Acorbat Revista de Tecnología y Ciencia

Control of black leaf streak disease without mancozeb in commercial plantations of Ivory Coast

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Contribución:
Tecnológica

Sección:
Fitosanidad

Recibido:
15 Diciembre, 2023
Aceptado:
15 Enero, 2024
Publicado:
16 Abril, 2024

Cita:
de Bellaire LL, Wyvekens A, Asara S, Ouattara M, Pugeaux A, Carlier J, Pagès C and Normand A. 2024. Control of black leaf streak disease without mancozeb in commercial plantations of Ivory Coast. *Acorbat Revista de Tecnología y Ciencia* 1(1): 30
https://doi.org/10.62498.ARTC.2430

RESUMEN

La Sigatoka Negra es el principal reto de la industria bananera, ya que el control de esta enfermedad es crucial para la exportación de banano. En la mayoría de los países el control de la SN se basa en aplicaciones semanales de fungicidas de contacto (mancozeb). Sin embargo, las limitaciones regulatorias del uso de ditiocarbamatos en la UE podrían bloquear el uso de este fungicida a muy corto plazo. Hemos evaluado nuevas estrategias excluyendo mancozeb en zonas húmedas de Costa de Marfil donde la resistencia a la mayoría de los fungicidas sistémicos está establecida. Esta estrategia se basa en observaciones semanales de los parámetros biológicos de la enfermedad para fundamentar las decisiones y en un uso más amplio del aceite mineral y de los fungicidas IBS del grupo 2. En las condiciones de esta evaluación, esta estrategia es mucho mejor en términos de rendimiento medioambiental y es al menos tan eficaz para el control de la enfermedad como la estrategia convencional basada en el uso de mancozeb, por lo que sigue siendo prometedora.

Palabras clave: *Pseudocercospora fijiensis*, preaviso biológico, Costa de Marfil, eliminación del mancozeb, Fitosanidad.

ABSTRACT

BLSD is the main challenge in the banana industry since the control of this fungal disease is crucial for banana exportation. In most countries, BLSD control relies on weekly applications of contact fungicides as mancozeb. Regulatory limitations of dithiocarbamates in the EU might soon block the use of this fungicide. Here we have evaluated new strategies excluding mancozeb in humid areas of Ivory Coast where fungicide resistance to most systemic fungicides is established. This strategy relies on weekly observations of biological disease parameters to inform decisions and on a wider use of mineral oil and fungicides from the SBI group 2. This strategy is better in terms of environmental performance and as performant for disease control as the conventional strategy based on mancozeb.

Keywords: *Pseudocercospora fijiensis*, forecasting strategy, Ivory coast, elimination of mancozeb, phytosanity.

INTRODUCTION

Black Leaf Streak Disease (BLSD) is a foliar disease of bananas caused by the ascomycetous fungi *Pseudocercospora fijiensis*. The disease is responsible for leaf spotting and reduces the photosynthetic area of bananas and by so might induce important yield losses up to 30% if not controlled (Mobambo et al., 1996). However, as long as 5-7 leaves are present at harvest, very few yield damages are expected (Vargas et al., 2009; Lassois et al., 2010), which is generally the case in most commercial banana plantations. Moreover, leaf spot disease of bananas (Yellow Sigatoka and BLSD) severely affect fruit conservation (Castelan et al., 2012; Castelan et al., 2013) and cause premature ripening that might prevent banana exportations or cause important commercial damages. For this reason, Black leaf streak disease is the most important threat to the banana industry (de Lapeyre de Bellaire et al., 2010) and requires important chemical treatments. In most tropical humid countries, chemical control relies on a massive use of mancozeb (Marin et al., 2003; Guzman et al., 2018) that is mostly used in a systematic strategy consisting in weekly applications in order to protect new leaves with this protectant fungicide. However, those strategies are not sustainable for technical, environmental, economic and regulatory reasons (de Lapeyre et a., 2009). Indeed, mancozeb has been banned in the EU in 2021 (Anonymous, 2020). From all fungicides of the dithiocarbamate group (thiram, propineb, maneb, ziram, metiram for which the same MRL should apply because of lack of specificity of detection methods), metiram is the only one authorized in UE having a MRL of 2 ppm. However, the MRL for mancozeb should been revised soon in the next year questioning on further use of this fungicide (and all contact fungicides of the dithiocarbamate group) for BLSD control. In this context it is vital to develop strategies excluding this contact fungicide.

