Original article

Necrotic leaf removal, a key component of integrated management of *Mycospaerella* leaf spot diseases to improve the quality of banana: the case of Sigatoka disease

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Necrotic leaf removal, a key component of integrated management of *Mycospaerella* leaf spot diseases to improve the quality of banana: the case of Sigatoka disease.

Abstract – Introduction. Bananas are harvested at the green preclimacteric stage prior to sale. The time between harvest and the initiation of the natural ripening process is called green life. Black Leaf Streak Disease and Sigatoka Disease are the main foliar diseases affecting banana production. Sigatoka Disease (SD) is due to *Mycosphaerella musicola*, which frequently causes early ripening in commercial banana plantations, considered as an important source of damage. Our work aimed at determining the effectiveness of the removal of necrotic leaves, one month before harvest, on recovering banana quality arising from SD-infested plants. **Materials and methods**. Banana plants (totaling 80) at the flowering stage were selected in an experimental plot. These plants were divided into two groups of SD infestation levels: level 1 (Severity Index at flowering < 10%) and level 2 (Severity Index at flowering > 25%). We divided each group into two treatments: a treatment with no necrotic leaf removal and a treatment with necrotic leaf removal. Fruits were harvested at the same physiological age, at 900 degree-days. Fruit quality was characterized by weight, diameter, % of ripe fruit in the field and fruit green life. **Results**. Our results showed that the removal of necrotic leaves causes a reduction in size parameters but leads to a strong reduction in SD effects on the fruit maturity, thereby preventing premature ripening. Banana plants whose necrotic leaves were removed produced fruit with a very long green life. **Discussion**. Removal of necrotic leaves allows thwarting the negative effect of SD on fruit physiology. This shows that the presence of necrosis during the last month of fruit growth is responsible for this fruit physiological modification characterized by early ripening. **Conclusion**. Removal of necrotic leaves one month before the date of harvest may be a technique that can limit production losses when the infestation level by SD is high.

Guadeloupe / *Musa acuminata* / plant diseases / *Mycosphaerella musicola* / postharvest ripening / fruits / quality

Élimination des feuilles nécrosées, un élément clé de la gestion intégrée des maladies foliaires à *Mycospaerella* pour améliorer la qualité de la banane : le cas de la maladie de Sigatoka.

Résumé – Introduction. Les bananes sont récoltées à un stade vert préclimactérique avant la vente. Le délai entre la récolte et le début du processus de maturation naturelle est appelée la vie verte. La maladie des raies noires et la cercosporiose sont les principales maladies foliaires affectant la production de bananes. La cercosporiose ou Sigatoka (SD) due à *Mycosphaerella musicola*, qui provoque souvent la maturation précoce en bananeraies commerciales, est considérée comme une importante source de dommages. Notre travail a cherché à déterminer l'efficacité de l'élimination des feuilles nécrosées, un mois avant la récolte, sur la récupération de la qualité de bananes infestées par SD. **Matériel et méthodes**. Des bananiers (80 plants) au stade de floraison ont été sélectionnés dans une parcelle expérimentale. Ces plantes ont été divisées en deux groupes de niveaux d'infestation SD : niveau 1 (indice de gravité à la floraison < 10 %) et le niveau 2 (indice de gravité à la floraison > 25 %). Nous avons divisé chaque groupe en deux traitements : un traitement sans enlèvement des feuilles nécrosées et un traitement avec enlèvement des feuilles nécrosées. Les fruits ont été récoltés au même âge physiologique, à 900 degrés-jours. La qualité des fruits a été caractérisée par le poids, le diamètre et le taux de fruits mûrs au champ et la durée de vie verte du fruit. **Résultats**. Nos résultats ont montré que la suppression de feuilles nécrosées entraîne une réduction des paramètres de taille, mais conduit à une forte réduction des effets de SD sur la maturité du fruit, ce qui empêche la maturation prématurée. Les bananiers dont les feuilles nécrosées ont été enlevées ont produit des fruits de vie verte très élevée. **Discussion**. La suppression des feuilles nécrosées permet de contrecarrer l'effet négatif de SD sur la physiologie des fruits. Cela montre que la présence de nécroses au cours du dernier mois de la croissance des fruits est responsable de la modification physiologique des fruits caractérisée par une maturation précoce. **Conclusion**. La suppression des feuilles nécrosées un mois avant la date de la récolte peut être une technique utile pour limiter les pertes de production lorsque le niveau d'infestation par SD est élevé.

Guadeloupe / *Musa acuminata* / maladie des plantes / *Mycosphaerella musicola* / maturation après récolte / fruits / qualité

1. Introduction

Bananas are climacteric fruits which must be harvested at the green pre-climacteric stage before shipping, commercialization and treatment with ethylene in commercial ripening rooms. The elapsed time between harvest and the start of the natural banana ripening is called green life [1]. The minimum green life required to enable commercialization is around 25 days for bananas produced in the French West Indies and exported to European countries [2].

