Original article

Adaptation of the forecasting system to control Black Leaf Streak Disease of banana in the specific conditions of Dominican Republic

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Adaptation of the forecasting system to control Black Leaf Streak Disease of banana in the specific conditions of Dominican Republic.

Abstract – Introduction. Black Leaf Streak Disease (BLSD) is the most important foliar disease affecting banana production worldwide. A forecasting system has been developed and implemented in various countries aiming at optimal control of BLSD through minimum applications of fungicide. In Dominican Republic, favorable dry climatic conditions contrast with serious organizational issues for BLSD control. Our objective was to evaluate the adaptation of this forecasting strategy in these specific conditions. **Materials and methods**. Fungicide resistance analyses were carried out in the northwestern region of Dominican Republic, in order to determine the appropriate spectrum of systemic fungicides for the forecasting strategy. Three field experiments were set up on commercial farms where disease evolution was monitored every week, on reference plots, in order to decide the pertinence of fungicide applications. **Results**. Fungicide resistance to QoI fungicides and strong sensitivity reduction to DMI (Demethylation Inhibitor) fungicides were detected in all farms. In spite of these limitations in the use of some fungicide groups, disease control was achieved with a limited number of fungicide applications (6–9), as compared with 13–26 applications in most commercial farms of Dominican Republic over the same period. **Discussion**. The calculation of an indicator of the efficiency of the chemical control confirmed the potential of the forecasting strategy, underlining the influence of crop management as well as the neighboring environment of the farms on its efficiency. The requirements for further generalization of this system to commercial farms of this country are discussed.

Dominican Republic / *Musa* / plant disease control / *Mycosphaerella fijiensis* / forecasting

Adaptation du système d'avertissement biologique de la maladie des raies noires dans les conditions spécifiques de la République dominicaine.

Résumé – Introduction. La Maladie des Raies Noires (MRN) est la maladie foliaire la plus importante affectant la production bananière à travers le monde. Un système d'avertissement biologique a été développé et mis en place dans plusieurs pays visant à un contrôle optimal de la MRN avec un nombre minimal d'applications de fongicides. En République dominicaine, les conditions climatiques sèches sont favorables au contrôle de la maladie, mais contrastent avec des lacunes organisationnelles importantes. Notre objectif a été d'évaluer l'adaptation de la stratégie d'avertissement dans ces conditions spécifiques. **Matériel et méthodes**. Des analyses de résistance aux fongicides ont été menées dans la région Nord Ouest du pays afin de déterminer la gamme de fongicides appropriée pour l'avertissement. Trois expérimentations ont été menées sur des plantations commerciales avec un suivi hebdomadaire de l'évolution de la maladie, sur des postes de référence, afin de décider de la pertinence de la réalisation d'un traitement. **Résultats**. Une résistance aux fongicides de type QoI et une forte réduction de la sensibilité aux DMI (*Demethylation Inhibitors*) ont été détectées dans toutes les plantations. Malgré ces limitations dans l'usage de certains groupes de fongicides, la maladie a pu être contrôlée avec un nombre limité de traitements (6 à 9), contre 13 à 26 traitements effectués dans la plupart des plantations commerciales du pays sur la même période. **Discussion**. Le calcul d'un indicateur d'efficacité du contrôle chimique a confirmé l'intérêt de la stratégie d'avertissement, soulignant l'influence des pratiques culturales ainsi que celle de l'environnement voisin de la plantation sur son efficacité. Les dispositions nécessaires à la généralisation de ce système aux plantations commerciales du pays sont discutées.

République dominicaine / *Musa* / lutte antimaladie des plantes / *Mycosphaerella fijiensis* / technique de prévision

1. Introduction

Bananas are one of the main agricultural products in the world, and the main fruit crop, with an annual production of about 110 Mt [1]. Production for international trade is limited to a small number of countries, but for which banana production is economically and socially important [2]. Black Leaf Streak Disease (BLSD) is the most costly and damaging disease affecting the banana industry worldwide [3], since the unique banana group of cultivars grown for exportation (*Musa acuminata*, AAA, Cavendish subgroup) is highly susceptible. This foliar disease is caused by the fungal pathogen *Mycosphaerella fijiensis*. The first symptoms are identified as reddish-brown streaks, becoming larger streaks eventually leading to leaf necrosis. In the absence of suitable control, the photosynthetic capacity of leaves is significantly reduced [4], which often results in a yield loss of 20% to 80% [5, 6]. However, the most important effect of BLSD is the reduction of fruit greenlife [4, 7]. Effectively, as a climacteric fruit, bananas are harvested still green, at the pre-climacteric stage. Fruit ripening is then artificially induced by ethylene treatment in commercial ripening rooms. In order to withstand maritime shipping, greenlife must be superior to the time between harvest and artificial ripening [8]. A direct relationship between disease severity and greenlife reduction has recently been shown [9–11].

With 18,300 ha, and more than 2,000 producers, banana production has major economic and social importance in Dominican Republic [12]. From the 600,000 t produced in 2010, 328,000 t (52%) were exported, generating a major source of foreign exchange (FOB value of USD$ 180 M). The rest of the production supplies local markets and domestic consumption as a food security crop [13]. Banana is also a source of permanent work for the rural population [13], generating more than 50,000 direct and indirect jobs [12]. From its origin in Southeast Asia, *Musa. fijiensis* was first detected in the Americas in the early 70s, in Honduras [3]. In Dominican Republic, BLSD was first reported in 1996, in the Montecristi province [14, 15]. It quickly spread to all the country, and now represents a major constraint to fruit exportation [16]. At the beginning, the BLSD impact on the banana industry was not severe, but it has increased over the years. Indeed, until 2008, six to ten chemical applications per year were necessary [16], compared with 16 to 20 cycles today, with some situations up to 36 cycles [12]. Furthermore, hundreds of farmers stopped exportation in 2011, due to severe BLSD, accounting for the loss of about 96 000 t of bananas, valued at more than USD$ 76 M. The cost of control also increased, up to an average of USD$ 1,135·ha^{-1}, and presently the control of BLSD has become a serious challenge for the banana industry in this country [12].

A warning system developed for the control of Sigatoka disease (SD, due to *M. musicola*) has been adapted for BLSD [17–20]. This strategy aims to optimize control of the disease, with minimum fungicide applications, which would result in a lower cost of control, lower risk of fungicide resistance and lower environmental negative impact. The forecasting system relies on several key factors such as (i) early disease detection, (ii) fast aerial spraying, (iii) a strong curative effect through the use of systemic fungicides in 15 L·ha^{-1} of mineral oil, (iv) fungicide resistance management, and (v) centralization and generalization of control over the whole banana-producing area [3, 20].

Since the 70s this forecasting system has allowed good control of both diseases through 5–8 (Sigatoka disease, in the French West indies) to 10–15 annual applications (Black Leaf Streak Disease, in Ecuador, Cameroon and Ivory Coast), when 30 to 60 systematic sprays are carried out in most Latin American countries [20]. However, the regular and intensive use of systemic fungicides has, in some situations, led to the emergence of resistant strains in *M. fijiensis* populations [20, 21]. Consequently, as in Cameroon, the control has progressively shifted to a systematic fungicide application of contact fungicides (more than 40 applications per year), showing that this strategy must be adapted to local conditions in order to ensure its sustainability [21].