BLSD has been detected for the first time in Ivory Coast in 1985 (Mourichon & Fullerton, 1990) and has replaced Sigatoka Disease in all banana commercial plantations in a about 10 years. At the beginning, only 14-16 applications of systemic fungicides (DMI, BMC and QoI) were used for BLSD control using a forecasting strategy (Fouré & Ganry, 2008). However, by 2008 the emergence of fungicide resistance to many products has led to a more important use of contact fungicides from which mancozeb was the mostly used (de Lapeyre de Bellaire 2008, 2013, 2017, 2018, Comm. Pers.).

The purpose of the work presented here has been to develop a new strategy excluding the use of mancozeb in commercial banana plantations where fungicide resistance was established. Effectively, forecasting strategies rely mainly on the use of systemic fungicides with high curative effect and the adaptation of this strategy in these conditions remains challenging. A forecasting strategy had been adapted in the dry tropical conditions of the Dominican Republic where fungicide resistance was established (Guillermet et al., 2014). However, the situation of larger plantations of Ivory Coast with a more humid climate (1500 mm/year versus 400 mm/year) requires other adaptations.

MATERIALS AND METHODS

Commercial farm selected. In 2020, a banana company of Ivory Coast decided to develop a partnership with Cirad to reduce fungicide use and specifically to find alternatives to mancozeb in order to anticipate upcoming regulatory issues. After several trials at small scale, two farms were selected to compare the conventional strategy based on mancozeb (farm 1) to a new strategy without mancozeb (farm 2). Farm 1 (473 ha) was joined with farm 2 (364 ha). Annual rainfall in both places is 1450 mm.

Evaluation of fungicide resistance. Fungicide resistance was evaluated in the two farms at two periods in 2019 and 2022. In each farm a fixed plot has been selected in the centre of the farm and samples were always collected in this plot. In each plot 30 plants were selected in various places of the plot and 30 pieces of leaves (25 cm x 25 cm) bearing stage 2-3 lesions of BLSD according to Fouré's scale (Fouré, 1988) were sampled and brought to the laboratory in order to isolate a population of 40 strains. Each strain was initiated from single conidia. For each lesion one single isolate has been selected and a maximum of 2 strains per leaf were selected in the final population. The evaluation of fungicide resistance was made from mycelial growth inhibition by spectroscopic optical densities measurements inspired from Chong et al. (2021). The population of 40 strains was grown under PDA for 10 days before growth inhibition measurements. A small colony of each strain was crushed to form a mycelial suspension used for further inoculations of liquid media. Mycelial growth was evaluated after 7 days incubation at 25°C in microplates in a liquid PBD medium amended or not with different concetrations of fungicides. For each fungicide, mycelial growth was evaluated on a broad range of active ingredient concentrations to estimate EC50. Optical densities measurements were made with a Tecan microplate reader at 690 nm.

For DMI fungicides, EC50 were assessed for propiconazole and difenoconazole. The range of concentration used was: 0; 0,004; 0,016; 0,04; 0,16; 0,64; 2,56 and 10,24 mg L−1. For each strain an EC 50 has been assessed and the mean EC50 of the population was compared to mean EC 50 of a fungal population never exposed to fungicides (reference population) in order to calculate a resistance factor of the population RF as Average EC50 plantation/Average EC50 of reference population.