The harvest date influences fruit attributes, such as green life and size. A way to control and standardize the harvest time of bananas is through the physiological age of the fruit, since green life closely follows the physiological age according to a negative exponential model [3]. Expressed in degree-days (dd), the physiological age of banana fruit (*Musa* AAA Cavendish sub-group cv. Grande Naine) is calculated as the sum of the mean daily temperatures during fruit filling with a threshold temperature of 14 °C. The physiological age correspondent to the harvest at marketable diameter is 900 dd [4], in which bananas must have a green life of approximately 25–30 days at 20 °C [3], when fruit pulp filling is not hampered by any cropping stress.

Mycosphaerella leaf spot diseases are the main foliar diseases of bananas; they are caused by two pathogenic fungi: *Mycosphaerella fijiensis* Morelet, which is responsible for Black Leaf Streak Disease (BLSD, also called black Sigatoka), and *Mycosphaerella musicola* Leach, which is responsible for Sigatoka Disease (SD, also called yellow Sigatoka). Cavendish cultivars, the types of dessert bananas that are grown for exportation, are highly susceptible to both diseases [5]. Unlike abiotic stresses, black Sigatoka and yellow Sigatoka diseases have a direct effect on the banana green life, resulting in premature ripening [6–8] and an alteration in pulp color [9, 10]. High infestation of yellow Sigatoka can cause the appearance of the phenomenon called yellow pulp [11, 12].

Control of the disease is usually managed through field fungicide applications that can be decided using a biological forecasting system [13, 14] or using a systematic control strategy [15]. However, leaf pruning is an important complementary method that is particularly used to limit ascospore inoculum sources in the case of BLSD [5, 16–18].

The main objective of our work was to determine if necrotic leaf removal one month before harvest can avoid early fruit ripening, and to evaluate how this cultural practice could contribute to exportation of bananas harvested on SD-infested plants. This work is a first step to understanding the incidence of deleafing practices in order to manage the effect of foliar diseases (BLSD and SD) on the quality of the fruit, and to propose alternatives that might enable exportation of diseased plants, which should be of interest particularly for Black Leaf Streak Disease, which is the most prevalent *Mycosphaerella* foliar disease in all banana-exporting countries.

2. Materials and methods

2.1. Plant material

Our experiment was conducted in Guadeloupe (FWI, 16° N, 62° W) in 2008, with *Musa acuminata* (AAA, cv. Grande Naine, Cavendish sub-group) bananas. The bananas tested were always cut from the third hand of the experimental bunches.

2.2. Evaluation of SD infestation rate

The experiment was conducted before the first detection of *Mycosphaerella fijiensis* and only *M. musicola* (SD) was present at that time. For each banana plant in the experiment, Sigatoka disease was quantified at the flowering date, on the basis of the severity index. The severity index (SI) was assessed according to the method developed by Stover [19] and modified by Gauhl *et al.* [20]. This method involves a visual estimation of the necrotic area per plant, scored on a scale of seven disease grades attributed to all plant leaves. Each leaf is scored according to the following scale: 0 = no necrotic lesion; 1 = less than 1% necrotic lesions; 2 = 1% to 5% necrotic lesions; 3 = 6% to 15% necrotic lesions; 4 = 16% to 33% necrotic lesions; 5 = 34% to 50% necrotic lesions; 6 = more than 50% necrotic lesions. The severity index is calculated as {SI = [(∑scores / 6) × TNL] × 100}, where ∑scores is the sum of the scores for all the leaves of the banana plant and 'TNL' is the number of leaves per plant.

2.3. Experimental design

The plot was selected in a commercial banana plantation in Montbelley, Capesterre Belle Eau (rainfall: 2500 mm per year, andosol, 180 m elevation; mean temperature 25.5 °C during the experiment), Guadeloupe, FWI. This plot was a second crop cycle, with a plant density of 1800 plants·ha^{-1}. It did not receive any fungicide treatment against Sigatoka disease after one month before the beginning of the experiment.

In the experimental plot, eighty banana plants were selected at the horizontal finger flowering stage; they were divided into two treatments:

– Treatment with no leaf removal: forty selected plants were classified into two different infestation levels (2 × 20 plants) based on the severity index (SI) at the flowering stage [level 1: low infestation (< 10%); level 2: high infestation (> 25%)].

– Treatment with leaf removal: forty selected plants were classified into two different infestation levels (2 × 20 plants) based on the severity index at the flowering stage [level 1: low infestation (< 10%); level 2: high infestation (> 25%)]. One month before harvest, all necrotic leaves (five leaves) were removed from the plant, using a sickle.