The objective of our work was to adapt this warning system method to the Dominican Republic, taking into account the specific conditions in this country: (i) favorable

Table I.
Fungicides used in the different culture media for the germination tests of *Mycosphaerella fijiensis* conidia.

Mode of action	Active ingredient	Concentration used ($\mu g \cdot mL^{-1}$)	Observations
DMI	Propiconazole	0.1	Length of germ tube
	Difenoconazole	0.1	Length of germ tube
Antimitotic	Methyl thiophanate	5	Morphology of the germ tube
Qoi	Azoxystrobin	10	Length of the germ tube
Control	None	0	Length of the germ tube

DMI: Demethylation Inhibitors.

climatic conditions for a forecasting system, because of annual precipitation lower than that in most of the banana production areas of the world (average 700 $mm \cdot year^{-1}$), but (ii) a difficult organizational context for BLSD control, since each banana grower (among more than 2000) implements his own strategy on his farm without any coordination [22]. This work was conducted in two steps. Firstly, fungicide resistance monitoring was embarked upon to identify the different systemic fungicides that could be used, ensuring a strong curative effect. Indeed, very few consistent data were available in this country. Secondly, three field trials were successively set up on three commercial farms with different agronomic and climatic conditions, in order to adapt the forecasting strategy, and to evaluate its performance under these specific conditions.

2. Materials and methods

2.1. Fungicide resistance analysis

2.1.1. Leaf sampling

Leaf samples were collected from two commercial farms, of (14 and 17) ha, located in Esperanza and Guayacanes, Valverde province, in the northwestern region of Dominican Republic. A limb fragment [(15 × 20) cm] bearing stage 2-3 lesions [23] was collected on each of 25 banana plants selected randomly on the same banana plot. Leaf samples were shipped to Montpellier, France, for further analysis. Leaf samples were collected in two periods: (i) before the experiment of the forecasting strategy (Esperanza and Guayacanes) and (ii) one year after the beginning of the experiment (Esperanza).

2.1.2. Fungal isolation and production of conidia

Conidia produced on the young stage 2-3 lesions were transferred to agar plates (30 $g \cdot L^{-1}$) and 50 single conidia were isolated (two isolates per leaf). Each isolate was further grown on V8 300sp medium (100 mL of V8 vegetable juice, 0.2 g of $CaCO_3$, 20 g of agar, 900 mL of water, and pH adjusted to 6) at 20 °C under continuous light to produce conidia that were used for germination tests.

2.1.3. Germination tests of conidia

After 10 days a fragment of each fungal colony grown on the V8 300sp medium was transferred into a microtube with 200 μL of sterile distilled water. After vortex agitation, a micro-drop of the conidial solution was transferred onto the various agar (20 $g \cdot L^{-1}$) media amended or not with different fungicide concentrations (*table I*).

Forty-eight hours after cultivation on the different agar + fungicide media, conidial germination was observed under a microscope [24]:

– For each isolate from cultivation with demethylation inhibitor (DMI) fungicides (propiconazole, difenoconazole) and QoI fungicides (azoxystrobin), the germ tube length of conidia was measured with a micrometer on the control (Lc) and on the fungicide-amended medium (Lf).

– For each isolate from cultivation with antimitotics (methyl thiophanate), the morphology of the germ tube was observed and

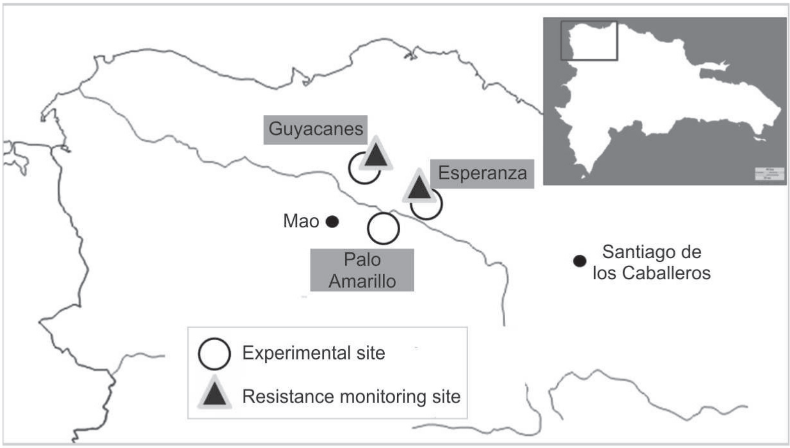

Figure 1.
Dominican Republic map with situation of sampling sites for the analysis of fungicide resistance and commercial farms where a forecasting strategy was tested.

classified as (i) normal (germination similar to the control); (ii) twisted (distortion of the germ tube); (iii) shorter (germ tubes not distorted, but length substantially shorter than the control), or (iv) not germinated.

The results were compared with those obtained from a non-treated banana farm in Cameroon (Cameroon baseline).

2.1.4. Data analysis

For each strain from cultivation with DMI fungicides (propiconazole, difenoconazole), the growth inhibition (GI) was calculated as {GI = [1 – (Lf / Lc)] × 100}. The average value of each population sampled was calculated and compared with the value of the baseline sample. The distribution of sensitivity of each strain using the following growth inhibition classes: [0–10[, [10–20[, [20–30[, [30–40[, [40–50[, [50–60[, [60–70[, [70–80[, [80–90[and [90–100] was represented, and the percentage of strains with less than 50% GI was also calculated in order to characterize the deviation of sensitivity of the fungal population analyzed (in baseline samples no strains have less than 50% GI).

For each strain from cultivation with QoI fungicides (azoxystrobin), the growth inhibition (GI) was calculated as {GI = [1 – (Lf / Lc)] × 100}. The average value of each population sampled was calculated and compared with the value of the baseline sample. The distribution of sensitivity of each strain using the following growth inhibition classes: [0–10[, [10–20[, [20–30[, [30–40[, [40–50[, [50–60[, [60–70[, [70–80[, [80–90[and [90–100] was represented. The percentage of strains with less than 40% GI was also calculated in order to estimate the percentage of resistance in each population (all strains with GI < 40% are resistant, but the proportion of resistant strains might be underestimated with this cut-off limit).

Resistant strains from cultivation with antimitotics (methyl thiophanate) are those with a normal or short germ tube. Therefore, for each population sampled, the percentage of resistant strains was calculated and compared with the baseline sample (0% of resistant strains).

2.2. Field experiments of the warning method on commercial farms

The experiments were set up (i) from April 2011 to March 2012 in Esperanza (14 ha), (ii) from April 2012 to April 2013 in Palo Amarillo (20 ha), and from April 2012 to April 2013 in Guayacanes (17 ha), all these farms being located in the Mao, Valverde province (*figure 1*).

2.2.1. Data collection

The development of Black Leaf Streak Disease was assessed every week through various parameters, following Fouré's method [3, 17].

The "Stage of Evolution of Disease" (SED) represents the rate of disease development, which reflects the climatic conditions, and the intensity of the infection [20]. It relies on the early detection of the first disease symptoms on the youngest banana leaves (ranks 1 to 4). This detection will allow blocking their evolution towards necrotic spots, and sexual sporulation, thanks to the early application of a curative fungicide. Decrease in the SED after an application therefore reflects its efficiency.

