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# REACTIONS OF WILD POPULATIONS OF *MYCOSPHAERELLA FIJIENSIS* TO THREE FAMILIES OF FUNGICIDES

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**ABSTRACT:** In order to know the origin of the resistance in order to develop a control strategy that respects the environment and human health, this study was initiated. More specifically, it should make it possible to determine the reaction of wild strains of *Mycosphaerella fijiensis* to systemic synthetic fungicides in areas of intensive cultivation of dessert bananas for export in Côte d'Ivoire. Samples of leaves from dessert and plantain banana trees affected by Black Leaf Streak Disease at stages 3 and 4 were taken at a distance of at least 10 km from the industrial plantations and then transported to the laboratory for fungicide sensitivity tests on agar medium according to the method proposed by **Van Den Berg Loridat in 1989** and modified by **Kobenan and *al* in 2008**. These tests revealed remarkable efficacy of all fungicides on all wild conidia of *Mycosphaerella fijiensis* regardless of their origin. For the triazoles the inhibition rates ranged from 67.45% to 75% for tebuconazole, 67.34% to 79.41% for epoxyconazole, and 70% to 80.14% for tebuconazole. As for methyl thiophanate, germination rates were between 0 and 11%. For azoxystrobin, germination rates were between 0 and 20 %. However, the same tests carried out in industrial plantations revealed losses in the effectiveness of certain fungicides of the triazole family in certain areas. This loss of efficacy observed in plantations regularly treated with fungicides is due to the repeated use of these fungicides without any real alternation (of the different families of active ingredients), which would have eliminated strains sensitive to these active ingredients and facilitated the proliferation of non-sensitive strains.

**KEYWORDS:** Reactions, Wild Populations, *Mycosphaerella Fijiensis*, Fungicides.

## 1 INTRODUCTION

Banana Black Leaf spot (Black Sigatoka) caused by *Mycosphaerella fijiensis* is ubiquitous in the banana plantations in Côte d'Ivoire **[1] (Koné *and al*., 2009**). It attacks the leaves, causes their deterioration and thus reduces their photosynthetic capacity, which reduces the number of functional leaves, resulting in a loss of yield that can be total **[2], [3] (Carlier *and al*., 2000; Ganry, 2010**). In the absence of resistant varieties, intensive cultivation of dessert bananas for export is only possible through strict chemical control. The introduction of synthetic systemic chemical fungicides of the benzimidazole family (anti-mitotics) in banana plantations dates back to the 1970s, following the oil boom, which led to an increase in weekly applications of contact chemical fungicides. Thus, treatments that had previously been curative became both preventive and curative, as the fungicide was able to protect the newly released leaves. With a reduction in the rate of applications, and thus a reduction in the budget for controlling this disease, the banana industry was able to maintain a relative competitiveness. Soon, repeated applications of this family of fungicides in banana plantations became less and less effective, due to the emergence of strains of the fungus resistant to this range of products. In the early 1980s, a new generation of fungicides (sterol biosynthesis inhibitors) was tested in plantations. The most well-known of these, propiconazole, proved more effective than antimitotics such as methyl thiophanate **[4], [5] (Fouré, 1983; Fouré, 1984a)**. Like the antimitotics, triazoles were quick to generate races of the fungus resistant to this range of fungicides. Indeed, the appearance of resistance zones has been observed in banana plantations in south-eastern Côte d'Ivoire for more than a decade. The reduction of susceptibility to propiconazole has been reported by some authors **[6] (De Lapeyre de Bellaire L., 1990**). Losses of propiconazole sensitivity in Banacomoé had already been reported in 2005 **[7] (N'guessan, 2008**), with inhibition rates of 46% at 0.1 µL/L and a CI 50 of 0.107 µL/L [8]. **(Essis *and al* (2010)** revealed cases of resistance in *Mycosphaerella fijiensis* populations in the Aboisso production area. The percentage of inhibition was 43%. As a result, new molecules belonging to the Strobilurins and Spiroketalamines have been approved for use alone or in alternation with existing molecules **[1] (Koné *and al*., 2009**).

The main reason for resistance results from poor implementation of recommendations for the use of synthetic chemical fungicides **[8]** (**Essis, 2010**). The use of synthetic fungicides also leads to higher production costs and contamination of bananas and the environment **[9], [10], [11]** (**Rodríguez & Jiménez 1985, Fullerton & Olsen, 1991; Mouliom, 1999**). In fact, we are witnessing an increase in the number of weekly fungicide treatments (40 to 50 treatments per year) and this accentuates the contamination of bananas and the environment. Banana Black Leaf Streak Disease monitoring analysis has enabled us to confirm that there is a loss of efficacy, especially with triazoles in Ivorian banana plantations **[12]** (**Kobenan *and al*., 2016**). This loss of fungicide efficacy can be attributed to several factors, including under-dosing of the fungicide itself, misuse by the phytosanitary teams on the plantations, the existence of resistant strains that have proliferated, or a combination of two or more of these factors.

Several decades after the introduction of benzimidazoles, followed years later by the introduction of triazoles, what about the "wild populations" of the fungus in areas producing bananas for export? The answer to this concern would arise from a series of studies in which this paper is part, which aims to investigate the sensitivity to systemic fungicides of the fungus *Mycosphaerella fijiensis* in its wild state (never treated with fungicides) in areas producing dessert bananas for export, in order to develop a control strategy that is respectful of the environment and human health.

## 2 Materials And Methods

### 2.1 Study Area

The sampling zone extends, on the one hand, between the 5th and 6th degrees of North latitude and, on the other hand, between the 3rd and 5th degrees of West longitude **[13]** (**Lassoudière, 1978**). This zone groups together the main industrial plantations of dessert banana trees (Figure 1). It is subdivided into administrative regions whose climatic characteristics are defined as follows; according to the site https://planificateur.a-contresens.net/afrique/cote_d_ivoire/:

- The South-Comoé region (Aboisso, Bassam and Bonoua) has forest vegetation, a savannah climate with a long dry season accompanied by hot, dry winds, an average temperature of 26.5°C and an average annual rainfall of 1266 mm;
- The Lagoon region (Anguédou) has a savannah climate with a long period of Harmattan. Over the year, the average temperature is 26.7°C and the average rainfall is 1466.4 mm;
- The region of Agneby-Tiassa situated at the savannah-forest border (Tiassalé, N'douci, Agboville and Azaguié) also has a savannah climate with a long dry season accompanied by hot, dry winds. Over the year, the average temperature is 26.7°C and rainfall averages 1466.4 mm.

