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# Distribution of *Fusarium s*pp. agents responsible for Fusarium Wilt of banana in Southern Benin

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## ABSTRACT
Fusarium wilt of banana caused by *Fusarium oxysporum* f. sp. *cubense* is one of the most important fungal diseases in banana growing areas. It constitutes a major problem for the intensification of banana production in Republic of Benin. This study analyzed the phenotypic and pathogenic diversity of Fusarium strains with a view to sustainable control. A survey was carried out in 72 fields located in eight (8) communities (Missrété, Sakété, Adja-Ouèrè, Avrankou, Adjara, Ifangni, Tori and Allada) to assess the prevalence of banana Fusarium wilt. The communities of Avrankou showed a low percentage (12.59%) of disease incidence while the highest (61.11%) was recorded in the commune of Missrété. During survey, 11 banana varieties were identified, such as, "Avlan", "Planta", "Ganhikokoé", "Akpagbo", "Gbogui", "Goukokoé", "Sotunmon", "PITA3", "Péripita", "CRBP755", "50C". The cultivar "Avlan" was found to be the most susceptible to Banana Fusarium wilt with 63.30% disease incidence. The phenotypic characterization performed on 86 isolates resulting from the samples of different origins, was able to identify two groups of isolates proving to be similar in terms of behavior towards the various tests carried out. These were *Fusarium oxysporum* with 77 isolates and *Fusarium graminearum* with 09 isolates. The results of the pathogenicity tests highlighted the symptoms of *Fusarium oxysporum* observed in the field. This is the first time that Fusarium wilt of banana has been reported in Benin.

Key words: Bananas, Benin, *Fusarium oxysporum*, Fusarium wilt, isolates

## RÉSUMÉ
La fusariose du bananier causée par *Fusarium oxysporum* f. sp. cubense est l'une des maladies fongiques les plus importantes dans les zones de culture de bananes. Elle constitue un problème majeur pour l'intensification de la production de bananes au Bénin. Cette étude a analysé la diversité phénotypique et pathogénique des souches de Fusarium en vue d'un contrôle durable. Une enquête a été réalisée dans 72 champs situés dans huit (8) communautés (Missrété, Sakété, Adja-Ouèrè, Avrankou, Adjara, Ifangni, Tori et Allada) pour évaluer la prévalence de la fusariose du bananier. Les communautés d'Avrankou ont montré un faible pourcentage (12,59 %) d'incidence de la maladie, tandis que le plus élevé (61,11 %) a été enregistré dans la commune de Missrété. Au cours de l'enquête, 11 variétés de bananes ont été identifiées, telles que "Avlan", "Planta", "Ganhikokoé", "Akpagbo", "Gbogui", "Goukokoé", "Sotunmon", "PITA3", "Péripita", "CRBP755", "50C". La variété "Avlan" s'est avérée être la plus susceptible à la fusariose du bananier avec une incidence de la maladie de 63,30 %.

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La caractérisation phénotypique réalisée sur 86 isolats provenant d'échantillons d'origines différentes a permis d'identifier deux groupes d'isolats présentant des comportements similaires aux différents tests effectués. Il s'agissait de *Fusarium oxysporum* avec 77 isolats et de *Fusarium graminearum* avec 09 isolats. Les résultats des tests de pathogénicité ont mis en évidence les symptômes de *Fusarium oxysporum* observés sur le terrain. C'est la première fois que la fusariose du bananier est signalée au Bénin.

Mots-clés : Bananes, Bénin, F*usarium oxysporum*, fusariose, isolats

## INTRODUCTION

The banana plant, originating from Southeast Asia (Gowen, 1994) with tropical requirements, is a fruit tree belonging to the Musaceae family whose fruits are generally bananas. In recent years, world banana production has increased from 108.7 million tonnes in 2010 to over 112 million tonnes in 2016, of which about 20% is traded internationally and the remaining 80% is consumed locally (FAOSTAT, 2017). As for Africa, a production of 20.8 million tons is estimated and comes in third place (17.5%) after Asia (55.1%) and Americas (25.3%) (FAOSTAT, 2017). In the Republic of Benin, banana is one of the main self-consumption crops of the population. The annual national production of this crop is estimated at 20756 tons in 2016 for a yield of 48048 hg/ha with a total harvested area of 4320 ha (FAOSTAT, 2017). Like cassava, rice, maize and palm oil, banana is an important source of household income (Dhed'a *et al*., 2011). Banana varieties grown in Benin are generally distinguished by their color, taste, size, fruit shape, etc. (Dhed'a *et al*., 2011). They are: "SOTOUNMON"; "DOHEZE"; "DANKOEKOE"; "TCHON"; "LIMU"; "GUNKOEKOE"; "SOKOEKOE"; "GBOGUI"; "HLO". They are cultivated all over the country.