The "Youngest Leaf bearing Streaks" (YLSt) is the rank of the youngest leaf bearing symptoms of the disease. It reflects the effect of the climatic conditions and fungicide spraying program on the incubation period of the disease. This parameter is complementary to the SED, as it enables one to understand the evolution of epidemiological conditions, especially when no symptom is observed on the first four leaves.

The "Youngest Leaf Spotted" (YLS) is the youngest leaf bearing necrotic lesions. Evolution of the YLS being a balance between the rate of necrotic formation and the foliar emission rate, its evolution allows understanding the efficiency of the fungicide control to reduce necrotic formation: (i) if the value of the YLS decreases, the chemical control is failing because necrotic formation is faster than leaf emission; (ii) if the YLS is increasing, the chemical strategy is efficient in slowing down the necrotic formation.

The "Number of Functional Leaves at Harvest" (NLH) is calculated as the sum of green portions of remaining leaves. It was evaluated every week on different banana trees at the harvest stage. It reflects the efficiency of the chemical strategy, and is used as a potential indicator for fruit exportability. Banana companies generally use an empirical threshold of the NLH (3–5 according to the company) in order to decide which bunches are exportable or not.

Daily rainfall was recorded during all the experiments in order to appreciate the potential influence of rainfall on ascospore release.

2.2.2. Timing of fungicide applications

A graphic representation of the SED, YLSt, YLS, NLH and precipitation was carried out weekly after field observations. The decision for a new application was a synthesis of all these parameters.

2.2.3. Fungicide applications

Once the decision was taken, the application was to be executed within 2 days. The type of fungicide used was a balance between a general principle of rotation of fungicides with different modes of action, and the need for a strong curative effect at the time of the decision. To ensure a strong curative effect of the applications [25], only systemic fungicides mixed in pure mineral oil were used. They were selected according to the results of the resistance monitoring carried out before the experiment. Only fungicides with an acceptable level of sensibility were to be used. Availability of the different products in Dominican Republic was then checked, as was their formulation, which had to be compatible for mixtures in pure mineral oil at a 15 L·ha^{-1} rate. Use of mineral oils strengthens the curative effect of the application because of their fungistatic effect [25]. The good quality of the oil is essential and must fit with the norms defined by Cuillé and Blanchet [26]. Viscosity 4° to 7° Engler at 20 °C and a sulfonation index lower than 85–90% are required.

During and after each application, the quality of the spraying was evaluated: (i) through the observation of droplet movement during the flight, and (ii) observation of oil deposits on the leaves, to evaluate product coverage in different plots of the farm.

3. Results

3.1. Fungicide resistance analysis before the field experiment

3.1.1. Demethylation inhibitor (DMI) fungicides

A strong loss of sensitivity to propiconazole was observed (*table II*). The mean percentage of growth inhibition was very low on both farms, 0% and 29% in Guayacanes and Esperanza, respectively, as compared with 74% in the untreated farm. A high proportion of the population in Guayacanes (100%) and Esperanza (74%) had a growth inhibition lower than 50% (no strains were inhibited less than 50% in the non-treated farm).

For difenoconazole, a strong shift in sensitivity was also observed. The mean percentage of growth inhibition was very low on both farms, Guayacanes (21%) and Esperanza (51%), as compared with 77% in the untreated farm. In addition, 40% of the strains showed low growth inhibition (< 50%) in Esperanza, and up to 95% in Guayacanes, whereas none of the Cameroon baseline strains were in this class.

3.1.2. QoI fungicides

For azoxystrobine, at least 18% of the strains from Esperanza and 30% from Guayacanes were resistant (growth inhibition < 40%) (*table III*), showing a strong QoI resistance in fungal populations of these two farms.

Table II.

Effect of demethylation inhibitor (DMI) fungicides (propiconazole and difenoconazole) at the rate of 0.1 μg·mL^{-1} on germ tube growth, in fungal populations of various Dominican Republic banana farms.

DMI fungicide	Farms studied	% growth inhibition	germ tube length (μm)	Classes of growth inhibition										% conidia with GI < 50%
				0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	
Propiconazole	Results 2011													
	Esperanza	29	158	30	16	16	8	4	4	2	6	6	8	74
	Guayacanes	0	378	100	0	0	0	0	0	0	0	0	0	100
	Results 2012													
	Esperanza	7	356	67	27	4	2	0	0	0	0	0	0	100
	Baseline Cameroon	74	61	0	0	0	0	0	0	24	66	10	0	0
Difenoconazole	Results 2011													
	Esperanza	51	150	13	4	8	2	13	15	27	19	0	0	40
	Guayacanes	21	294	10	40	45	0	0	0	0	0	5	0	95
	Results 2012													
	Esperanza	44	215	4	14	22	18	14	0	0	8	16	2	73
	Baseline Cameroon	77	52	0	0	0	0	0	0	10	62	28	0	0

GI: Growth Inhibition.

Table III.

Effect of QoI fungicide (azoxystrobin) at the rate of 10 μg·mL^{-1} on germ tube growth, in fungal populations of various Dominican Republic banana farms.

Azoxystrobin	% growth inhibition	germ tube length (μm)	Classes of growth inhibition										% conidia with GI < 40%
			0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	
Results 2011													
Esperanza	84	36	4	2	6	4	2	0	0	0	6	76	18
Guayacanes	43	213	0	0	0	30	60	10	0	0	0	0	30
Results 2012													
Esperanza	84	60	0	0	6	4	2	4	6	4	4	69	12
Baseline Cameroon	92	24	0	0	0	0	0	0	2	6	27	65	0

GI: Growth Inhibition.

Table IV.
Percentage of resistant strains of *Mycosphaerella fijiensis* to methyl thiophanate (5 µg·mL^{-1}) in various Dominican banana farms.

Methyl thiophanate	% Resistant	% Susceptible	Number of strains
Results 2011			
Esperanza	2	98	50
Guayacanes	0	100	15
Results 2012			
Esperanza	0	100	49
Baseline Cameroon	0	100	50

Table V.
List of fungicides that can be potentially used for the adaptation of forecasting strategies for Black Leaf Streak Disease (BLSD) control in banana farms of Dominican Republic.

Mode of action	Potential products	Curative effect	Resistance risk	Resistance in Dominican Republic	Potential use in the forecasting experiment
DMI	Tilt 25 EC Sico 25 EC Opal 7,5 EC Silvacur 30 EC Folicur 25 EW	++++	+++	Yes	Limited
QoI	Tega 25 SC	++++	+++++	Yes	No
Antimitotic	Topsin M70 OD Callis	+++	++++	No	Yes (no oil formulation available in Dominican Republic)
SBI class II	Volley 88 OL Impulse 80 EC	++	+/-	nd	Yes
Pyrimidin	Siganex 60 SC	++	+	nd	Yes
SDH inhibitors	Cumora Reflect 125 EC	+++	nd	nd	Yes (not available until November 2012)

DMI: Demethylation Inhibitors.
nd: not determined.

3.1.3. Antimitotic fungicides

For methyl thiophanate (*table IV*), almost all strains had twisted morphology, characteristic of susceptible populations as encountered on non-treated farms. None of the strains from Guayacanes and only 2% of the strains in Esperanza were resistant.

