 25 days to be long enough for shipping duration (from French West Indies or Africa) and market liquidity.

GL is highly temperature dependent (Blake & Peacock, 1971) but is also closely related to the physiological age of the fruit, as calculated by the sum of mean daily temperatures, according to a negative exponential model (Jullien, 2000). When fruit pulp filling is not hampered by any cropping stress, bananas harvested at 900 dd (at 14°C, i.e. the zero growth threshold for cv Grande Naine) reach a marketable diameter (Ganry & Meyer, 1975) and a GL that is sufficient to withstand maritime shipping (Jullien, 2000). However, it has been shown that bananas harvested from plants that have undergone stress during the fruit pulp filling phase (reduced number of leaves at flowering, excess moisture) have lower diameter growth but similar GL (Chillet *et al*, 2006; Daniells *et a*l, 1987).

*Mycosphaerella* leaf spots diseases (MLSD) due the two pathogenic fungus *Mycosphaerella fijiensis,* responsible for Black leaf streak disease (BLSD), and *Mycosphaerella musicola* responsible for Sigatoka disease (SD) are the main foliar diseases of bananas, especially for exportation of dessert bananas very susceptible to both diseases (Jones, 2000). Heavy infestations of this leaf spot disease can lead to a considerable reduction in the photosynthetic leaf area of the plant, and thus in the bunch weight (Ramsey et al. 1990).

MLSD control (by frequent fungicides applications) is essential for exportation because this disease is highly detrimental to fruit quality. It has been shown that severe SD infestations have a marked effect on banana ripeness at harvest (Meredith, 1970; Wardlaw, 1972). Ramsey *et al.* (1990) found that the GL of bananas harvested from highly infected plants was substantially shortened. However, in these experiments, as bananas were harvested at a commercial grade (¾ full) without any indication on their physiological age, the authors could not conclude to the origin of the reduction in GL.

Thus, it is essential to integrate the notion of physiological age of bananas at harvest in order to be able to differentiate a trophic effect of MLSD, i.e. delayed growth leading to fruit harvesting at an advanced physiological age; from a direct effect of SD on fruit physiology.

This paper aims to study and precise the effect of MLSD on fruits quality mainly characterise by their greenlife.

## Material and methods

The effect of MLSD on fruit quality has been assessed through two complementary approaches: survey and experimentation

### 1. Study design

#### *Survey approach*

Two surveys were realised in commercial plots in Guadeloupe (against SD, in December 2004) and in Cameroon (against BLSD, in 2007).

A susceptible variety (Grande naine) was cultivated in two main banana-growing areas of Guadeloupe (2500 mm/year rainfall) and in Cameroon (2400 mm/year rainfall) both on ferralitic soil at low elevation (between 30 and 50 m) and a mean temperature at 28°C. Control practices against MLSD were applied on these plots they consisted in frequent fungicide aerial applications and frequent deleafing of necrotic surfaces for BLSD. Other crop

management strategies were similar: blue polyethylene bunch sleeving, monthly fertilization, and crop protection treatments.
Two plots were selected according two level of disease pressure (high and low). The plots belonged to two different private growers in Guadeloupe whereas in Cameroon, plots were selected in the same sector (Mbomé 5) of a private plantation.

***Experimental approach***

An experimentation was conducted in Guadeloupe (against SD between September 2006 and August 2007) by comparing the behaviour of two plots of susceptible variety (Grande naine) in the Neufchâteau research station (3500 mm/year rainfall; andosol; 250 m elevation; mean temperature 26°C). The first plot (plot C) had no fungicide application (=maximum SD infestation level) whereas the second plot had undergone a regular SD control treatment with fungicide spraying and deleafing (= very low SD infestation level). The experiment was replicated through three consecutive cropping cycles.

***Sampling***

Between 12 to 20 bananas were studied by location and modality. In Guadeloupe, bananas were selected at the horizontal finger flowering stage whereas in Cameroon, bananas were selected at harvest.