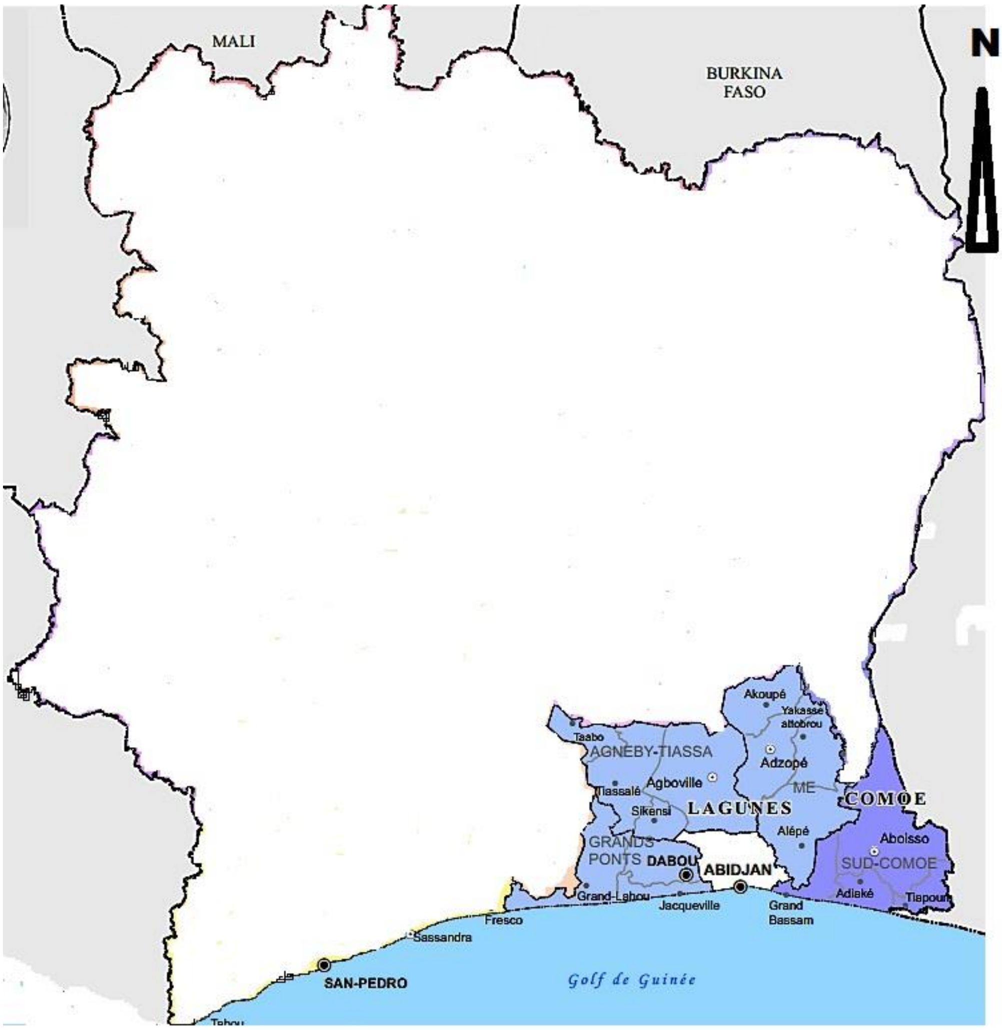


*Fig. 1. Map showing the study area*

### 2.2 Plant Material

The plant material used for this study consisted of banana leaves and plantain from village plantations in export banana production areas and trials established in Anguédou, showing symptoms of Black Leaf Streak disease at stages 2 to 4 **[14]** (**Fouré, 1984 b**).

### 2.3 Fungal Material

The fungal material consists of conidia of *Mycosphaerella fijiensis* from the leaves of banana and plantain trees bearing the symptoms of Black Leaf Streak disease from the localities of Aboisso, Bonoua, Agboville, Azaguié, Tiassalé, N'douci and Anguédou.

### 2.4 Fungicides Used

The fungicides used for conidial susceptibility testing are unisite (systemic) and belong to three families of fungicides used in the banana plantations of Côte d'Ivoire (Table 1). These fungicides were supplied by the phytosanitary teams of the plantations.

***Table 1. Different fungicides used***

| Family | Trade name | Active ingredient | Formulation | Dose |
|---|---|---|---|---|
| Triazoles | | **Sterol Biosynthesis Inhibitor, DM** | | |
| | Folicur 250 EW/Junior | Tébuconazole | 250 g/l | 0,4 l/ha |
| | Opus 075 EC | Epoxiconazole | 75 g/l | 1 l/ha |
| | Sico 250 EC | Difenoconazole | 250 g/l | 0,3-0,4 l/ha |
| Benzimidazoles | Peltis/Calis | **Antimitotic, MBC**<br>Methyl-thiophanate | 400 g/l | 320 g/ha |
| Strobilurins | Bankit | **Breathing inhibitor, IQ**<br>Azoxystrobin | 400 g/l | 0,4 l/ha |
| | Téga 075 EC | Trifloxystrobin | 75 g/l | 75g/ha |

### 2.5 METHODS

#### LEAF REMOVAL

The essential material for our study is conidia of the fungus *Mycosphaerella fijiensis*. To obtain it, samples of bananas leaves (Banana and plantains) are taken from village plantations or in trials. These leaves must show symptoms of stages 3 or 4 of Black Leaf Streak disease. They are taken at a distance of at least 10 km from the industrial plantations and then packed in plastic packaging with labels, which constitutes a sample. The samples obtained are transported to the laboratory for analysis.