Globally, banana (which includes the dessert and plantain type bananas) is the main fresh fruit subject of important international trade. Its socio-economic and nutritional importance is considerable because, far from being a simple dessert, bananas play an essential role in the food security of over 400 million people in tropical countries. It is also a source of employment and income for local populations (Arias *et al*., 2003). Although there has been an increase in global banana production, bananas are subject to numerous parasitic constraints, among which fungal diseases contribute significantly to the decline in yields in different types of production (Daniells, 2009). These losses are observed through the net reduction of plant growth, size and weight of the bunch. For example, when plants are severely affected, the reduction of the bunch weight can be as high as 78% (Kangire, 1998). The pathogens responsible for the disease can be of various origins (Carlier *et al*., 2003).

Fusarium wilt caused by *Fusarium oxysporum* f. sp. cubense (Zambrano *et al*., 2007), is considered as one of the most important banana fungal diseases (Lassoudière, 2007). Four biovars have been identified in *Fusarium oxysporum* by their ability to infect a specific banana cultivar (Groenewald, 2006). The symptoms are, uniform yellowing of older to youngest leaves, necrosis of the stipe and browning of the vascular, root and rhizome systems (Do *et al*., 2001). Fusarium wilt was first described in 1876 in Australia on the cultivar Gros Michel (Carlier *et al*., 2003) and in 1952, in Kenya on the cultivar Bluggoe (Kung'u, 1995). In the Republic of Benin, banana fusarium wilt has never been reported, but characteristic symptoms of this disease have been observed in several Beninese banana plantations. However, abiotic factors can promote the development of such symptoms (Van Ee, 1999). More so, these symptoms can be confused with those of bacterial infection caused by race 2 of *Ralstonia solanacerum* (Ploetz *et al*,. 2003). Faced with the threat posed by Fusarium

species, it is important to establish the Fusarium species in Republic of Benin in order to set up adequate disease control strategies against this pathogen. This study therefore aimed to constitute a large collection of Fusarium isolates relating to the spread of Fusarium wilt in banana plantations in Southern part of Republic of Benin and to analyze the phenotypic and pathogenic diversity of Fusarium strains with a view to sustainable control.

## MATERIAL AND METHODS

**Study areas and sampling**. The various surveys were carried out in eight main banana production zones (communes) in the Republic of Benin (Fig 1). Nine banana fields in each commune were chosen, about 25 kilometers apart. In total, 60 banana fields were chosen for systematic surveys. On each of these plots, 30 banana trees representative were chosen at random and used for the collection of different data.

**Plant material and soil**. Fragments from leaves and root were taken at random from five infected plants with pruning shears (Figs. 2 and 3). About 5 g of soil samples were taken at the base of the five infected bananas, at a depth of 20 to 30 cm, over a radius of 2 m (Fig 4). The samples were taken while maintaining aseptic conditions as much as possible and avoiding direct contact between the different samples. Each sample was placed in a paper envelope bearing indications relating to its origin (date of sampling, variety, density, etc.). The organs thus harvested were transferred to the laboratory for isolation.