These results led us to draft a list of the potential fungicides to be used for the experiment, limited to the few families and few products available in Dominican Republic (*table V*). Since QoI resistance was demonstrated, these products could not be used in the forecasting strategy. The significant shift in sensitivity to DMI fungicides led us to limit the use of these fungicides drastically, ordinary important components of the strategy. If used, these fungicides would be mixed with another fungicide (SBI class II) in order to reinforce their curative effect. Antimitotics could be used, but unfortunately no for-

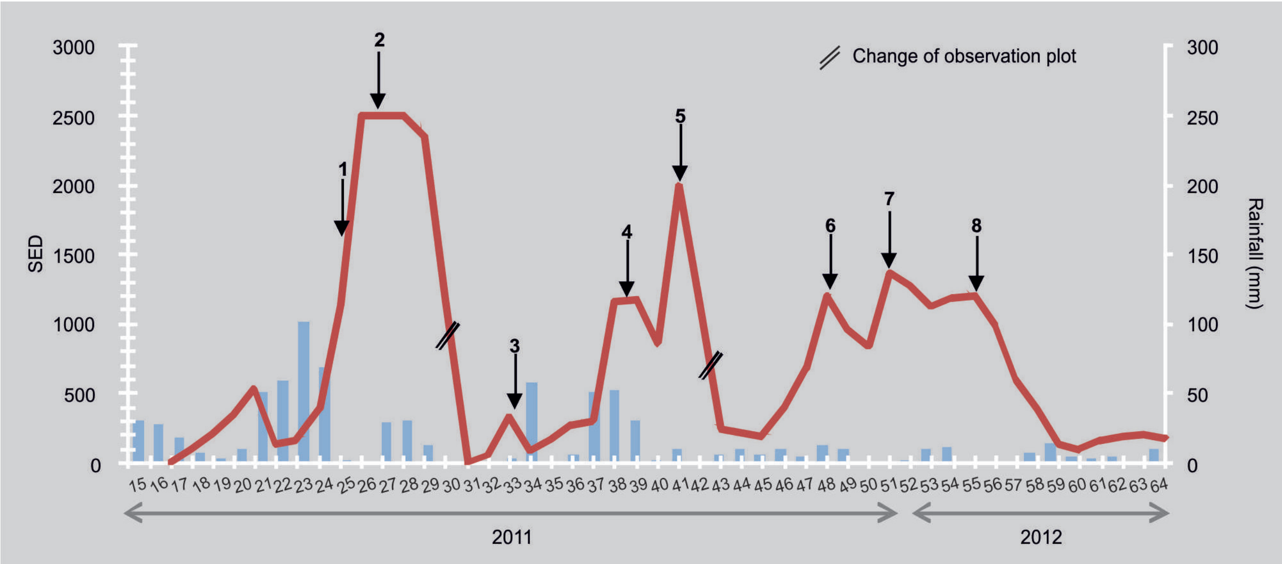

Figure 2.
Weekly variations of the Stage of Evolution of the Disease (SED) in Esperanza (Dominican Republic).

mulation compatible with oil was available in Dominican Republic. The SBI class II and pyrimidins could be used without restriction because these fungicides are generally not affected by fungicide resistance. However, they have a limited curative effect and cannot be used efficiently in some situations. The new family of the SDH inhibitors could be used, but were not available at the beginning of the experiment.

3.2. Disease control over a one-year period

Generally, a new fungicide application was decided when a significant increase in the Stage of Evolution of Disease (SED) occurred. However, this decision was adapted to the specific climatic conditions of this country (shown by rainfall data) and the evolution of the Youngest Leaf bearing Streaks (YLSt) and also to the efficiency of the applications in the context of fungicide resistance [shown by SED evolution after the fungicide application and by the evolution of the Youngest Leaf Spotted (YLS)]. For instance, during dry periods, slight increases in the SED did not lead to a new fungicide application when the YLS and YLSt remained at high values. On the other hand, the fungicide application was not considered to be efficient when the SED did not decrease rapidly and the YLS started to decrease significantly. In this case a new fungicide application was decided.

3.2.1. In Esperanza

Over a one-year period, eight fungicide applications were carried out according to the variations of disease evolution (*figures 2*, *3*, *table VI*). During this experiment, the delay between the decision and application varied from (2 to 10) days (*table VI*), depending on several factors such as (i) grower reactivity, (ii) some delays in product purchase, (iii) product availability and (iv) delays of the spraying company. These delays are far from the requirements of the forecasting strategy, and the efficiency of some applications (very low control duration for treatments 1 and 6, *table VI*) was altered by being carried out too late. Out of eight applications, seven were undertaken in optimal climatic conditions. One application (treatment 6) was carried out later in the morning, when the temperature was higher than 28 °C, and poor coverage of the product was observed. For other applications, the quality of the coverage proved to be acceptable.

More specifically, the first fungicide application decided on at week 23 was carried out 10 days later, and did not allow good disease control. A second application (mixture of a DMI with a SBI class II) was necessary to achieve further SED decrease.

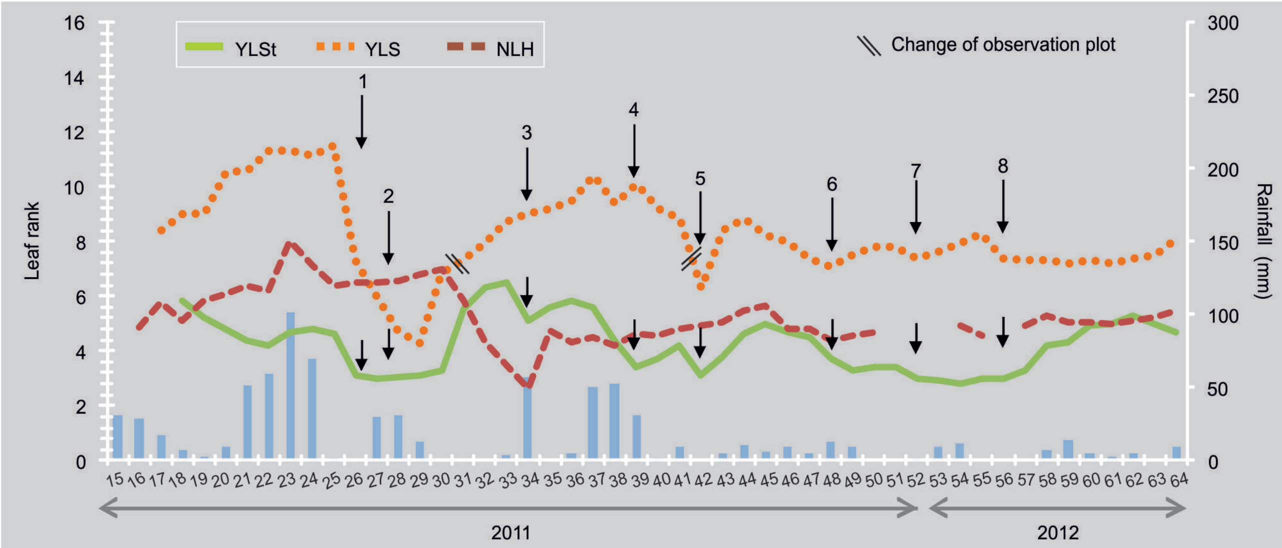

Figure 3.
Weekly variations of the Youngest Leaf Bearing Streaks (YLSt), Youngest Leaf Spotted (YLS) and Number of Leaves at Harvest (NLH) in Esperanza (Dominican Republic).