For MBC fungicide we used thiabendazole at 0; 0,1; 1; 10; 100 mg L^{-1}. Susceptible strains never grew over 1 mg L^{-1}. Each strain could be identified as resistant or susceptible and a percentage of resistant strain was calculated for each population analysed. For QoI fungicide we used azoxystrobin at 0,03; 0,1; 0,3; 1; 3; 10; 100 mg L^{-1}. Resistant strains grew up to 10-100 mg L^{-1}. Each strain could be identified as resistant or susceptible and a percentage of resistant strain was calculated for each population analysed.

Disease monitoring, strategy of decisions and fungicide applications. Each week various disease parameters were assessed in order to get all the biological information needed for biological forecasting system developed at Cirad (Fouré and Ganry, 2008). In each farm 10 plants were selected on a plot and monitored every week until flowering. Data were collected every week on 16 plots in farm 1 and 14 plots in farm 2.

The biological information consisted in:

- SED as calculated from all symptoms observed in leaves 2 to 4 and leaf foliar emission as proposed in Fouré and Ganry, 2008.
- YLSt, the youngest leaf bearing streaks has been assessed as proposed in de Lapeyre de Bellaire et al., 2010. YLSt is an estimation of incubation period and is useful to understand epidemic conditions.
- YLS, the youngest leaf spotted has been assessed as proposed in de Lapeyre de Bellaire et al., 2010. YLS is an estimation of total epidemic cycle duration and its evolution is a balance between leaf emission and disease growth which is useful to understand efficiency of control.
- NLH, the number of leaves at harvest was evaluated on 20 banana plants bearing a bunch ready for harvest as proposed in de Lapeyre de Bellaire et al., 2010.
- NLF was evaluated on 20 banana plants just flowering a new flower bunch like for NLH.
- Density score was the sum of all occurrence of large intensities of inoculum: i.e., more than 50 lesions observed on a leaf during SED assessment as scored by a +

notation in Fouré and Ganry, 2008. This score reaches a maximum value of 30 for observations in leaves 2, 3, and 4 on 10 plants.

Each week, decisions were taken according to graphical reporting of all disease parameters. In Farm 1, the conventional strategy program relied on almost weekly applications of mancozeb in alternation with some systemic fungicides mainly used in the rainy season. Decisions were made by the company itself. In farm 2, decisions were discussed between Cirad and the company. Only some systemic fungicides (DMI, QoI, MBS) could be applied according to the results of sensitivity tests. The strategy included also the use of mineral alone (12 l/ha) that has a light curative effect generally inferior to two weeks (Guyot & Cuillé, 1955; Delatouche et al., 2023) and also the use of Sterol biosynthesis Inhibitors of group 2 (morpholines, amines, spiroketalamines). The evolution of SED, YLSt and density score was particularly followed to decide application of systemic fungicides or mineral oil. Applications were always separated at least by two weeks. The evolution of YLS was monitored to evaluate the efficiency of the strategy. All fungicide applications in a farm were realised the same day with an airplane.

Evaluation of the performance of the strategy. Various parameters have been established for the whole duration of the year to compare the performance of both strategies:

- The limitation of disease symptoms in the top of the canopy. This was assessed as the limitation of SED below 1500. For all observations made on the 16 (farm 1) or 14 (farm 2) plots over the 52 weeks of the year we have summed the occurrence of SED values > 1500.
- The limitation of large densities of inoculum. This was assessed from the density score value obtained from SED measurements. For all observations made on the 16 (farm 1) or 14 (farm 2) plots over the 52 weeks of the year we have summed the occurrence of density score values > 10.
- The limitation of bunches with very few leaves. For all observations made on the 16 (farm 1) or 14 (farm 2) plots over the 52 weeks of the year we have summed the occurrence of average NLH <3.
- The limitation of risky bunches. In the farms, a specific tracking of risky bunches was made at field level before harvest. A risky bunch is harvested on plants reaching harvest stage with less than 3 functional leaves. According to company criteria, a functional leaf is a leaf bearing more than 1/3 green area. All risky bunches are counted in packing stations and a percentage ok risky bunches has been calculated on each farm for all the year. This data is very important and

discriminatory because it is assessed over all banana bunches harvested on the farms.