**2. MLSD evaluation**

For surveys, disease levels were evaluated for each selected banana in Guadeloupe (SD) at flowering and harvest with a global visual estimation of SD level (plot H, highly infested ; plot L barely infested) and at harvest with the leaf number classified in 2 grades (<1 or >5) in Cameroon (BLSD).

For the experimentation, SD level was quantified at flowering and at harvest with 2 parameters: (i) youngest leaf spotted (YLS) according to the method of Stover and Dickson (1976) and (ii) the severity index (SI) according to Gauhl *et al.* (1993) which consists in estimating visually (with grades) the necrotic surface per plant.

**3. Fruits characterisation**

***Harvest***

Bunches were harvested at a constant physiological age according to the method described by Ganry and Meyer (1975), i.e. when the mean daily temperature sum at the 14°C threshold ($ST_{base\ 14}$) between flowering and harvest reached 900 dd. Temperature captors were used to determine the harvest dates.
For each bunch, the median internal fruits of the third hand were washed and treated with thiabendazol (500 ppm).

***Green life***

The green life (GL) was measured at 20°C and corresponded to the duration between harvest and the climacteric crisis. It was detected through daily gas analyses ejected by fruits and corresponded to the peak of CO2 production as described by Chillet *et al*. (2008).

***Physico-chemical analyses***

The grade (diameter in mm) of fruits was measured using a slide gauge in Guadeloupe and in Cameroon.
The weight of fruits (in gr) was measured also in Cameroon.

**4. Data analysis**

The fruit grades and weight, and GLs were compared according to the production conditions and MLSD levels. Analyses of variance followed by Newman-Keuls tests (5% threshold) were carried out to compare the fruits quality of different plots (representing high or low

MLSD level) in the experiments using the STAT-ITCF software package (1991). The parameters were correlated via linear regressions using Excel software (2003 version).

## Results and discussion

### 3.1. Evidence of the effects of *Mycosphaerella* leaf spot diseases on the greenlife of bananas

The effects of MLSD on the bananas greenlife were studied successively through two different approaches.

- **Commercial conditions**

In commercial conditions, the greenlife of bananas harvested at 900 dd in plots heavily infected with MLSD was significantly lower than for bananas harvested in plots controlled against MLSD (figure 1). Indeed, the greenlife of bananas well controlled against MLSD was 28 days whereas the greenlife of bananas highly infected with SD in Guadeloupe or with BLSD (with less than one leaf at harvest) was respectively 17,3 days and 23 days (figure 1).
As bananas were harvested at a same physiological stage (900 dd) in any plot (with different level of diseases), these results demonstrated the direct effect of the impact of MLSD on the reduction of bananas greenlife.
This reduction was higher for SD than for BLSD for which its incidence is known to be higher. This result could be explained by a natural decrease in BLSD pressure during the dry season (study period) and/or by the absence of a precise quantification of the 2 diseases in the 2 surveys.
It is noteworthy that bananas harvested on plots with a low SD or BLSD infestation rate had an equivalent GL (28 days) which is sufficient with respect to the shipping time and is close to that previously measured for bananas harvested at 900 dd (Chillet et al, 2006).

- **Experimental conditions**

Like in the commercial conditions, the greenlife of bananas harvested at 900 dd was 28 days in the plot controlled against SD (table 1). However, it reached only 6,1 days in the plot not controlled against SD. This difference is highly significant. This important reduction of the greenlife can be explained by a high disease severity in the untreated plot. Indeed SD severity varied from 35% to 60% on the untreated plot (assessed at the flowering stage) and from 0% to 20% on the treated plot (data not shown).
Correlations between GL and the SD quantification parameters were calculated (figure 2). The three calculated correlation coefficients were high (> 0.7), with the highest being obtained for the SD severity index at flowering ($r^2 \approx 0.86$), whereas the lowest (0.7) was obtained with the YLS parameter at flowering (data not shown). This difference could be explained by a better disease quantitative assessment with the severity index than with YLS.