Table VI.
Description of the applications carried out for control of Black Leaf Streak Disease (BLSD) in a banana farm in Esperanza (Dominican Republic).

Number of the application	Days between decision and application	Efficiency of the application (weeks)	Product	Active ingredient	Mineral oil (15 L·ha^{-1})
1	9	2	Volley 0.5 L·ha^{-1}	Fenpropimorph	Orange Oil
2	5	7	Volley 0.5 L·ha^{-1} + Opal 1 L·ha^{-1}	Fenpropimorph + epoxiconazole	Spraytex
3	2	5	Siganex 0.5 L·ha^{-1}	Pyrimethanil	Orange Oil
4	2	3	Topsin 300 g·ha^{-1}	Methyl thiophanate	Spraytex
5	3	7	Sico 0.4 L·ha^{-1} + Volley 0.5 L·ha^{-1}	Difenoconazol + fenpropimorph	Spraytex
6	10	3	Siganex 0.4 L·ha^{-1}	Pyrimethanil	Banole
7	6	4	Topsin 300 g·ha^{-1}	Methyl thiophanate	Banole
8	5	> 9	Volley 0.5 L·ha^{-1} + Opal 1 L·ha^{-1}	Fenpropimorph + epoxiconazole	Banole

As a consequence of bad disease control, the YLS decreased strongly between week 25 and week 29, and this decrease was later observed after week 30 for the number of functional leaves at harvest (NLH) (*figure 2*). In two other situations, we decided to use a mixture of a DMI with a SBI class II, as the SED did not decrease significantly after previous fungicide applications (applications 5 and 8).

During our experiment, poor deleafing practices and poor crop management resulting in a low foliar emission rate (mean 0.85 leaf over 10 days' duration) were performed by the grower. As a consequence, the YLS and NLH remained in a low position since the leaf emission rate did not compensate for disease development, and the inoculum was persistent in necrotic stages. In spite of this, the conservation of

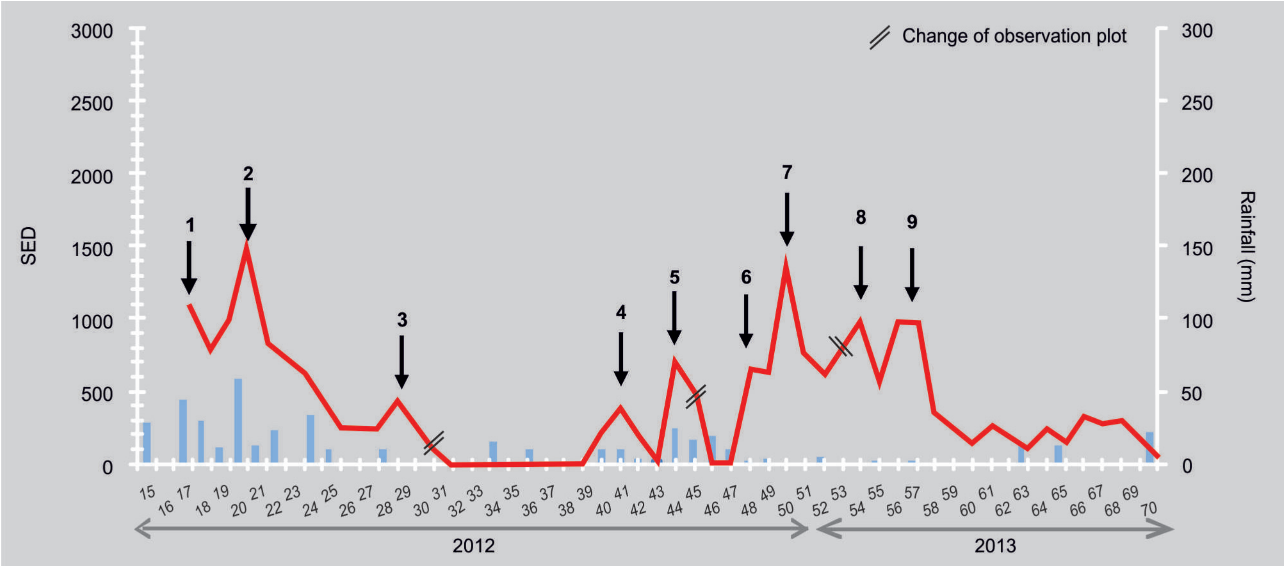

Figure 4.
Weekly variations of the Stage of Evolution of the Disease (SED) in Palo Amarillo (Dominican Republic).

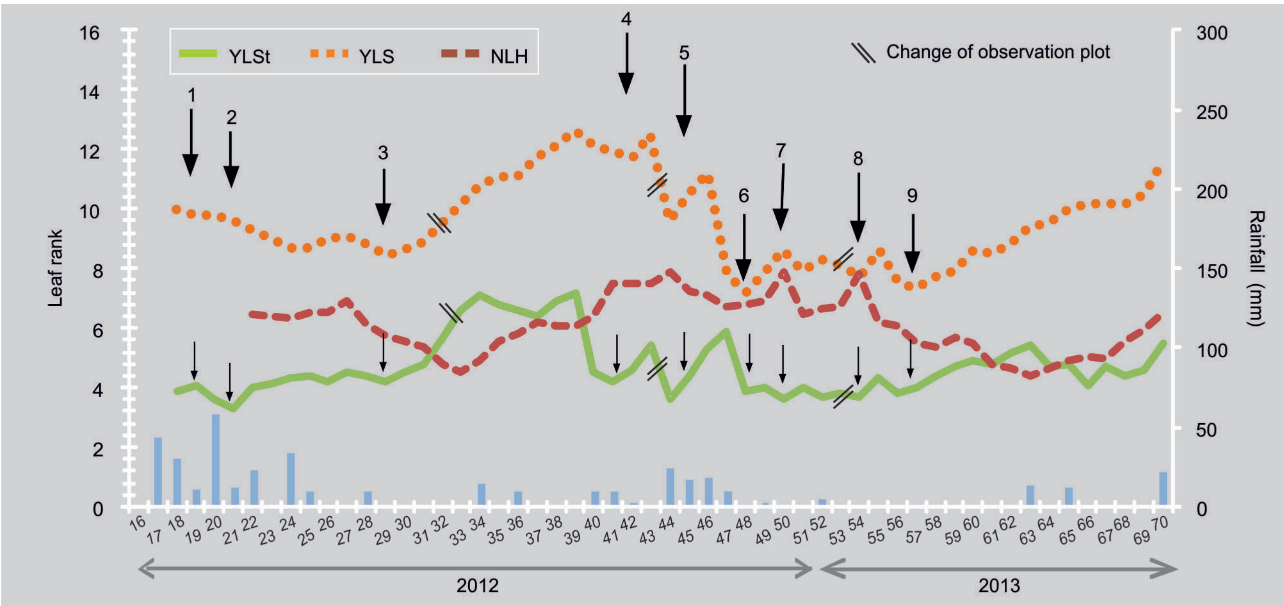

Figure 5.
Weekly variations of the Youngest Leaf Bearing Streaks (YLSt), Youngest Leaf Spotted (YLS) and Number of Leaves at Harvest (NLH), in Palo Amarillo (Dominican Republic).

exported fruit was not affected during the experiment.