Treatments and replicates were totally randomized in the field. The soil was homogeneous. The crop management strategies were similar and conformed to normal commercial practices for all the banana plants: a colored band was tied to each plant to mark the flowering date; bunches were covered with a blue polyethylene sleeve; monthly fertilization [100 g per plant (NPK + Mg) (15-4-30 + 8) per month] and crop protection treatments (apart from fungicide sprays) were applied.

2.4. Calculation of physiological age of the fruit

Temperature probes (Gemini Data Loggers Ltd., West Sussex, UK) were placed under a forecast shelter on the experimental plot. Mean daily temperature was calculated on the basis of hourly temperatures. The physiological age of the fruit [expressed in degree-days (dd)] was calculated by the method described by Ganry and Meyer [4].

2.5. Measurement of fruit green life, fruit dimensions and ripe fruit percentage in the field

Bunches were harvested at a physiological age of 900 dd according to the method described by Ganry and Chillet [21] and green life was measured at 13 °C according to the method described by Chillet *et al.* [22], by measuring the evolution of the respiratory intensity (IR) during storage. The size diameter and weight of each banana were also measured with a calliper and an analytical balance. We also assessed the percentage of ripe fruits in the field, which is the number of bunches whose maturation was initiated while fruits were still on the plant (in this case green life = 0), divided by the total number of bunches (× 100).

2.6. Data analysis

Analyses of variance followed by Newman-Keuls tests (5% threshold) were carried out to compare the two treatments using the XLStat software package (2010).

3. Results

3.1. Effects of removing necrotic leaves on fruit pomological parameters

Our results showed that fruits harvested from plants (whatever the infestation level) with no leaf removal had a significantly higher weight and diameter than fruits harvested from plants with leaf removal (*table I*).

Table I.
Weight and diameter of fruits from banana plants with leaf removal and no leaf removal before harvest and fruits from diseased plants (Sigatoka disease) with low-infested and high-infested levels. Fruits are harvested at 900 degree-days. Values are means ± standard deviation.

Level	Weight (g)		Diameter (mm)	
	No leaf removal	Leaf removal	No leaf removal	Leaf removal
Low infested	182.1 ± 24.3 a	147.7 ± 23.2 b	35.7 ± 1.8 a	32.2 ± 1.8 b
High infested	166.5 ± 24.3 a	140.5 ± 35.4 b	34.2 ± 1.8 a	32.0 ± 2.6 b
F Test	9.3		15.0	
Probability	< 0.0001		< 0.0001	

a, b, c indicate uniforms group defined by the analysis of variance (at 5%) according to Newman-Keuls test.

Table II.
Green life and % of fruits ripe in the field for fruit of banana plants with leaf removal and no leaf removal before harvest and fruits from diseased plants (Sigatoka disease) with low-infested and high-infested levels. Fruits are harvested at 900 degree-days. Values are means ± standard deviation.

Level	Green life (days)		% of ripe fruits	
	No leaf removal	Leaf removal	No leaf removal	Leaf removal
Low infested	44.8 ± 14.4 b	65.0 ± 6.4 a	0.0	0.0
High infested	7.7 ± 11.1 c	45.8 ± 11.1 b	55.0	0.0
F Test	92.44		–	
Probability	< 0.0001		–	

a, b, c indicate uniforms group defined in the analysis of variance (at 5%) according to Newman-Keuls test.

3.2. Effects of removing necrotic leaves on fruit ripening process

The percentage of ripe fruits before the harvest date (900 dd) for each disease level (low-infested and high-infested levels) and for both treatments showed that highly infested banana plants without leaf removal had a high proportion of ripe fruit in the field (55.0%) (*table II*). On the contrary, when the necrotic leaves were removed, there was no ripe fruit before the harvest date whatever the Sigatoka disease infestation level.

In each experimental plot, the mean green life value of fruits from treatment of plants without leaf removal and highly infested plants showed a very short period (7.7 days) as compared with fruits of plants with the same treatment with a low disease level (44.8 days) (*table II*). On the other hand, leaf removal greatly increased fruit green life in fruit from low-diseased plants (65 days), and especially in fruit from highly diseased plants (45.8 days). As expected, fruits from low-infested plants also revealed a significant increase ($P < 0.0001$) in fruit green life (from 44.8 days to 65.0 days).