Even differences in the plant physiological status due to different crop management strategies can induce variation in the greenlife (Bugaud and Lassoudiere, 2005), the results presented in this paper highlighted for the first time, that MLSD have a direct effect on the extent of ripeness of bananas harvested at a constant physiological age. They showed that the reduction in GL observed for bananas harvested from highly MLSD infected plants resulted from a direct effect of this leaf spot disease on fruit development in green and was not associated with late harvesting of bananas whose growth would have been slowed down. Bananas harvested at a constant physiological age (900 dd) from plots with a high MLSD infestation rate therefore did not have the GL predicted by the model described by Jullien (2000), contrary to bananas harvested from plots with a low MLSD infestation rate.

### 3.2. Effects of *Mycosphaerella* leaf spot diseases on the physico-chemical characteristics of bananas

Few physico-chemical characteristics of bananas were measured in commercial and experimental conditions.

Results showed that fruit grades were similar (no significant difference) for bananas harvested at 900 dd in plots with low or high disease pressure and for both diseases (tables 1 and 2). Indeed, fruit diameter varied from 34,2 to 35,8 in any case.

However, fruit weight was significantly lower from bananas highly infested with BLSD in comparison with fruits from bananas barely BLSD infested (table 2). Indeed, it was respectively 169,6 g and 191,5 g. This weight reduction could be explained by the low photosynthetic rate of highly infected plant consequently to their low leave number per plant.

These studies showed the absence of effects of MLSD on fruit grade and the effect on fruit weight. The reduction in the photosynthetic area due to a heavy MLSD infestation did not lead to a reduction in the diameter of fruit but a reduction of weight.

Interestingly, some preliminary results on postharvest ripening suggest that fruits harvested on bananas highly infested with SD in Guadeloupe showed a higher sucrose rate than fruits from uninfected bananas. Because sucrose results from starch degradation, this data although preliminary, suggests a putative effect of SD on starch to sucrose conversion during postharvested ripening.

## Conclusion

The survey and experimental results obtained in this study revealed that bananas harvested at a same physiological age from plots with a high MLSD infestation rate had a much shorter GL than those from healthy plots, whereas they had the same fruit diameter but a lower weight.

Our results showed a close correlation between the level of SD infestation of banana plants at flowering and GL measured on bananas harvested at a constant physiological age. Further experiments are required to clearly determine the nature of this relation (linear or with threshold effects) to improve control practices against MLSD. Indeed, it is important to determine the maximum MLSD infestation rate without effect on GL in order to obtain a duration acceptable for export.

The effect of MLSD infestation on bananas harvested at a constant physiological age on the reduction the GL of bananas with a high MLSD infestation rate and not on fruits diameter suggest not a trophic effect but rather a direct effect of this leaf spot disease on green physiological development stages and on the extent of fruit ripeness.

These results also question about the physiological mechanisms involved in this relationship between banana leaf diseases and their direct effect on fruit physiology. They can have various origins such as fungal metabolites implicated in pathogen aggressiveness, secondary plant metabolites involved in resistance or hormones. Further experiments should be undergone to understand this relation.

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**Table 1**: Fruit diameter (mm) and greenlife (days) of bananas harvested from experimental plots with a high and low Sigatoka disease infestation rate.

| | **Plot with a high SD infestation rate** | **Plot with a low SD infestation rate** |
|---|---|---|
| **Fruit diameter (mm)** | **34.2** ± 1.9 NS | **34.7** ± 1.1 NS |
| **Greenlife (days)** | **6.1** ± 3.2 ** | **28.0** ± 3.7 ** |

Each value is the mean of triplicate measurements. Fruits were harvested at 900 dd. ** indicates that the statistical test was significant at the 5 % probability level. NS indicates that there was no significant difference at the 5 % level.