### 2.6 METHOD OF ANALYSIS IN THE LABORATORY

The analytical method used to test the sensitivity of *Mycosphaerella fijiensis* to these three families of fungicides is a modification of the one described by **[15] Van Den Berg (1989)** and modified by **[16] Kobenan *and al* (2008).** It involves the following steps:

#### 2.6.1 PREPARATION OF STOCK SOLUTIONS

Stock solutions are obtained by dissolving raw fungicides in sterile distilled water. Dissolve 0.1 ml methyl thiophanate (benzimidazoles), 0.16 ml difenoconazole, epoxiconazole, and tebuconazole (triazoles) and azoxystrobin (strobilurins) in 40 ml sterile distilled water to give a concentration of 1000 µL/L for each fungicide. The others (100, 10, 5 µL/L....etc.) are obtained by successive dilutions. All these solutions are kept in the refrigerator at 4° C.

#### 2.6.2 PREPARATION OF THE CULTURE MEDIUM

The agar media with 2% agar (i.e. 20 g of agar for 1 L of distilled water) are prepared in Erlenmeyers and are autoclaved at 121°c for 20 minutes at a pressure of 1 bar. After autoclaving, depending on the desired concentration, dilutions are made. The mixture is then well homogenised with the help of a stirrer and poured into Petri dishes at a rate of 10 to 15 ml per dish. The doses studied are 5 µL/L of methyl thiophanate (Benzimidazoles) and 0.1 µL/L of difenoconazole, epoxyconazole and tebuconazole (Triazoles) followed by 1 µL/L of azoxystrobin and 0.1 µL/L of trifloxystrobin (Strobilurins). The control is a 2% fungicide-free agar medium (0 µL/L).

#### 2.6.3 CONIDIAL INOCULATION AND INCUBATION

Fragments of diseased leaves that have produced many conidia are cut into fragments of 1 to 2 $cm^2$ using a pair of scissors. The underside of the fragments is applied to the surface of the freshly prepared agar medium (16 leaf fragments on average per Petri dish) and amended with fungicide.

Incubation is done in Petri dishes for 48 hours at room temperature to allow the fungus to grow.

#### 2.6.4 MICROSCOPIC READING

The reading is made with an optical microscope (Leitz Laborlux k) 48 hours after the conidia have been in contact with the medium containing or not containing the fungicide. It consists of:

- For benzimidazoles, to observe the normal germination of the conidia (without any deformation) or abnormal (with deformation of the germ tubes) and to consider also the non-germinated conidia. The counting is done with the help of a mechanical counter (ferrari-statitest, typ: 8 nr. 84110281) and an optical microscope with magnification g x 10. A total of 50 conidia are observed for each concentration (5 µL/L). After counting the individual conidia, the normal germination rate is determined by dividing the number of germinated conidia by the total number of observed conidia. The laboratory threshold for reporting resistance is 20% normal germination at the 5 µL/L concentration.

$$TGN = \frac{N_{GN}}{N_{TO}} \times 100; \quad TD = \frac{N_D}{N_{TO}} \times 100; \quad TNG = \frac{N_{NG}}{N_{TO}} \times 100$$

*TGN= Normal germination rate; TD= Rate of deformed conidia; TNG = Rate of ungerminated conidia; NTO = total number of conidia observed; NGN = number of conidia with normal germination; NNG = number of ungerminated conidia and ND = number of deformed conidia.*

- For triazoles and strobilurins, the length of the reduced and normal (fungicide-free) germ tubes of the conidia was measured using an optical microscope at g x 40 magnification with a micrometer-object graduated in 0.01 mm increments, which was placed on the stage of the device. For each concentration, an average germ tube length of 50 conidia is determined. The rates of growth reduction compared to the control on a fungicide-free medium are thus determined. The growth rates were calculated according to the following formula:

$$TI = \frac{LMo - LM_T}{LMo} \times 100, \quad \text{et } TC = 100 - TI = \frac{LM_T}{LMo} \times 100$$

*TI=inhibition rate; LMO= average germ tube length (controls); LMT= average germ tube length of the different treatments, TC= growth rate.*

The laboratory threshold for reporting resistance is set, for Triazoles at 35% growth rate at 0.1 ppm and for Strobilurins at 75% of the control, according to the FRAC recommendations **[17]** (**Knight *and al.*, 2002**).

### 2.7 METHODS OF STATISTICAL ANALYSIS

Conidial germ tube lengths (without fungicide), inhibition rates at 0.1 ppm triazoles and germination rates with benzimidazoles and strobilurins were treated by analysis of variance (ANOVA) at 5% significance using the statistical software STATISTICA 7.0. The NEWMAN KEULS test was used to separate the means. EXCEL software was used to plot the graphs.

## 3 RESULTS

### 3.1 GROWTH OF CONIDIAL GERM TUBES ON FUNGICIDE-FREE AGAR MEDIUM

The growth of conidial germ tubes from plantations never treated with fungicides is not uniform (Figure 2). Some plantations show similarities. The length of conidial germ tubes varies from 81 µm to 102 µm depending on the locality. Thus, three groups of germ tube lengths were statistically distinguished.

The germ tubes of conidia taken from the leaves of dessert banana trees belonged to all three groups, but conidia taken from the leaves of plantain trees had germ tubes of average size intermediate between slow and fast growing tubes.