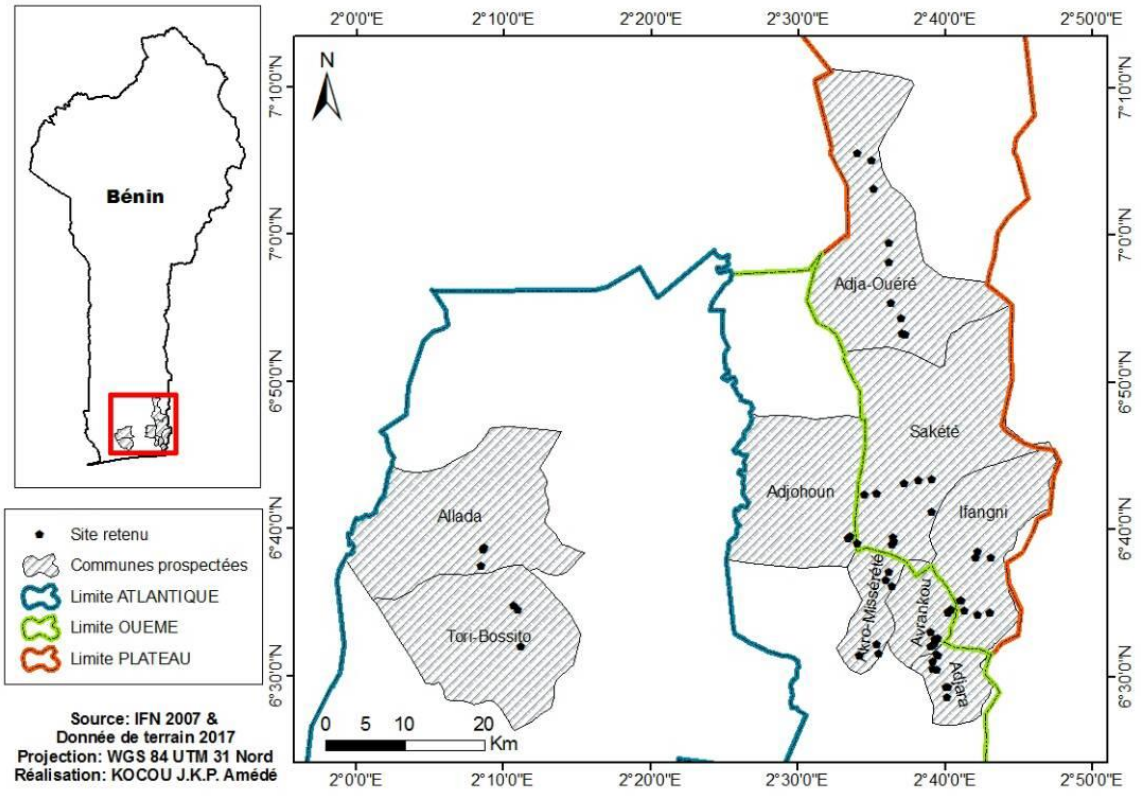


**Fig.1 :** Study areeas

**Fig. 2 :** Infected banana root

**Fig. 3 :** Infected banana leaft

**Fig. 4 :** Soil of the infected banana

Subsquently, samples were taken for morphological, physiological and pathogenic characterizations of the fungal collection obtained.

**Isolation of fungi from banana leaves and roots**. The fungi were isolated from fragments of infected banana leaves and roots. Fragments of 4 to 7 mm, disinfected with 70 alcohol, were cut and then deposited on PDA (Potato Dextro Agar) culture medium contained in 9 cm Petri dishes and incubated at 25 ± 2°C for 5 to 8 days. After development of the fungal colonies, the purification step was carried out using the successive sub-culturing technique, based on macro morphological cultural characters.

The soil adhering to the roots of the infected banana plants was collected, dried at 30°C and ground in a sterilized mortar. The fungi were isolated by taking 5 g of soil to which 3 ml of sterile distilled water was added. Then, 7 deposits of the peat obtained were made using a toothpick on PDA medium. The pure cultures were obtained by the purification process indicated above and the experiment was repeated three times.

**Purification and identification of isolates**. The purification of the isolates consisted of having a pure culture by successive subculturing of an isolated colony. After 48 to 72 hours of incubation, fragments in form of mycelial discs were cut out under sterile conditions on the growth front of the previous culture media and sub cultured onto new PDA culture media under the same conditions. These subcultures were repeated until colonies of pure appearance were obtained. The identification of isolates was carried out according to morphological characteristics using the criteria of the identification key described by Djerbi (1990).

The various fungal colonies obtained from cultures aged seven days on PDA culture medium, incubated at 25 ± 2°C. were described macroscopically and then observed under an optical microscope X40 resolution in order to identify them. The microscopic observation took into account the shape, the size of the spores, the presence or absence of chlamydia spores, the partitioning and the branching or not of mycelium. The isolation frequency (IF) of isolates of *Fusarium* spp. is estimated by the number of isolates obtained from a sample (NI) out of the total number of isolates obtained (NTI).

$$IF = \frac{NI}{TNI} x\ 100 \quad (1)$$

**IF:** Isolation Frequency

**NI:** Number of Isolates in a sample

**TNI:** Total Number of Isolates obtained

**Pathogenicity tests**. The inoculum was prepared from the 7-days old culture of *Fusarium* spp. The surface charged with conidia and immersed with 10 ml of distilled water was scraped using a Pasteur pipette then transferred to a test tube before being brought to the vortex to homogenize well. The suspension thus obtained was filtered through a muslin sheet and diluted with distilled water containing 0.05% Tween 20 and 5% gelatin so as to obtain a final concentration of 106 conidia / ml using a Malassez slide. The plant material consisted of 60 in vitro plantlets of bananas of two varieties "Avlan" and "Sotounmon" (30 plants each) provided by the Niaouli Agronomic

Research Center (CRA-Niaouli), INRAB. The fungal material was composed of nine (9) isolates of *Fusarium oxysporum* and Nine (9) isolates of *Fusarium graminearum*.

The inoculation consisted of putting 20 ml of spore suspensions (106 spores / ml) of Fusarium isolates to the roots and leaves. The suspension was injected using a sterile syringe into two main roots and the main vein of three leaves per plant. These young plants were placed in a greenhouse using a Fischer block device for six (06) weeks. Young plants inoculated with distilled water were considered as controls. This experiment was repeated three times. The green and yellow leaves present on each cultivated banana plant were counted weekly during the six (06) weeks. The incidence and severity of the disease were determined after the percentage inoculation of plants that developed symptoms of internal and external wilt of *Fusarium* spp. by the sixth week, the coloring of the mature leaves and the condition of the root system of each young plant were noted. The search for necrosis was also made through longitudinal sections made in the rhizome of each infected young plant. The diagnosis of this disease was made at the level of the roots and leaves of infected young plants, according to the isolation method of Davet (1997) on PDA culture medium and identified according to the identification keys.

**Symptom assessment**

Symptoms of Fusarium wilt were rated using the following rating scale:

0 : healthy leaves

1 : Yellowing of the lower leaves

2 : Yellowing of all lower leaves and slight discoloration of younger leaves

3 : Developed stage of infection :Wilting of leaves

4 : Death of the plant.