**Table 2**: Characterisation of fruits (grade and weight) harvested at 900 dd from the two plots with low and high level of Black leaf streak disease (BLSD) in Cameroon

| | **high BLSD** | **low BLSD** |
|---|---|---|
| **Fruit grade (mm)** | **34.6** ± 0,12 NS | **35.8** ± 0,16 NS |
| **Weight (gr)** | **169,6** ± 16 ** | **191,5** ± 20 ** |

Disease level was evaluated with the number of leaves at harvest >5 or <1. Each value is the mean of 11 to 15 measurements. ** indicates that the statistical test was significant at the 5 % probability level. NS indicates that there was no significant difference at the 5 % level.

**Figure 1 :** Green Life of bananas harvested at 900 degrees days in plots with 2 different levels of Sigatoka disease in Guadeloupe or Black leaf streak disease in Cameroon

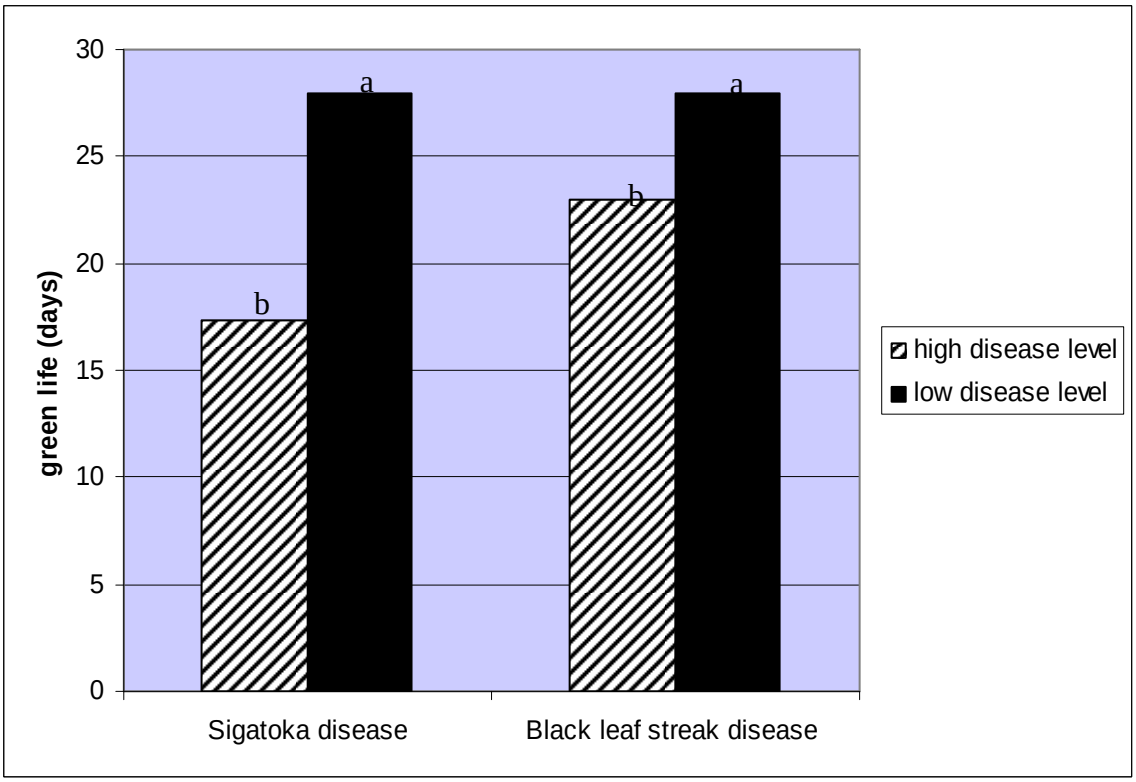


**Figure 2** : Relationship between banana greenlife and SD severity measured at the flowering stage for bananas harvested at 900 dd in the two experimental plots (♦)