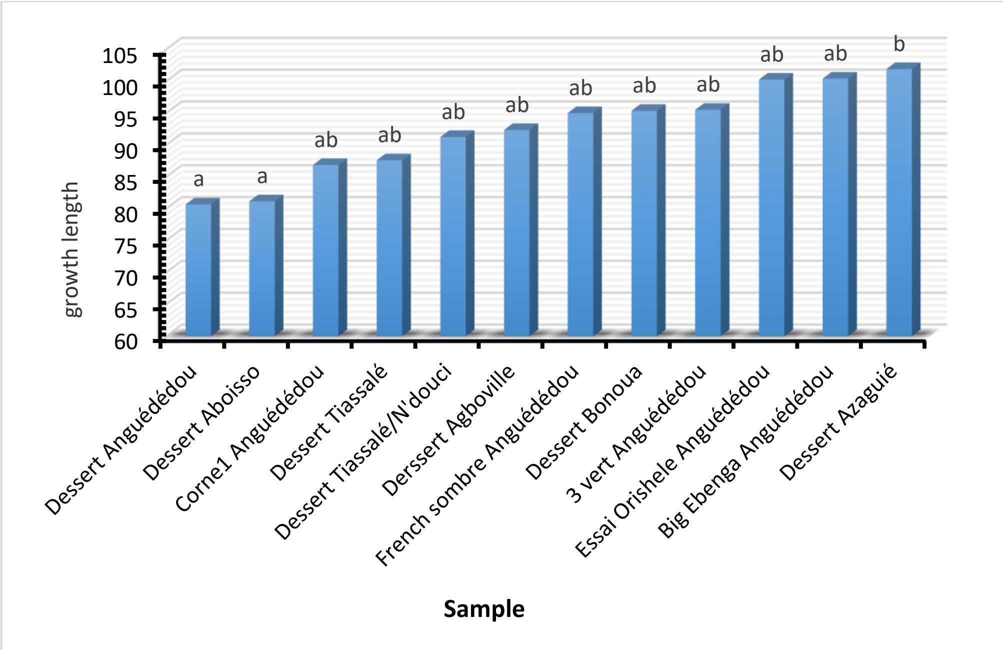


***Fig. 2. Average growth of conidial germ tubes of wild populations of Mycosphaerella fijiensis on agar medium without fungicide from export banana production areas in Côte d'Ivoire***

### 3.2 Sensitivity Of Mycosphaerella Fijiensis Conidia To Fungicides

#### 3.2.1 Sensitivity To Triazoles

In triazoles, there was no significant variation in the rates of conidial germ tube growth inhibition between the different active ingredients.

- **Sensitivity of conidia of *Mycosphaerella fijiensis* to difenoconazole**

Germination inhibition rates of *Mycosphaerella fijiensis* conidia treated with the active ingredient Difenoconazole (Figure 3) vary from 70% to 80%.

However, three groups were statistically distinguished:

- The group with inhibition rates close to the resistance threshold comprising the samples of the Anguédou dessert (70%), 3 green Anguédou (72%), Azaguié dessert (73%) and that of the Tiassalé/N'douci axis (73%).
- The group of intermediate inhibition rates between groups "a" and "b" includes the samples of Anguédou horn (74%), Agboville dessert, French Sombre and Orishele Anguedededou (75%) then those of Big Egenga Anguédou (75%), Aboisso and Bonoua dessert (76%). In this group we have inhibition rates far from the resistance threshold.
- And the group of high inhibition rates with the sample of Tiassalé dessert (80%) with the highest inhibition.

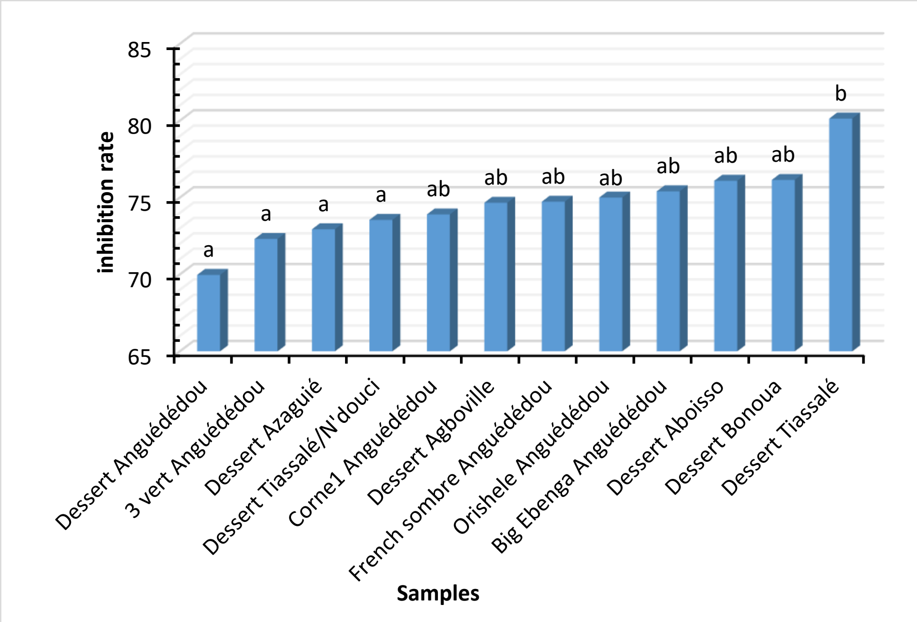


***Fig. 3. Figure 3: Germination inhibition rate of wild conidia of Mycosphaerella fijiensis on agar medium amended with Difenoconazole in export banana production zones in Côte d'Ivoire***

- **Sensitivity of *Mycosphaerella fijiensis* conidia to epoxiconazole**

For this epoxiconazole active ingredient (Figure 4) the range of values is approximately the same as for Difenoconazole, they are all above 65% and vary from 67% to 79% depending on the samples. On the other hand, at the statistical level, five groups can be distinguished:

- The "a" group composed of conidia from the Anguédou dessert (67 %) and "ab" group composed of conidia from the Tiassalé dessert (68 %) and Azaguié (70 %) with inhibition rates slightly higher than the resistance threshold (65 %) with average inhibition.
- The "abc" group intermediate between average inhibition and strong inhibition comprising the samples French Sombre, Corne and Orishele (72 %), 3 vert and Aboisso (73 %),
- Finally, the group of strong inhibitions which includes the Big Ebenda sample from Anguédou (76%) and Agboville (79%) with the highest inhibition rate for this active ingredient.