- An Index of Treatment Frequency (ITF) calculated as the sum of all fungicide reference rates used /ha/year. For each fungicide the rate has been compared to a reference rate. The ITF has been calculated for each fungicide application taking into account that in the case of mixture of two active ingredients, the ITF value takes into account both fungicides. The annual value is the sum for all fungicides applied.
- The quantity of active ingredient of fungicides used/ha/year
- The total number of aerial application or cycle.

RESULTS AND DISCUSSION

Fungicide resistance. In 2019, only a moderate shift of sensitivity to DMI fungicides was noticed on both farms with low Resistance Factors < 20 (Table 1). No resistance to QoI fungicides was noticed and only a small proportion of strains resistant to MBC fungicide could be noticed in farm 2. In 2022, an important proportion of strains resistant to QoI fungicides could be noticed on both farms and those fungicides are not used since the end of 2022 in both farms. No resistant strains to MBC were observed in both farms. Those fungicides are not used for many years and it has been proposed to use them again at the end of year 2022 (1 to 2 applications/year) even if possible low level of resistance could have been reported in the past. The sensitivity shift to DMI fungicides was more pronounced in 2022 with RF to difenoconazole > 100. DMI were not used more than twice a year and only mixed with SBI of group 2.

Table 1. Sensitivity to different groups of systemic fungicides assessed in 2022 in farm 1 and farm 2.

Fungicide	Farm 1 2019	Farm 2 2019	Farm 1 2022	Farm 2 2022
% resistance to MBC	0	3%	0	0
% resistance to QoI	0	0	28%	23%
Resistance Factor to Propiconazole	20	13	71	42
Resistance Factor to Difenoconazole	14	9	133	121

Those results limit the use of DMI fungicides which are the most effective for BLSD control. From these results, control should rely on a combination of mineral oil, SBI fungicides of group 2, a strong limitation of DMI fungicides, a possible limited use of MBC.

Performance of the strategies. In terms of chemical use both strategies were strongly different. The use of fungicides was more intensive in the conventional strategy displayed in farm 1 as shown by ITF values that were reduced by 34% in farm 2 (20 in farm 2 versus 30,4 in farm 1, Table 2). These differences are mainly due to the fact that fewer aerial applications were made in farm 2 (25 versus 38 in farm 1, table 3) and also because of more numerous mineral oil applications without fungicide were applied in the strategy without mancozeb (figures 1 and 2). Considering the quantity of active substance/ha/year the difference between both strategies is wider because 76% reduction could be achieved in the strategy without mancozeb (6,7 kg/ha/year in farm 2 versus 27,8 kg/ha/year in farm 1, table 2). This difference is mainly due to the fact that mancozeb treatments require generally about 1kg ai/ha/treatment and weekly applications. Then the elimination of mancozeb significantly reduces chemical load.

Table 2. Comparison of the conventional strategy with mancozeb performed on farm 1 (473 ha) and the strategy excluding mancozeb on farm 2 (364 ha) during all the year 2022. Disease data were collected weekly over 16 plots in farm 1 and 14 plots in farm2.