4. Discussion

Our results show that the removal of necrotic leaves allows limiting the negative effect of Sigatoka disease on fruit green life when harvested at 900 dd. Although leaf removal could cause a loss of production (in our experiment, weight and diameter were lower), this technique allows producing fruit with a green life sufficient to withstand the transit time to an exportation area. However, depending on the amount of leaves that have been cut, there could be an effect on the fruit filling. If leaf removal is very intense (three leaves remaining), there will be a loss in fruit weight, as has already been shown in previous work [23, 24]. If leaf removal is low (five or more leaves remaining), there should not be any loss of production [25, 26]. Since the differences between weight and diameter were not significant between the two disease levels (low-infested and high-infested levels) for each treatment (no leaf removal and leaf removal) , we can also conclude that the effect of Sigatoka disease is not significant on weight and fruit diameter, as reported previously by Chillet *et al.* [7] and Castelan *et al.* [8]. The last five leaves therefore have a photosynthetic activity sufficient to enable correct filling of fruit.

This technique of leaf removal can be applied to support more conventional methods of treatment against Sigatoka disease, especially when there is a deficiency in the application of conventional treatment (delay, for instance).

Our results raise questions about the physiological mechanisms involved in the relationship between Sigatoka disease and fruit green life. They suggest the importance of the presence of necrotic stages in the last month before harvest. During this period, there could be biochemical exchanges between necrotic leaves and fruits that could affect the maturation mechanisms. Metabolites synthesized by the pathogenic fungus could have an impact on fruit physiology. For BLSD, it has been shown that *M. fijiensis* secretes a toxin that can induce necrotic lesions on leaves [27]. An equivalent toxin may also be secreted by *M. musicola*, and might be involved at the beginning of a cascade of biochemical events that affects fruit preclimacteric life. Similarly, secondary plant metabolites involved in resistance mechanisms may also have an impact on this relationship. These secondary metabolites could be transported to the fruit by the phloem and have a stimulatory effect on ripening [28]. Finally, hormones, such as AIA and GA3 [29], or ABA [30, 31], may also be involved in the communication between necrotic leaves and fruits. Experiments must be carried out to understand this relationship between leaf necrosis by *M. musicola* and early-ripening fruit better.

5. Conclusion

Our experiment highlighted that the removal of necrotic leaves is a simple and effective cultural practice which can greatly limit the effects of early ripening caused by Sigatoka disease when the severity of the disease is high. Likewise, similar experiments should also be conducted with BLSD to see if the effect of leaf removal is confirmed with this other disease. Nevertheless, the results of our experiment are particularly interesting for an integrated management of Sigatoka disease without increasing pesticide use.

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Eliminación de las hojas necrosadas, un elemento clave en la gestión integrada de las enfermedades foliares con *Mycospaerella* para mejorar la calidad del plátano: el caso de la enfermedad de Sigatoka.

Resumen – **Introducción**. Los plátanos se recogen en un estado verde preclimatérico antes de venderse. El periodo entre la cosecha y el principio de su proceso de maduración natural se denomina "vida verde". La enfermedad de las rayas negras y la cercosporiosis son las principales enfermedades foliares que afectan a la producción de los plátanos. La cercosporiosis o Sigatoka (SD) debida a *Mycosphaerella musicola*, que a menudo provoca la maduración precoz de los plataneros comerciales, se considera una importante fuente de daños. Nuestro trabajo se encaminó a determinar la eficacia de eliminación de las hojas necrosadas un mes antes de su cosecha en la recuperación de la calidad de los plátanos infestados por SD. **Material y métodos**. Se seleccionaron bananeros (80 plantas) en estado de floración en una parcela experimental. Dichas plantas se dividieron en dos grupos según su nivel de infestación de SD: nivel 1 (índice de gravedad en la floración < 10%) y nivel 2 (índice de gravedad en la floración > 25%). Se dividió cada grupo en dos tratamientos: un tratamiento en el que no se retiraron las hojas necrosadas y otro en el que sí se retiraron. Las frutas se recogieron con la misma edad fisiológica a 900 grados-día. La calidad de las frutas se determinó por el peso, el tamaño y la proporción de fruta madurada en el campo frente y la duración de la vida verde. **Resultados**. Según nuestros resultados, la supresión de las hojas necroadas implica una reducción en cuanto a tamaño, pero conlleva una gran disminución de los efectos de la SD en la maduración de la fruta, lo que impide una maduración prematura. Los bananeros en los que se retiraron las hojas necrosadas produjeron frutas con una vida verde muy elevada. **Discusión**. Retirar las hojas necrosadas permite contrarrestar el efecto negativo de la SD en la fisiología de las frutas, lo que demuestra que la presencia de necrosis a lo largo del último mes de crecimiento de las frutas es responsable de la modificación fisiológica de las mismas por una maduración precoz. **Conclusión**. Retirar las hojas necrosadas un mes antes de la cosecha podría ser una técnica útil para limitar las pérdidas de producción cuando el nivel de infestación de SD es elevado.

Guadalupe / *Musa acuminata* / enfermedades de las plantas / *Mycosphaerella musicola* / maduración en postcosecha / frutas / calidad