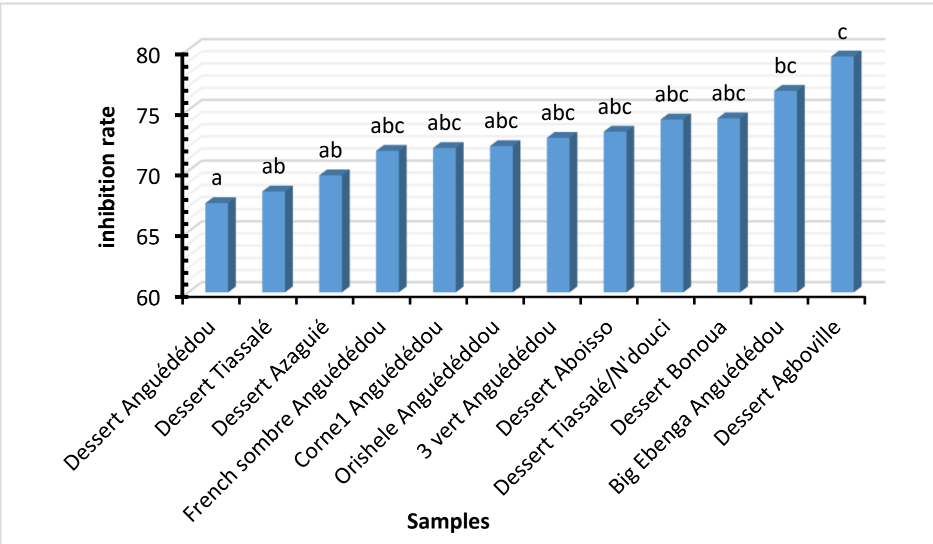


***Fig. 4. Germination inhibition rate of wild Mycosphaerella fijiensis conidia on epoxiconazole-amended agar medium from export banana production areas in Côte d'Ivoire***

- **Tebuconazole sensitivity of *Mycosphaerella fijiensis* conidia to tebuconazole**

For the active ingredient tebuconazole (Figure 5) the germ tube growth inhibition rates are slightly below those of the other active ingredients of the same family. However, they remain above the resistance threshold (65%). These rates range from 67% in the Anguédou horn test to 75% in the Big Ebenga test. For this fungicide the inhibition rates represent a homogeneous group within which there is no significant difference.

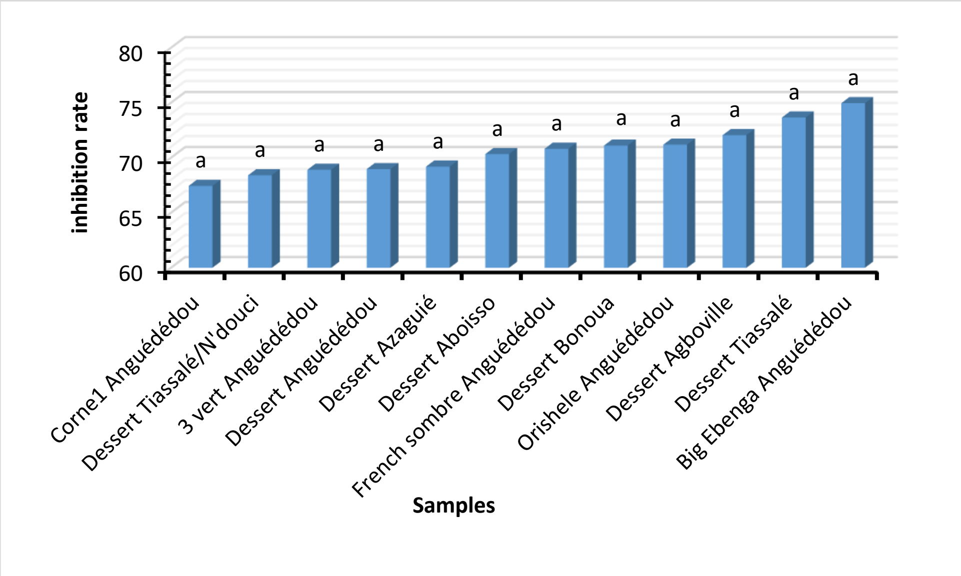


***Fig. 5. Germination inhibition rate of wild Mycosphaerella fijiensis conidia on agar agar amended with tebuconazole in export banana production areas in Côte d'Ivoire***

### 3.2.2 Partial Conclusion

The growth inhibition rates of the germ tubes varied according to the active ingredients and the production areas. All conidia, regardless of their origin, were sensitive to triazoles, but to different degrees. Conidial germ tube growth inhibition rates were higher for difenoconazole and epoxiconazole than for tebuconazole. Also the conidia from the leaves of Anguédou dessert banana trees behaved in a special way; in addition to having the lowest germ tube growth rate on agar medium without fungicide, they had the lowest inhibition rates for difenoconazole and epoxiconazole and even for tebuconazole the inhibition rate was among the lowest even if statistically no difference appeared between these rates.

### 3.2.3 Sensitivity Of Mycosphaerella Fijiensis Conidia To Benzimidazoles

Treatment of conidia with methyl thiophanate at 5 µL/L gave the following results (Figure 6):

- Germination rates are generally nil except for Big Ebenga d'Anguédou 1%, Orishele 2% and French Sombre d'Anguédou 4%, followed by the Tiassalé zone with 11%.
- Non-germination rates vary considerably; from 2% for the Anguédou dessert to 93% for the Bonoua sample.
- Deformation rates range from 7% for the Bonoua area to 97% for the Anguédou dessert test.

Normal germination rates are below the threshold (20%) of resistance in all zones.