Evaluation of the strategy	Conventional strategy with mancozeb (farm1)	Strategy excluding mancozeb (farm2)
[a]Occurrences of SED > 1500	61/833	70/728
	7,32 %	9,62%
[b]Occurrences with densities >10	7/833	13/728
	0,84%	1,79%
[c]Occurrences with NLH < 3	121/833	26/728
	14,5%	3,54%
[d]Risky bunches	31435/2172615	2716/1605619
	1,47%	0.17%
[e]ITF	30.4	20
[f]kg a.i./ha/year	27.8	6.7
[g]Number of cycles	38	25

[a]umber of weekly data with SED > 1500; [b] Number of weekly observations with more than 10 observations showing a high density of lesions; [c] Number of weekly observations with an average value of NLH < 3; [d] Number of bunches harvested and tagged as risky over the year 2022. A risky bunch had less than 3 functional leaves (a functional leaf had at least 1/3 of green area); [e] Sum of reference rates of all fungicides used / ha /year; [f] Sum of active ingredients of fungicides used per ha/year; [g] Number of plane applications per year.

The prevalence of high leaf infection was slightly higher in the strategy without mancozeb as shown by the occurrence of SED > 1500 (9,6% in farm 2 versus 7,3% in farm 1, table 3) and by the occurrence of density scores > 10 (1,8% in farm 2 versus 0,8% in farm 1, table 3). However, the performance in terms of number of leaves at harvest and risky bunches was better in the strategy without mancozeb as shown by the occurrence of NLH< 3 (3.5% in farm 2 versus 14,3% in farm 1) and the percentage of risky bunches (0,17% in farm 2 versus 1,47% in farm 1). This result shows that there is not a direct correlation between leaf infection and leaf reduction which is probably the consequence of using more curative products in the strategy without mancozeb (SBI of group 2 and mineral oil).

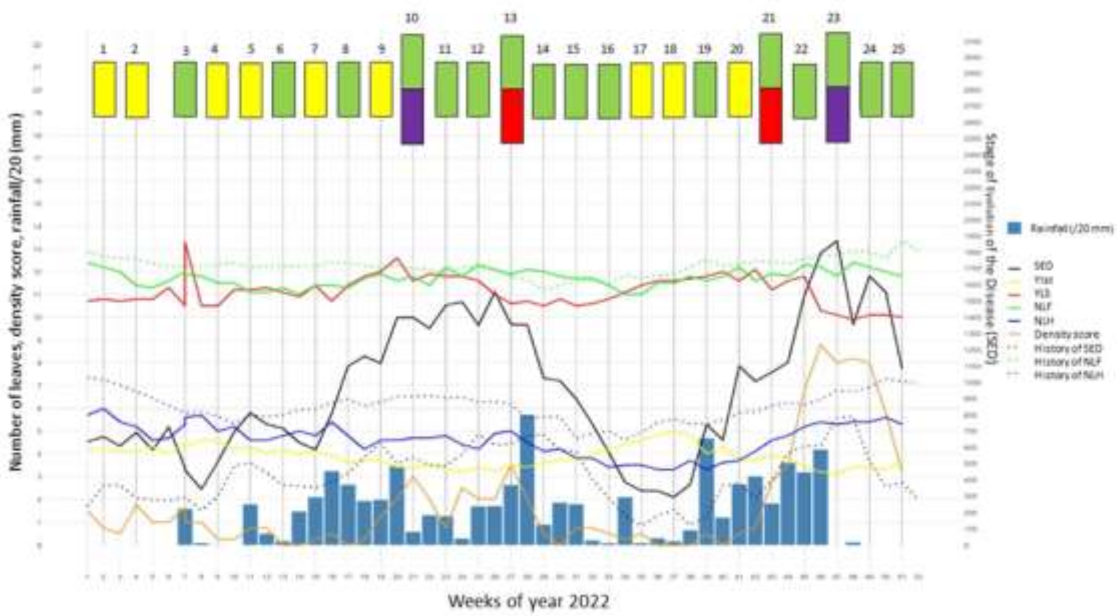

Figure 1. Weekly evolution of disease parameter in the strategy without mancozeb in farm 2. Average values of 14 plots. Treatments are indicated with bars: yellow mineral oil; green: SBI of group 2 (morpholine); red: DMI; Purple: QoI. The number of spray cycle in the year is indicated over the bars.