The infection index was evaluated for each plant of each replicate, at each stage of growth according to the following formula (Carlier, 2002):

$$I = \frac{\sum nb}{(N-1)T} x\ 100 \quad (2)$$

**Where:**

**I:** Incidence of the disease

**b :** degree of scale

**n :** number of sheets for each degree of the scale

**T :** total number of leaves evaluated

**Data analysis**. The data collected during the evaluation of the various parameters were subjected to an analysis of variance (ANOVA with on factor) using the STATISTICA software version 7.1. In case a significant difference was observed, the means were compared using the Student-Newman-Keuls test at the 5% threshold in order to distinguish groups according to the mean values of the tested variables.

## RESULTS

**Distribution of Fusarium wilt in the Communes**. The surveys carried out in the eight study areas (Missrété, Sakété, Adja-Ouèrè, Avrankou, Adjara, Ifangni, Tori and Allada) in 72 banana plantations (Table 1), revealed the presence of symptom of Fusarium observed on the leaves and manifested as yellowing and drying that progress from the base upwards. The yellowing was accompanied by sagging of the leaves at the level of the petiole or more generally towards the base of the midrib and hang down forming a “skirt” of dead foliage around the pseudo-stem (Fig 5). The symptoms differed to some extent according to the locality, the cultivar attacked and the growing conditions. The surveys carried out in the eight (08) communes of southern Benin made it possible to observe the characteristic symptoms of Fusarium wilt. The distribution of the disease varied from one locality to another. In the communes of Missrété, Sakété, Tori, Ifangni and Allada, surveys revealed that, in several banana plantations, the disease was present and caused significant damage. In contrast, in the Avrankou area, the disease was rarely observed and sometimes absent.