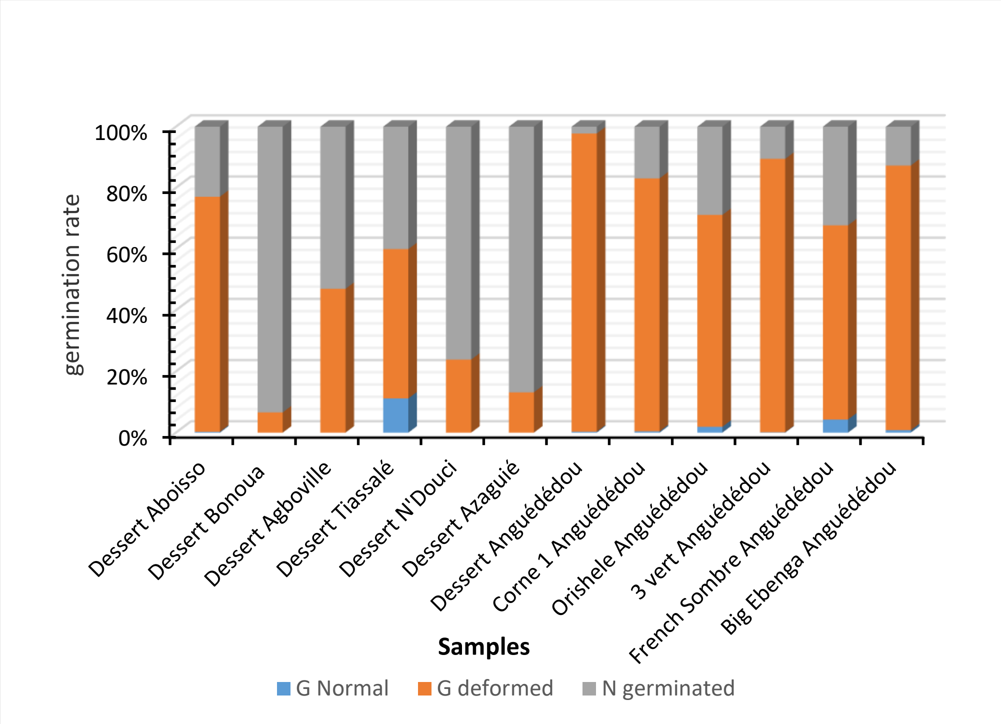


***Fig. 6. Germination rate of wild conidia of Mycosphaerella fijiensis on agar agar amended with methyl thiophanate from export banana production areas in Côte d'Ivoire***

### 3.2.4 Sensitivity Of Mycosphaerella Fijiensis Conidia To Strobilurins

Conidia treated with 1 µL/L of Azoxystrobin gave the following results (Figure 7):

- Normal germination rates were zero for conidia from the Agboville, N'douci, Azaguié and Anguédou dessert trials, whereas they were 2% for conidia from Bonoua, 5 % for Big Ebenga, 6 % for Orishele and the Aboisso sample, 7 % for the Tiassalé sample, 13 % for horn and 20 % for conidia taken from the French Sombre d'Anguédou test.
- Conidial deformation rates are nil for 7 samples (Agboville, N'douci and the dessert, horn, Orishele, 3 green and French Sombre d'Anguédou tests), for the rest we have 2% for Tiassalé, 4% for Aboisso, 5% for Bonoua, 7% for Azaguié and 43% for the Big Ebenga d'Anguédou test.
- Concerning the non-germination rates, they are the highest and range from 50 % for the Big Ebenga test to 100 % for the Agboville, N'douci and Anguédou dessert samples.

However, normal germination rates are below the threshold of strobilurin resistance.

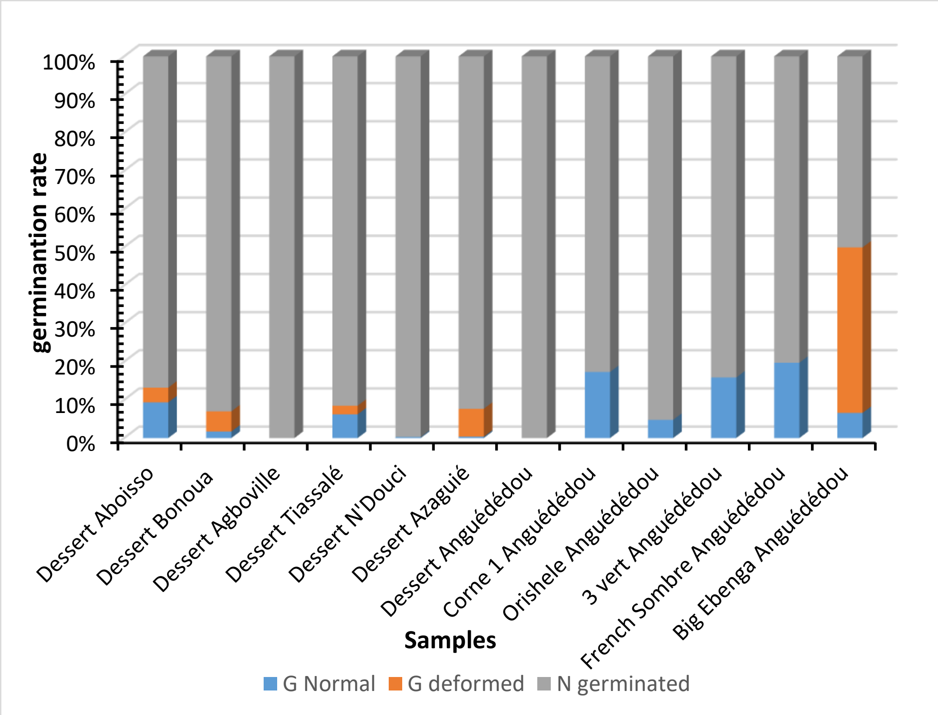


***Fig. 7. Germination rate of wild conidia of Mycosphaerella fijiensis on agar agar amended with Azoxystrobin from export banana production areas in Côte d'Ivoire***

### 3.3 Comparison Of Sensitivities Of Wild (Untreated) And Non-Wild Conidia In Regularly Treated Plantations

#### 3.3.1 Average Comparative Germ Tube Growth Of Wild Conidia And Regularly Treated Plantations

The average growth of conidial germ tubes is the same in treated and untreated village plantations in zones such as Aboisso and Tiassalé, (Aboisso zone 81 µm and Tiassalé-N'douci zone 87 µm for the TIABAM sector and 91 µm for the SIAPA sector). But for the other zones that host EGLIN plantations, such as Bassam (treated 88 µm and untreated 95 µm), Agboville (treated 81 µm and untreated 92 µm) and Azaguié (treated 75 µm and untreated 102 µm), the conidia of treated plantations have a slower average growth of germ tubes than those of untreated village plantations (Figure 8).