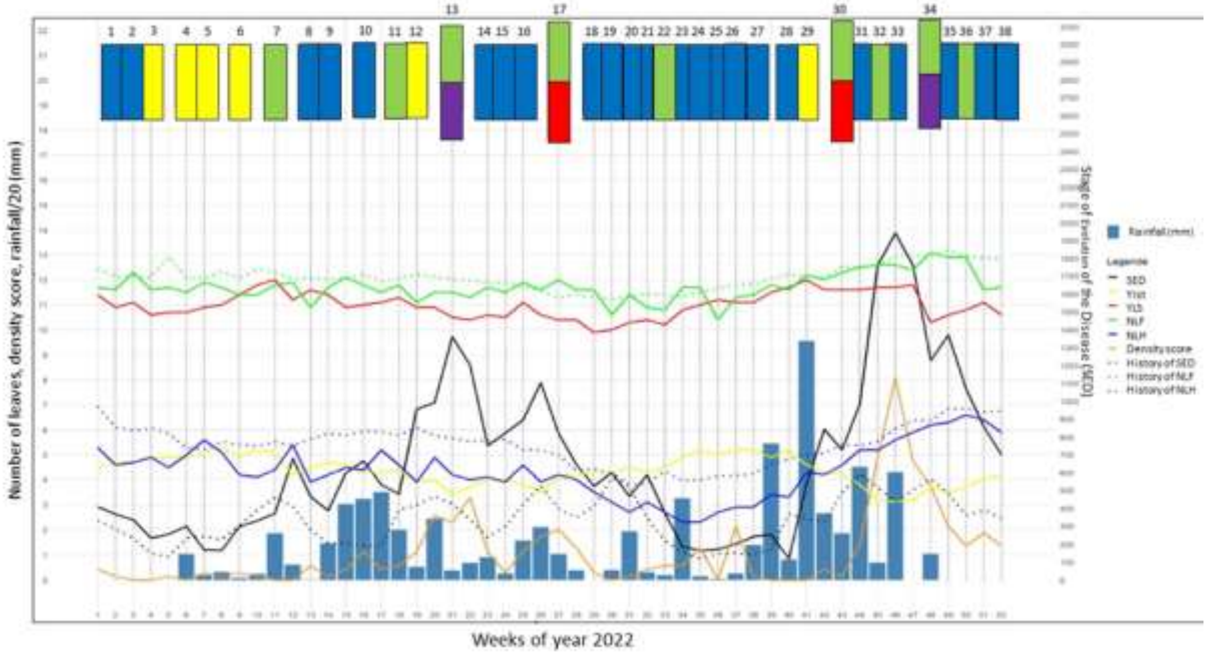

Figure 2. Weekly evolution of disease parameter in the conventional strategy with mancozeb in farm 1. Average values of 16 plots. Treatments are indicated with bars: blue: mancozeb; yellow mineral oil; green: SBI of group 2 (morpholine); red: DMI; Purple: QoI. The number of spray cycle in the year is indicated over the bars.

CONCLUSIONES

The evaluation of alternative strategies excluding mancozeb use has been satisfactory during a whole year season in conditions with established resistance to most systemic fungicides, particularly the most curative ones, the DMI. This strategy relies on weekly monitoring of biological information, decisions made on this biological information, broader use of mineral oil alone and SBI group 2 fungicides. The performance of such strategy is very good in terms of environmental impact since 75% reduction in chemical load has been achieved. This reduction does not alter crop losses because risky bunches as well occurrence of bunches harvested with few leaves were not more important and were even less frequent. From these results such strategy is now carried over more than 3000 ha and in the future the challenge will be to reduce the number of SBI group 2 treatments introducing more mineral oil applications and new groups of fungicides (pyrimidins, dodine, MBC). Indeed, even if the develop of resistant strains to SBI group 2 fungicides is unlikely, this risk should be mitigated.

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