**Table 1. Location, characteristics of the investigated sites and number of samples**

| Communes | Sites | Longitude | Latitude | Altitude (m) | Varieties | Number of samples |
|---|---|---|---|---|---|---|
| MISSRETE | Katagon 1 | 002 36'42.1'' | 06 36'12.4'' | 31 | Avlan | 30 |
| | Katagon 2 | 002 36'01.5'' | 06 36'53.8'' | 36 | Akpagbo | 30 |
| | Katagon 3 | 002 36'21.5'' | 06 37'08.5'' | 48 | Planta | 30 |
| | Vakon 1 | 002 35'52.9'' | 06 31'54.5'' | 9 | Sotunmon | 30 |
| | Vakon 2 | 002 35'38.9'' | 06 32'19.5'' | 30 | Gbogui | 30 |
| | Vakon 3 | 002 34'18.7'' | 06 31'43.0'' | 39 | Avlan | 30 |
| | Zoungbomè 1 | 002 34'04.9'' | 06 39'05.6'' | 73 | Planta | 30 |
| | Zoungbomè 2 | 002 33'42.3'' | 06 39'39.9'' | 75 | Planta | 30 |
| | Zoungbomè 3 | 002 33'58.1'' | 06 39'53.2'' | 78 | Avlan | 30 |
| ADJARA | Adjara centre 1 | 002 39'53.8'' | 06 31'42.7'' | 36 | Avlan | 30 |
| | Adjara centre 2 | 002 39'47.3'' | 06 31'46.0'' | 36 | Goukokoé | 30 |
| | Adjara centre 3 | 002 39'54.3'' | 06 31'44.7'' | 32 | Goukokoé | 30 |
| | Honvié 1 | 002 39'19.4'' | 06 31'02.0'' | 29 | Goukokoé | 30 |
| | Honvié 2 | 002 39'18.8'' | 06 30'54.4'' | 37 | Avlan | 30 |
| | Honvié 3 | 002 39'46.4'' | 06 30'46.7'' | 39 | Avlan | 30 |
| | Malanhoui 1 | 002 40'26.7'' | 06 29'29.6'' | 12 | Goukokoé | 30 |
| | Malanhoui 2 | 002 40'12.3'' | 06 29'30.6'' | 19 | Avlan | 30 |
| | Malanhoui 3 | 002 40'16.6'' | 06 28'59.1'' | 18 | Avlan | 30 |
| SAKETE | Sakété centre 1 | 002 38'24.5'' | 06 43'29.9'' | 69 | Avlan | 30 |
| | Sakété centre 2 | 002 39'09.4'' | 06 43'40.2'' | 71 | Ganhikkokoé | 30 |
| | Sakété centre 3 | 002 39'13.0'' | 06 41'20.1'' | 69 | Avlan | 30 |
| | Takon 1 | 002 36'49.1'' | 06 39'43.5'' | 26 | Ganhikkokoé | 30 |
| | Takon 2 | 002 36'57.8'' | 06 39'15.3'' | 38 | Avlan | 30 |
| | Takon 3 | 002 36'41.2'' | 06 39'00.0'' | 39 | Avlan | 30 |
| | Yoko 1 | 002 34'56.9'' | 06 42'34.4'' | 89 | Avlan | 30 |
| | Yoko 2 | 002 35'41.1'' | 06 42'44.3'' | 84 | Avlan | 30 |
| | Yoko 3 | 002 37'25.8'' | 06 43'14.9'' | 70 | Avlan | 30 |
| ADJA-OUERE | Ikpinlè 1 | 002 37'07.6'' | 06 53'29.7'' | 119 | Avlan | 30 |
| | Ikpinlè 2 | 002 37'28.7'' | 06 53'21.8'' | 129 | Ganhikkokoé | 30 |
| | Ikpinlè 3 | 002 37'01.0'' | 06 54'31.5'' | 107 | Planta | 30 |
| | Adja-ouèrè 1 | 002 36'36.7'' | 06 55'38.4'' | 131 | Goukokoé | 30 |
| | Adja-ouèrè 2 | 002 36'20.5'' | 06 58'13.6'' | 132 | PITA 3 | 30 |
| | Adja-ouèrè 3 | 002 36'22.4'' | 06 59'44.6'' | 1O7 | PITA 3 | 30 |
| | Masse 1 | 002 35'16.7'' | 07 03'15.6'' | 53 | Planta | 30 |
| | Masse 2 | 002 35'01.1'' | 07 05'05.3'' | 45 | Avlan | 30 |
| | Masse 3 | 002 34'07.3'' | 07 05'53.5'' | 43 | Avlan | 30 |
| IFANGNI | Tchaada 1 | 002 41'08.9'' | 06 35'17.9'' | 41 | Avlan | 30 |
| | Tchaada 2 | 002 41'16.8'' | 06 35'14.3'' | 37 | Avlan | 30 |
| | Tchaada 3 | 002 41'35.8'' | 06 34'46.6'' | 40 | Avlan | 30 |
| | Daagbé 1 | 002 42'24.6'' | 06 34'19.3'' | 32 | Avlan | 30 |
| | Daagbé 2 | 002 43'09.7'' | 06 34'35.0'' | 35 | Avlan | 30 |
| | Daagbé 3 | 002 43'10.8'' | 06 34'32.1'' | 35 | Avlan | 30 |
| | Banigbé 1 | 002 42'10.8'' | 06 38'04.9'' | 51 | Avlan | 30 |
| | Banigbé 2 | 002 43'11.4'' | 06 38'04.5'' | 42 | Avlan | 30 |
| | Banigbé 3 | 002 42'24.0'' | 06 38'51.5'' | 50 | Avlan | 30 |
| AVRANKOU | Ouanho 1 | 002 39'08.4'' | 06 32'04.1'' | 29 | Avlan | 30 |
| | Ouanho 2 | 002 39'16.5'' | 06 32'14.2'' | 33 | Avlan | 30 |
| | Ouanho 3 | 002 39'33.3'' | 06 32'21.9'' | 11 | Avlan | 30 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | Gbozounmè 1 | 002 40’26.8’’ | 06 34’34.6’’ | 44 | Avlan | 30 |
| | Gbozounmè 2 | 002 40’24.0’’ | 06 34’38.5’’ | 43 | Planta | 30 |
| | Gbozounmè 3 | 002 40’41.5’’ | 06 34’52.1’’ | 32 | Avlan | 30 |
| | Sédjè 1 | 002 39’07.7’’ | 06 33’04.4’’ | 19 | Planta | 30 |
| | Sédjè 2 | 002 39’36.2’’ | 06 32’55.4’’ | 29 | Planta | 30 |
| | Sédjè 3 | 002 39’54.7’’ | 06 32’59.4’’ | 34 | Planta | 30 |
| TORI | Gbégoudo | 002 11’01.4’’ | 06 34’50.0’’ | 62 | Avlan | 30 |
| | Goussa | 002 10’70.3’’ | 06 34’74.6’’ | 72 | Péripita | 30 |
| | Cada | 002 11’20.2’’ | 06 32’00.4’’ | 52 | CRBP755 | 30 |
| ALLADA | Allada 1 | 002 08’60.9’’ | 06 38’61.4’’ | 95 | 50C | 30 |
| | Allada 2 | 002 08’49.6’’ | 06 37’48.5’’ | 92 | Avlan | 30 |
| | Allada 3 | 002 08’69.0’’ | 06 38’75.3’’ | 98 | Avlan | 30 |
| Total | 1800 | | | | | |


**Figure 5. External and internal symptoms characteristic of Fusarium wilt observed on banana plants. a: Banana field attacked by Fusarium wilt; b: Cross section of the stipe with necrosis at the periphery of the main axis; c: Uniform yellowing of mature and young leaves of an infected banana plant; d: Necrotic root system; e: Cross section of the stripe showing necrosis of the main axis; f: Dead banana plant**

The results showed that the infection rate varied from 12.59 to 61.11% for all the plots studied. This rate, calculated as the ratio of diseased plants to all of bananas evaluated, varied between localities, and within localities. The locality of Avrankou had a low percentage (12.59%) of diseased plants compared to the other seven localities surveyed. The locality of Missrété had a higher incidence (61.11%) (Fig 6). The results indicated varied response: "Avlan" (63.30%), "Sotounmon" (42.86%), "Planta" (33.85%). The cultivars "Gbogui" (0%) and "Akpagbo" (0%) showed no symptoms of Fusarium in the 72 banana plantations surveyed (Fig 7). According to farmers, these cultivars were free from the typical symptoms of Fusarium.

**Isolation and identification of Fusarium isolates from plant and soil**. The results of the disease progression on infected banana plants are shown in Figure 5. All samples of infected roots and leaves revealed the presence of Fusarium.