3.2.2. In Palo Amarillo

Over one year, nine applications were carried out (*figures 4*, *5*, *table VII*). Delays between the decision and application were reduced to (1 to 3) days, thanks to improvements in farmer involvement, product supply and agreement with the spraying company. Fungicide mixtures were carried out in tanks equipped with a rotor, allowing optimum mixing. All of the nine applications were undertaken in good climatic conditions, and the quality of the coverage

Table VII.
Description of the applications carried out for control of Black Leaf Streak Disease (BLSD) in a banana farm in Palo Amarillo (Dominican Republic).

Number of the application	Days between decision and application	Efficiency of the application (weeks)	Product	Active ingredient	Mineral oil
1	2	3	Volley 0.5 $L \cdot ha^{-1}$	Fenpropimorph	Spraytex[1]
2	3	8	Volley 0.5 $L \cdot ha^{-1}$ + Opal 1 $L \cdot ha^{-1}$	Fenpropimorph + epoxiconazole	Banole[1]
3	2	12	Volley 0.5 $L \cdot ha^{-1}$	Fenpropimorph	Banole[1]
4	1	3	Siganex 0.5 $L \cdot ha^{-1}$	Pyrimethanil	Banole
5	2	4	Cumora 0.3 $L \cdot ha^{-1}$	Boscalid	Banole[2]
6	2	2	Volley 0.5 $L \cdot ha^{-1}$ + Opal 1 $L \cdot ha^{-1}$	Fenpropimorph + epoxiconazole	Banole[1]
7	2	4	Impulse 0.3 $L \cdot ha^{-1}$	Spiroxamine	Banole[1]
8	2	3	Volley 0.5 $L \cdot ha^{-1}$	Fenpropimorph	Banole[1]
9	1	> 13	Reflect 0.6 $L \cdot ha^{-1}$	Isopyrazam	Banole[1]

[1]: mineral oil at 15 $L \cdot ha^{-1}$.
[2]: mineral oil at 9 $L \cdot ha^{-1}$.

proved to be good at field level. As a consequence, fungicide applications generally allowed good disease control, as shown by disease-free periods of up to 8 weeks with the SED values equal to zero, or long periods of up to 12 weeks where fungicide applications were not necessary. Here again, the use of a mixture of a DMI with a SBI class II allowed good control (treatment 2), but not in all circumstances (treatment 6). It is also noteworthy that, in dry conditions, the use of a SBI class II or pyrimidins could allow good disease control (treatments 3 and 4). SDHi proved to be effective in BLSD control, particularly in treatment 9 with Reflect, whose formulation is more miscible in oil than Cumora (treatment 5). Lastly, adequate field management allowed a good leaf emission rate (mean 1.1 to 1.3 leaf over 10 days' duration) and generally YLS and NLH evolution did not show a failure in disease control (*figure 5*).

3.2.3. In Guayacanes

Over 12 months, six applications were carried out (*figures 6, 7, table VIII*). As in Palo Amarillo, applications were carried out within optimal time scales (2 to 3 days), with good mixing and climatic conditions. As a consequence, it allowed good to excellent control, with up to 8 weeks with SED values equal to 0 and long periods up to 19 weeks where fungicide applications were not necessary. As in Palo Amarillo the use of a mixture of a DMI with a SBI class II allowed good disease control in some instances (treatment 5) but not in others (treatment 1). The use of SDHi proved to be effective (treatment 3), as well as the use of a SBI class II in dry conditions (treatments 2 and 6). Good field management allowed a good leaf emission rate (mean 1.1 leaf over 10 days' duration), and YLSt and YLS evolution showed overall good disease control (*figure 7*).

3.3. Fungicide resistance analysis after the field experiment

Fungicide resistance was re-evaluated at the end of the experiment carried out in Esperanza. No changes were observed for QoI and antimitotics. For azoxystrobine (*table III*), sensibility was almost unchanged, and resistant strains were still observed even if no QoI fungicides were used over the last year. Finally, sensibility to benzimidazoles proved to be still very good, even after the two applications carried out (*tables IV, VI*). However, the susceptibility to DMI

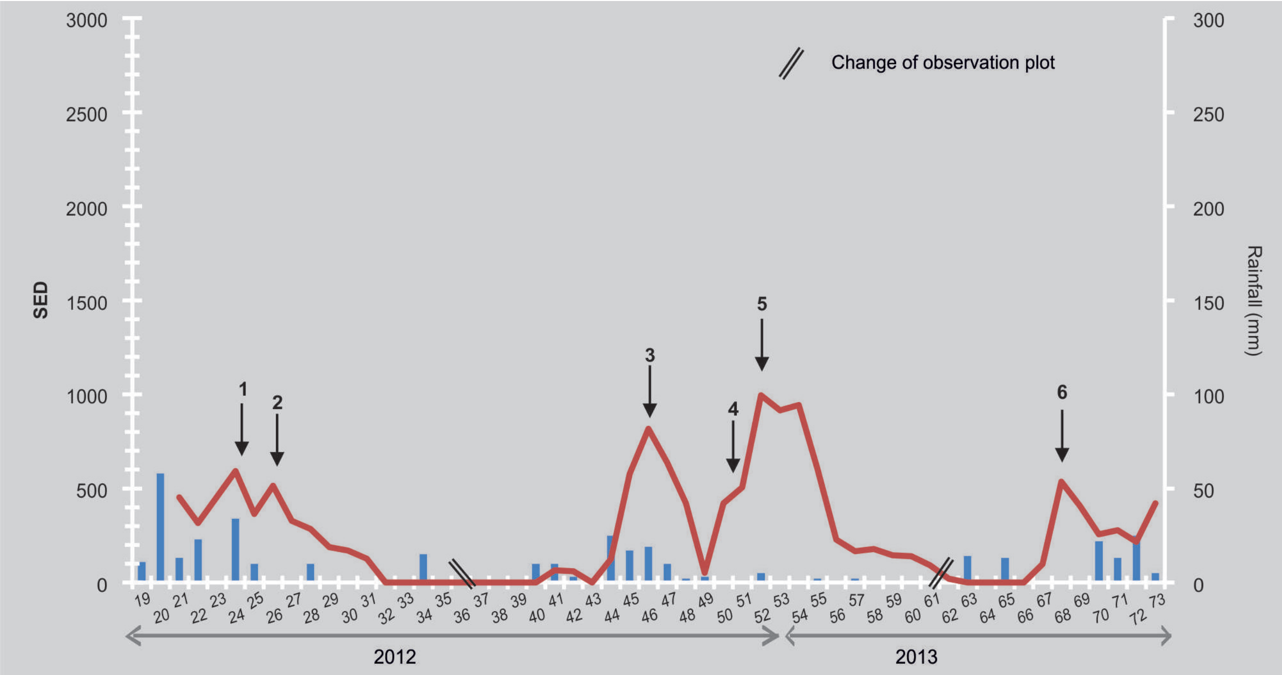

Figure 6.
Weekly variations of the Stage of Evolution of the Disease (SED) in Guayacanes