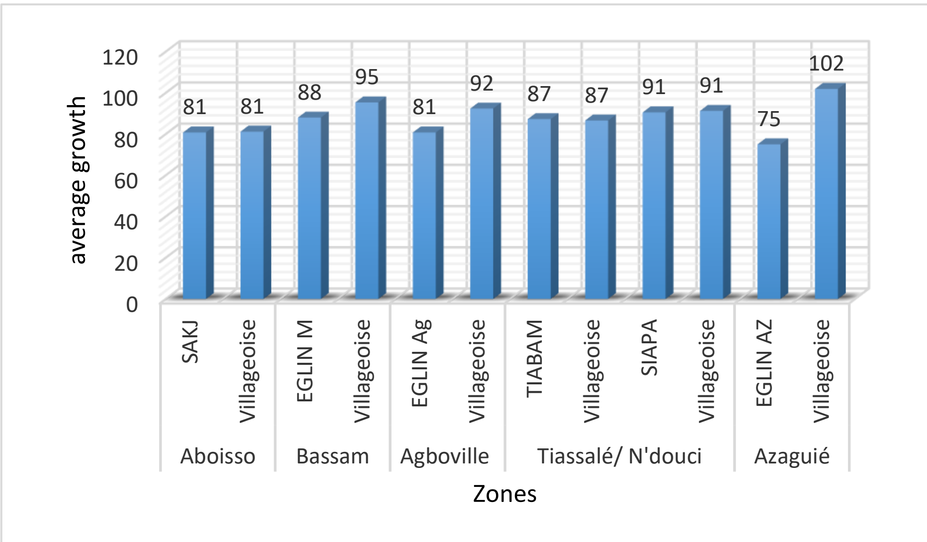


***Fig. 8. Average germ tube growth of wild populations of Mycosphaerella fijiensis conidia and non-wild populations of Mycosphaerella fijiensis conidia in export banana production areas in Côte d'Ivoire***

### 3.3.2 Reaction To Triazoles Of Conidia Collected From Village Plantations Without Fungicide Applications And From Industrial Plantations Regularly Treated With Fungicides.

The comparison of reactions at the level of triazoles shows that:

- For difenoconazole, (Table. 3) the growth inhibition rates of the germ tubes of wild conidia are well above the resistance threshold (from village or untreated plantations), whereas treated conidia (from industrial or treated plantations) are very close to or equal to the threshold, except for those from EGLIN Azaguié (74%) and Motobé (70%), which are higher. As for the sample of EGLIN Agboville it has an inhibition rate (60%) lower than the threshold which is 65%. The biggest differences were observed in the zones of Aboisso (11 %), Agboville (15 %) and Tiassalé (12 % and 9 %).
- For epoxiconazole the germ tube inhibition rates of wild conidia are well above the resistance threshold (Table. 2). However, for treated plantations such as EGLIN Motobé and SIAPA, they are equal to it, while other treated plantations have slightly higher rates, none of which are below the threshold. The differences are significant for Bassam (10%), Agboville (14%) and Tiassalé SIAPA (10%).
- For tebuconazole (Table 2), the germ tube inhibition rates of wild conidia are above the resistance threshold, whereas those of conidia in industrial plantations are all below the threshold. For this active ingredient, the differences were very significant in Aboisso (17%), Bassam (21%), Agboville (15%) and Tiassalé TIABAM (12%).

***Table 2. Comparison of germination inhibition rates of conidia taken from village plantations without fungicide applications and industrial plantations regularly treated with fungicides***

| Areas | Plantations | Difénoconazole | gap | Époxiconazole | gap | Tébuconazole | gap |
|---|---|---|---|---|---|---|---|
| Aboisso | SAKJ | 65 | 11 | 68 | 6 | 54 | 17 |
| | Wild | 76 | | 74 | | 71 | |
| Bassam | EGLIN M | 70 | 7 | 65 | 10 | 51 | 21 |
| | Wild | 77 | | 75 | | 72 | |
| Agboville | EGLIN Ag | 60 | 15 | 66 | 14 | 58 | 15 |
| | Wild | 75 | | 80 | | 73 | |
| Tiassalé/ N'douci | TIABAM | 68 | 12 | 69 | 2 | 63 | 12 |
| | Wild | 80 | | 71 | | 75 | |
| | SIAPA | 65 | 9 | 65 | 10 | 63 | 6 |
| | Wild | 74 | | 75 | | 69 | |
| Azaguié | EGLIN AZ | 74 | 1 | 68 | 2 | 62 | 7 |
| | Wild | 73 | | 70 | | 69 | |

### 3.3.3 Reaction To Strobilurins And Benzimidazoles In Conidia Collected From Village Plantations Without Fungicide Applications And Industrial Plantations Regularly Treated With Fungicides

In all areas the normal germination rates of wild conidia at 1 µL/L of Azoxystrobin are very low (they do not exceed 10%). As for non-wild conidia, in the plantations of ELIN Azaguié (6%), SIAPA (5%) and THIABAM (4%), normal germination rates are also very low, but they are higher in the plantations of SAKJ (12%), EGLIN Agboville (17%) and EGLIN Motobé (38%). However, all normal germination rates are below the threshold of strobilurin resistance (Table 3).

***Table 3. Comparison of azoxystrobin germination rates of conidia collected from village plantations without fungicide application and from industrial plantations regularly treated with fungicide***

| | | **Azoxystrobin 1 µL/L** | | |
|---|---|---|---|---|
| Areas | Plantations | G Normal (%) | G Deformed (%) | N Sprouted (%) |
| Aboisso | SAKJ | 12 | 13 | 75 |
| | Wild | 9 | 4 | 87 |
| Bassam | EGLIN M | 38 | 14 | 47 |
| | Wild | 2 | 5 | 93 |
| Agboville | EGLIN Ag | 17 | 2 | 82 |
| | Wild | 0 | 0 | 100 |
| Tiassalé/ N'douci | TIABAM | 4 | 6 | 90 |
| | Wild | 6 | 2 | 92 |
| | SIAPA | 5 | 9 | 85 |
| | Wild | 0 | 0 | 100 |
| Azaguié | EGLIN AZ | 6 | 12 | 82 |
| | Wild | 0 | 7 | 92 |

For 5 µL/L of methyl thiophanate, the normal germination rates of wild conidia are zero, except for the Tiassalé area in the THIABAM sector, which is 11 %. For conidia from treated plantations these rates are low and are between 0% and 25% for the sample from the EGLIN Motobé plantation, which is higher than the resistance threshold (20%) (Table 4).