The result of the disease incidence among the communities and varieties are presented in Figures 6 and 7. From the result it was observed that Missrete and Ifangni had the highest disease incidence of 60% while Avrankou had the lowest disease incidence of 10%. Also, from the result it was observed that Avlan variety had the highest disease incidence while Gbogui and Akpagbo had the lowest disease incidence.

The result of the isolation frequency per sample source and among communities are shown in Figures 8 and 9. From the result, it was observed that about 51.78% of the isolates were isolated from soil, 29.83% from the roots and 18.38% from the leaves. Statistical analyzes showed that these frequencies were significantly different. Also, the soil sample had the highest number of Fusarium.

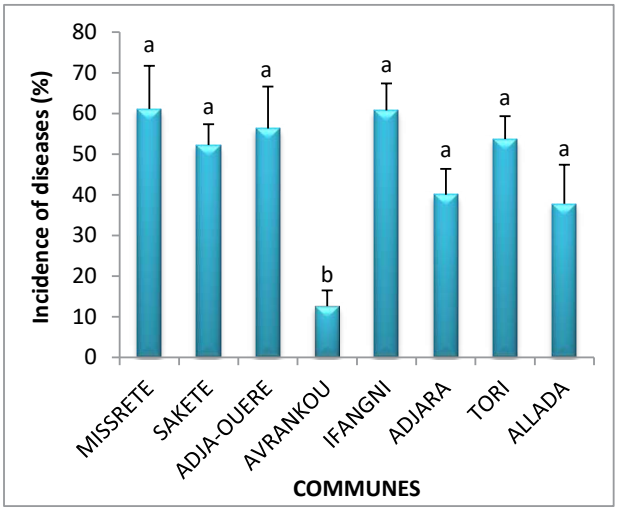


**Fig. 6.** Incidence of *Fusarium* wilt by locality

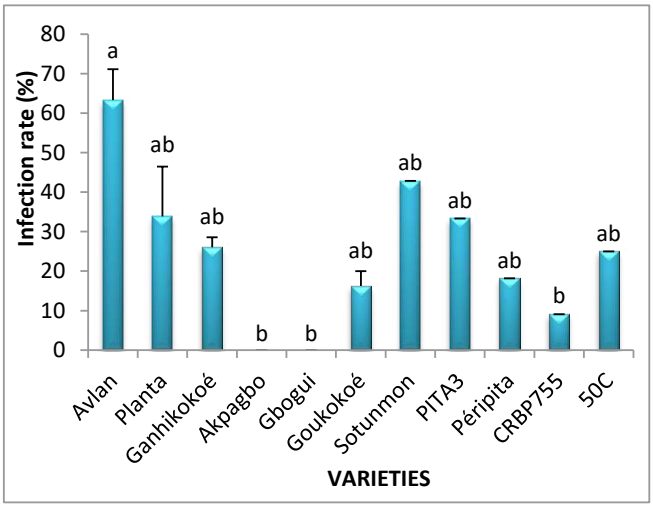


**Fig. 7.** Incidence of *Fusarium* wilt on infected banana cultivars

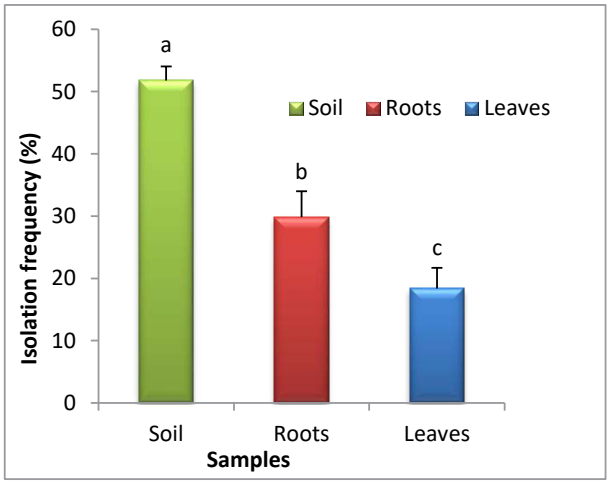


**Fig. 8.** Isolation frequencies of *Fusarium* spp. according to samples

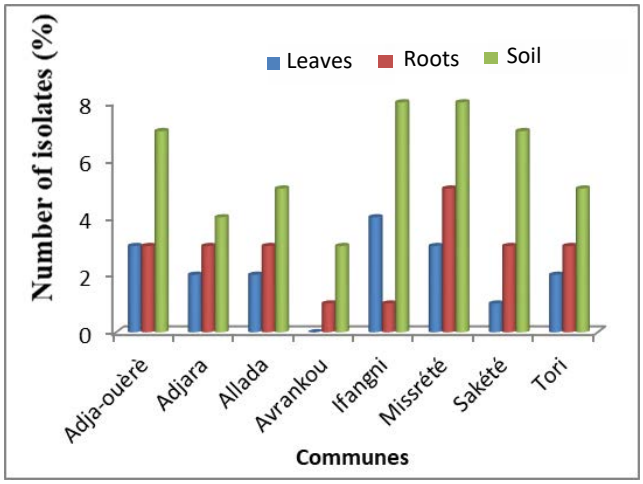


**Fig. 9.** Distribution of *Fusarium* spp. per locality sample

The appearance of the mycelium and the color of the colonies on PDA culture medium made it possible to classify the isolates into two homogeneous groups:

1. Group 1. Colonies of fluffy white color, fine, curly, showing a cottony, aerial and scanty mycelium at the periphery with a slow growth (8.6 cm in diameter in 9 days). The number of isolates carrying this type was 77 (Fig 10a).