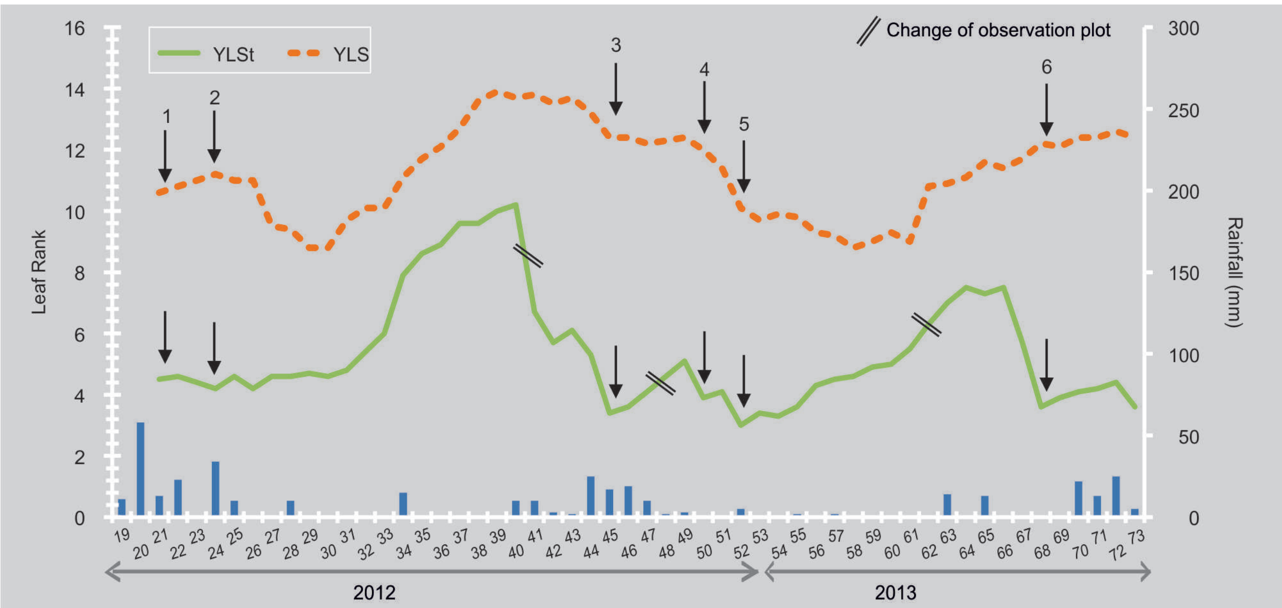

Figure 7.
Weekly variations of the Youngest Leaf Bearing Streaks (YLSt) and Youngest Leaf Spotted (YLS) in Guayacanes (Dominican Republic).

fungicides, especially to propiconazole, decreased as a consequence of three applications that were carried out during the experiment with DMI mixed with a SBI class II (*tables II, VI*). This decrease can be illustrated by the very low average value of the % of growth inhibition (GI = 7%) and by the significant proportion of strains that were slightly inhibited (100% of strains had a GI < 50%).

Table VIII.
Description of the applications carried out for control of Black Leaf Streak Disease (BLSD) in a banana farm in Guayacanes (Dominican Republic).

Number of the application	Days between decision and application	Efficiency of the application (weeks)	Product	Active ingredient	Mineral Oil
1	3	3	Volley 0.5 $L \cdot ha^{-1}$ + Opal 1 $L \cdot ha^{-1}$	Fenpropimorph + epoxiconazole	Banole[1]
2	2	19	Volley 0.5 $L \cdot ha^{-1}$	Fenpropimorph	Banole[1]
3	2	5	Cumora 0.3 $L \cdot ha^{-1}$	Boscalid	Banole[2]
4	2	1	Impulse 0.3 $L \cdot ha^{-1}$	Spiroxamine	Banole[1]
5	2	16	Volley 0.5 $L \cdot ha^{-1}$ + Sico 0.4 $L \cdot ha^{-1}$	Fenpropimorph + difénoconazole	Banole[1]
6	2	> 6	Volley 0.5 $L \cdot ha^{-1}$	Fenpropimorph	Banole[1]

[1]: mineral oil at 15 $L \cdot ha^{-1}$.
[2]: mineral oil at 9 $L \cdot ha^{-1}$.

4. Discussion and perspectives

Our study carried out in three very different sets of conditions at the farm level allows demonstrating that it is possible to adapt the forecasting method to control Black Leaf Streak Disease (BLSD) in the specific conditions of the Dominican Republic: climatic conditions adverse for disease development but strong limitations in the use of some systemic fungicides. Moreover, our study allowed us to highlight critical points to be considered before the implementation of this strategy on a larger scale.

4.1. A significant reduction in the number of fungicide applications

The forecasting strategy allowed a significant reduction in the number of fungicide applications in all situations: six applications per year were carried out in Guayacanes, eight in Esperanza and nine in Palo Amarillo. In comparison, in the meantime, most farms located in these areas applied systematic application programs [12] with cycles every (2 to 4) weeks (13 to 26 applications per year). Moreover, our results confirm that the dry season conditions, unfavorable to disease development, provide very favorable conditions for the forecasting strategy. Indeed, disease monitoring showed that it was useless to carry out a new fungicide application for periods as long as 12 weeks in Palo Amarillo, and 19 weeks in Guayacanes.

4.2. Factors limiting the success of the forecasting strategy

To evaluate and compare the efficiency of the strategy over the different locations, we calculated an indicator of the efficiency of the chemical control (ECC): ECC = (SEDy) × Ny, where SEDy is the average of the weekly values of the SED over one year in a location and Ny is the number of fungicide treatments applied in one year in this same location [3]. The lower the ECC, the more efficient the chemical control strategy, as it means that a good control of BLSD was achieved (low average value of the SED), with a low number of fungicide treatments.

These calculations show that the best efficiency of the forecasting strategy was achieved in Guayacanes, with an ECC lower than 2000, and the worst in Esperanza (*table IX*).

4.2.1. Influence of farm environment

Since the Guayacanes and Palo Amarillo experiments were performed over the same climatic period, these situations might be

Table IX.
Efficiency of the chemical control for three experiments of Black Leaf Streak Disease (BLSD) control in Dominican Republic.

Experiment	Mean SED over 1 year	Number of applications over 1 year	ECC
Esperanza	767	9.2 (8 applications over 45 weeks)	7087
Palo Amarillo	410	9	3692
Guayacanes	266	6	1598

SED: Stage of Evolution of the Disease. ECC: Efficiency of the Chemical Control.

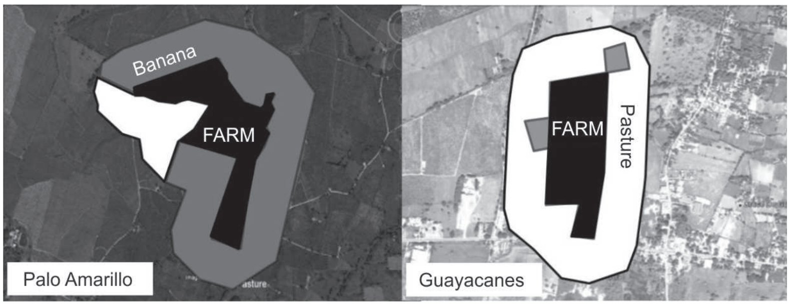

Figure 8.
Environment of the commercial farms where a forecasting strategy was tested in 2012. Palo Amarillo (left) and Guayacanes (right). Various geographical features situated in a 200-m border are indicated: [gray: banana or plantain; white: pasture or other crop (except banana); black: experimental farm].

compared and the better performance of the strategy in Guayacanes (*table IX*) might be explained by geographical features. Since ascospores of *M. fijiensis* disperse at long distances [27], and on average at 200 m, we considered the geographical features within a border of 200 m around each banana plot. This analysis showed that Guayacanes farm is quite isolated: 82% of this border is grown with bananas in Palo Amarillo, *versus* only 5% in Guayacanes (*figure 8*). Furthermore, many of the bananas grown in the border of the Palo Amarillo farm suffer from bad phytosanitary control, making them many potential inoculum sources of ascospores.