***Table 4. Comparison of methyl thiophanate germination rates of conidia collected from village plantations without fungicide application and industrial plantations regularly treated with fungicides***

| | | **5 PPM** | | |
|---|---|---|---|---|
| Zone | Plantations | G Normal (%) | G deformed (%) | N germinated (%) |
| Aboisso | SAKJ | 1 | 43 | 57 |
| | Wild | 0 | 77 | 23 |
| Bassam | EGLIN M | 25 | 39 | 36 |
| | Wild | 0 | 7 | 93 |
| Agboville | EGLIN Ag | 5 | 75 | 20 |
| | Wild | 0 | 47 | 53 |
| Tiassalé/ N'douci | TIABAM | 5 | 57 | 38 |
| | Wild | 11 | 49 | 40 |
| | SIAPA | 0 | 92 | 8 |
| | Wild | 0 | 24 | 76 |
| Azaguié | EGLIN AZ | 0 | 76 | 24 |
| | Wild | 0 | 13 | 87 |

## 4 DISCUSSION

Conidia from wild strains of *M. fijiensis* grown on fungicide-free agar medium; had varying lengths of germ tube growth. This variation could be associated with variability within the species, but also with the variety of banana tree attacked and the environment where the samples were taken. According to **[18] Etebu *and al* (2005),** growing areas with high humidity and low light levels are favourable for rapid conidial growth in *M. fijiensis*. Moreover, the comparison between wild (untreated) and treated populations shows that some local populations (from the same area) may be identical, as can be seen in the Aboisso or Tiassalé-N'douci areas where wild and non-wild conidia showed identical average growth lengths. These differences could also be explained by the proximity or otherwise of the wild sample collection areas to the treated plantations. With regard to the reaction to triazoles, conidia from wild populations have shown a good level of sensitivity to all the active ingredients of this family. This could be explained by the fact that these populations have never been exposed to these active ingredients. The rates of germ tube growth inhibition varied according to the active ingredients and the production areas. According to **[1]**

**Koné *and al* (2009),** the rates of reduction of mycelial growth of fungal parasites in banana were variable depending on the active ingredients and the species considered. Also, variability in sensitivity to triazoles was observed in different industrial plantations in Cameroon **[19]** (**Onautshu, 2013**). The active ingredient difenoconazole was found to be more effective than the other two triazoles; similar results were obtained by **[20] N'guessan *and al* (2016).** On the other hand, the active ingredient tebuconazole was less effective. Studies have shown that differences in sensitivity of *M. fijiensis* strains could be explained by differences in the intrinsic activities of the active ingredients **[21]** (**Gisi *and al.,* 2000**). Comparison of the response of 'wild' conidia and conidia from regularly treated plantations showed that inhibition rates are higher in 'wild' conidia; except for the active ingredient difenoconazole from the Azaguié area. The recurrent use of fungicides without alternating the active ingredients could reduce the sensitivity of conidia to its fungicides. The work of **[20] N'guessan *and al* (2016),** showed that the variability of the sensitivity of the *M fijiensis* strains tested to the different active ingredients seems to be related to the number of treatments carried out with these ingredients in their original plantations. Thus the selection pressure exerted by fungicide treatments could lead to a gradual decrease in the sensitivity of populations **[22], [23]** (**Wolfe, 1982; Parisi *and al.,* 1994**). The Benzimidazoles and Strobilurins tests included a fungicide-free control with zero conidial deformation rates, with germination rates ranging from 44% for the Bonoua zone to 99% for the Horn and Big Ebenga trials of Anguédou. The non-germination rates ranged from 1% for the Anguédou Horn test to 56% for the Bonoua zone. The absence of deformation is explained by the fact that the conidia were not in contact with fungicides, which are the cause of the deformation. The low germination rate of methyl thiophanate in wild conidia could be explained by the mode of action of this active ingredient, which leads to deformation or lack of conidial germination. However, there are a few rare germinations that are linked to the existence of populations that are not sensitive to this active ingredient, which could possibly proliferate if this active ingredient is used regularly in these areas without alternating with other fungicide families. Comparison of the reactions of wild conidia and conidia from areas regularly subjected to fungicide treatments showed very low normal germination rates on both sides. Fungicides of the Benzimidazole family have a very remarkable *in vitro* efficacy on the germination of *Mycosphaerella fijiensis conidia in* all collection areas. The sensitivity of this fungus to this fungicide (methyl-thiophanate) is due to the fact that this family (Benzimidazoles) is rarely used in the majority of plantations studied **[24]** (**Joseph *and al.*, 2020**). However, at the level of industrial plantations, precisely in Eglin Agboville, the normal germination rate is higher than the resistance threshold for benzimidazoles. This active ingredient would have lost its efficacy, which would be linked to a constant use of this active ingredient in the said plantation. For azoxystrobin, normal germination rates of wild conidia vary between 0% and 20%, so wild conidia are sensitive to this active ingredient. However, rates of 16 % for conidia taken from the leaves of 3 Green, 17 % for horn conidia and 20 % for conidia taken from French Sombre d'Anguédou are observed, which would be due to the presence of resistant strains in these wild conidia populations. In all areas normal germination rates for wild and non-wild conidia are low except for some samples where germination rates do not exceed the resistance threshold. Indeed, like azoxystrobin, trifloxystrobin (two strobilurins), a respiration inhibitor has been shown to be effective on radial growth compared to triazoles **[1]** (**Koné *and al.,* 2009**). However, special attention is needed as losses in efficacy could occur if the use of these active ingredients is not monitored in these areas.

## 5 Conclusion

At the end of this work, which consisted of studying in the laboratory the reactions of wild conidial populations to fungicides commonly used in industrial plantations, all the active ingredients were effective on all conidia regardless of their area of origin. Since the different fungicidal active ingredients act on different metabolisms of the fungus *Mycosphaerella fijiensis*, it is possible that some fungicides may not have an effect on certain strains of the fungus that have mutated, which is why the efficacy of these fungicides is not 100%. This would be explained by the existence of strains of conidia that are resistant to certain fungicides due to mutations. But these strains would exist in very small numbers because they compete with the other strains. The second phase, which compared the reactions of wild conidia with those of conidia in treated plantations, shows that the repeated action of fungicides is the cause of the proliferation of resistant strains, which explains the loss of efficacy of certain fungicidal active ingredients in industrial plantations. In view of all the above, it is necessary, in order to avoid the loss of fungicide sensitivities, that the phytosanitary teams of the industrial plantations strictly comply with the fungicide alternation prescriptions.

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