2. Group 2. Colonies were salmon pink in color, showing abundant, redaerial mycelium upside down from the petri dish with rapid growth (8.2 cm in diameter in 6 days). The number of isolates carrying this type was 9 (Fig 10b, c).

Microscopic observations showed septate, branched and hyaline mycelium for all isolates. However, we noted the presence of a false head on the micro phialids which are of short in length. The micro conidia were very numerous, ovoid, unicellular with dimensions of the order of 1.2 - 4.8 µm. Macro conidia had an almost straight, thin shape with three to four partitions, a basal cell in the shape of a foot and a curved and tapered apical cell. They were abundant, spindle shaped and produced from phyalids on conidiophores. Similar to the density of macro conidia, chlamydospores were generally scarce, but sometimes numerous in some isolates, especially those isolated from soil. They had a globular shape with thick walls. They were formed of hyphae (Fig 11).

**Pathogenicity test of Fusarium isolates**. The pathogenicity experiment was performed in a greenhouse to test the effect of Fusarium spores on young potted banana plants. The onset of leaf symptoms was observed from the 10th day after inoculation. They were characterized by yellowing and chlorosis that initially appeared on older leaves, followed by general wilting and then wilting of the plant. The yellowing of the leaves began along the margin and progressed to the vein to reach the entire leaf surface and the leaf margins turned a grayish-brown color (Fig 12).

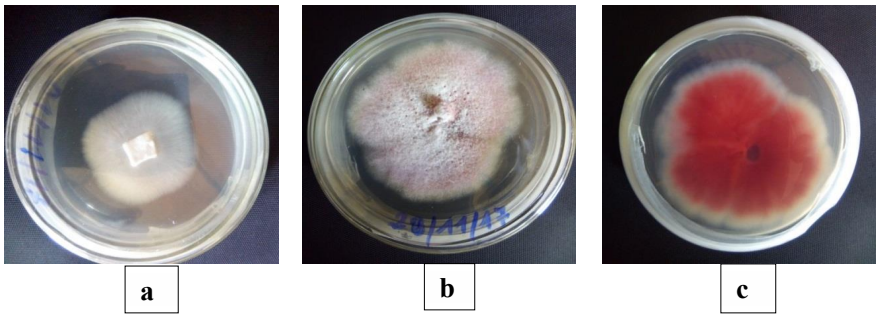


**Fig. 10 .** Morphotype of the fusarium thallus. **a:** white fluffy morphotype, thin; **b:** Morphotype salmonpink, showing a white, aerial and abundantmycelium; **c:** Red back of the salmonpink morphotype

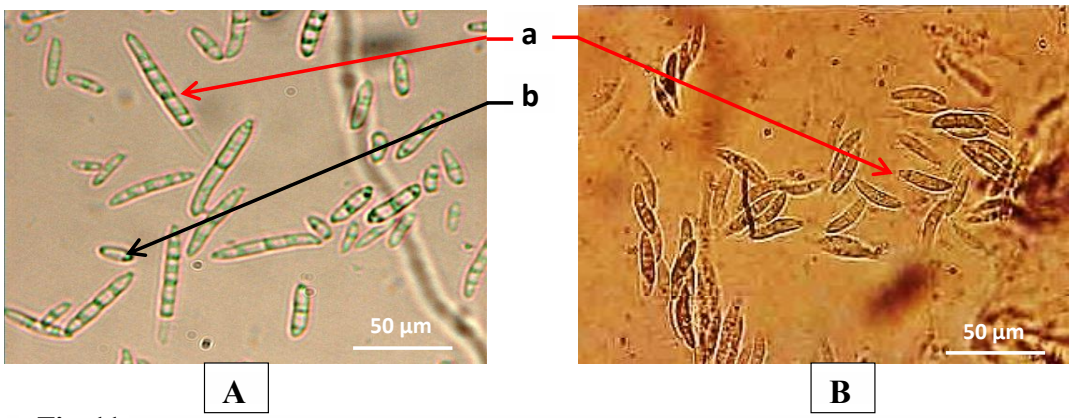


**Fig. 11.** Microscopic observation or micrograph of *Fusarium*. **A:** Microscopic observation of *Fusarium oxysporum* f. sp. cubense;**B:**microscopic observation of *Fusarium graminearum*; **a:**macroconidia; **b:**microconidia