4.2.2. Influence of agronomic practices

The worse efficiency of the strategy in the Esperanza farm (*table IX*) confirms that better plant growth management, with adequate fertilization and irrigation, is essential to optimize the efficiency of the chemical strategy. Indeed, the agronomic management was very poor in the Esperanza farm as compared with the two other farms. Particularly, good agronomic practices are essential to optimize the leaf emission rate (LER), and compensate for disease reduction of the leaf area. It is stated that a minimum LER of one leaf a week should be targeted. Overall, better management of the field inoculum needs to be achieved, in order to limit new contamination inside the same farm, and between close farms, as their influence proved to be significant in the Palo Amarillo experiment.

4.2.3. Influence of timing in decisions and carrying out the applications

Significant delays in applications (over 48 h) proved to impact the efficiency of the applications in the Esperanza farm significantly, especially at the beginning of the experiment. Shortening delays in the decision and undertaking of fungicide applications is a key component to the success of the forecasting strategy [19, 20]. Thus, as expected in the Palo Amarillo and Guayacanes farms, a better efficiency of the strategy was achieved thanks to specific agreements with the spraying company that allowed a faster carrying out of fungicide applications, within 48 h, and in the best meteorological conditions (early in the morning when the temperature does not exceed 27 °C).

4.2.4. Influence of fungicide resistance

Considering the dry climatic conditions in the Dominican Republic, good control of BLSD with a lower number of fungicide applications should be expected, since, in similar climatic conditions in Ghana, only 5-6 applications per year are necessary for very good BLSD control (*Compagnie Fruitière*, pers. commun.). One of the objectives of our study was to evaluate whether the forecasting strategy could still be efficient in a context where the use of some systemic fungicides is limited by fungicide resistance. Our results showed that, in spite of a greater number of fungicide applications than expected, the forecasting strategy was still efficient in the conditions of the Dominican Republic. Particularly, we showed that the use of DMI fungicides mixed with a SBI class II allowed efficient control of the disease in farms where sensitivity to this group of fungicides was significantly reduced. This alternative was necessary because other fungicides with a strong curative effect (antimitotics, QoI, SDH inhibitors) were not available or could not be used. Indeed, the curative effect of the SBI class II and pyrimidines is too low to allow good disease control in high outbreak situations. Nevertheless, we also showed that this use of DMI fungicides, even if limited, could worsen the level of fungicide resistance to these fungicides. The use of DMI fungicides must therefore be very limited. The registration of a new group of fungicides, the SDH inhibitors, in Dominican Republic (isopyrazam, fluopyram, boscalid) provide a new alternative as high curative effect fungicides as they do not have cross-resistance with other groups. This was confirmed by the applications carried out in Palo Amarillo and Guayacanes, especially using Reflect. Furthermore, availability of antimitotic products usable in oil should also be investigated in Dominican Republic, since fungal populations of this country remain susceptible to this group of fungicides, which is known as an efficient tool for BLSD control [21].

4.3. Considerations for a future extension of this strategy to banana farms of Dominican Republic

While the forecasting method proved to be efficient in controlling disease on a single farm scale, consequent organizational issues need to be arranged within the different producers' associations before further implementation on a larger scale:

1) Banana farms should be sectored into functional spraying areas (100–200 ha) where disease development is homogeneous. These areas would include various farmers that would follow the same disease control strategy. To determine these areas, it will be essential to take into account the particularity of this country where organic and conventional farms are closely located in the same vicinity.

2) Decisions must be centralized within a technical team, responsible for decisions of fungicide applications and for the selection of the most adequate products, based on technical criteria, instead of the current individual decisions.

3) The logistics of aerial applications must be reorganized to avoid long delays in their undertaking, and to ensure that applications are undertaken in optimal climatic conditions for all farmers located within the same spraying area.

4) High curative effect fungicides must be selected. These products must be compatible for a mix in pure mineral oil, as its use will reinforce the curative effect of the treatment.

5) The different products must be used rationally in order to manage fungicide resistance. The general guideline is to alternate fungicides with different mode of actions, and the strategy must be driven by regular fungicide resistance monitoring.

6) The strong heterogeneity in the implementation of agronomic practices to eliminate inoculum sources and to optimize the leaf emission rate must be considered to evaluate the efficiency of the chemical strategy.

Therefore, significant changes in the organization of the Dominican banana sector are required before further large-scale implementation of this strategy in this country. Nevertheless, without this reorganization, BLSD will keep challenging the evolution of the banana industry, as its control will be more and more difficult and expensive, potentially compromising its sustainability.

Acknowledgments

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Adaptación del sistema de aviso biológico de la enfermedad de las rayas negras en las condiciones específicas de la República Dominicana.

Resumen – Introducción. La enfermedad de las rayas negras (ERN) es la enfermedad foliar que afecta a la producción bananera más importante en el mundo. Se desarrolló un sistema de aviso biológico y se puso en marcha en varios países con el objetivo de controlar de forma óptima la ERN con un número mínimo de aplicación de fungicidas. En la República Dominicana, las condiciones climáticas secas son favorables para controlar la enfermedad, pero contrastan con los importantes vacíos organizacionales. Nuestro objetivo pretendió evaluar la adaptación de la estrategia de aviso en estas condiciones específicas. **Material y métodos**. Se realizaron análisis de resistencia a los fungicidas en la región del noroeste del país con el fin de determinar la gama de fungicidas apropiada para el aviso. Se ejecutaron tres experimentos en plantaciones comerciales con un seguimiento semanal de la evolución de la enfermedad, en puestos de referencia, para decidir la pertinencia de la realización de un tratamiento. **Resultados**. Se detectaron una resistencia a los fungicidas de tipo QoI y una fuerte reducción de la sensibilidad a los DMI (*Demethylation Inhibitors*) en todas las plantaciones. A pesar de estas limitaciones en el uso de ciertos grupos de fungicidas, la enfermedad pudo controlarse con un número limitado de tratamientos (6 a 9), frente a 13 a 26 tratamientos efectuados en la mayoría de las plantaciones comerciales del país en el mismo periodo. **Discusión**. El cálculo de un indicador de eficacia del control químico confirmó el interés de la estrategia de aviso, subrayando la influencia de las prácticas culturales, así como la del entorno vecino a la plantación en su eficacia. Se discuten las disposiciones necesarias para la generalización de este sistema en las plantaciones comerciales del país.

República Dominicana / *Musa* / control de enfermedades de plantas / *Mycosphaerella fijiensis* / técnicas de predicción