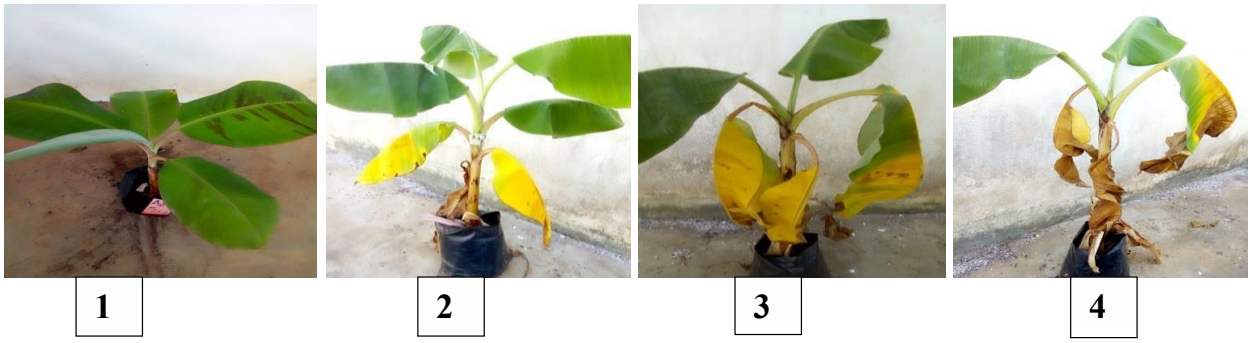


**Fig. 12.** *Fusarium* wilt rating scale based on external and internal symptoms in the Greenhouse. **1:** No symptoms; **2:** initial yellowing mainly on the lower leaves; **3:**Yellowing of all lower leaves with some discoloration of younger leaves; **4:**Withering and wilting of leaves

After three months, no early leaf yellowing was observed on the control plants (T). In contrast, all "Avlan" and "Sotounmon" banana plants inoculated with *Fusarium oxysporum* and *Fusarium graminearum* developed leaves which turned yellow early. Plants infected with these isolates also showed browning in the vascular tissue of the stipe. In the control plants, after six weeks and without the isolates, the average number of green leaves (NMFV) decreased from 4.60 to 5 for the variety "Avlan" and from 3.76 to 4.93 for "Sotounmon" and the average number of yellow leaves (NMFJ), from 0.33 to 2.60 and 0.20 to 2, respectively, for "Avlan" and "Sotounmon" (Fig 13 a and b). In young plants of the "Avlan" variety and in the presence of isolates, the evolution of NMFV and NMFJ varied with time.

From the 1st to the 6th week, the NMFV gradually decreased from 4.46 to 0.13 while the NMFJ increased from 0.2 to 3.4 (Fig 14 a). In addition, in young plants of the variety "Sotounmon", the NMFV dropped from 4.6 to 1.6 while the NMFJ increased from 0.5 to 3.46 (Fig 14b). The analysis of the change in the average number of leaves after inoculation according to the Newman Keuls test revealed significant differences between the number of yellow leaves and the number of green leaves as a function of time.

The results shown in Fig 15 represent the severity of the disease on the leaves of the banana plants, six weeks after inoculation with the Fusarium species tested. For the "Avlan" variety, the severity of the disease was 86% for young plants treated with *Fusarium oxysporum* and 43.16% for those treated with *Fusarium graminearum*. For the variety "Sotounmon", the severity of the disease was 62.97% for the seedlings treated with *Fusarium oxysporum* and 36.50% for those treated with *Fusarium graminearum*. The results of analyzes showed that there was a significant difference between the severity of the two Fusarium species after inoculation.

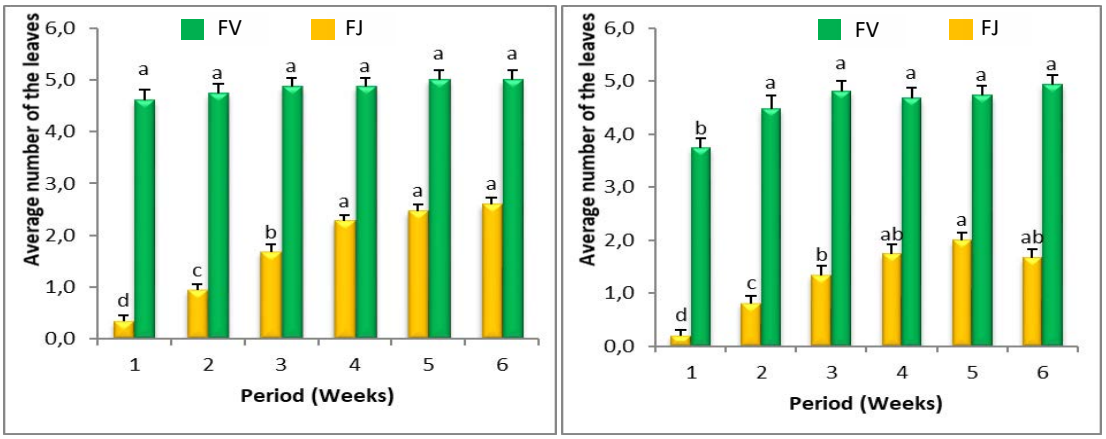


**Fig. 13.** Evolution of the average number of uninfected leaves as a function of time. **a:**Variety "Avlan"; **b:**Variety "Sotounmon" **FJ:** Yellow leaves ;**FV:** Green leaves

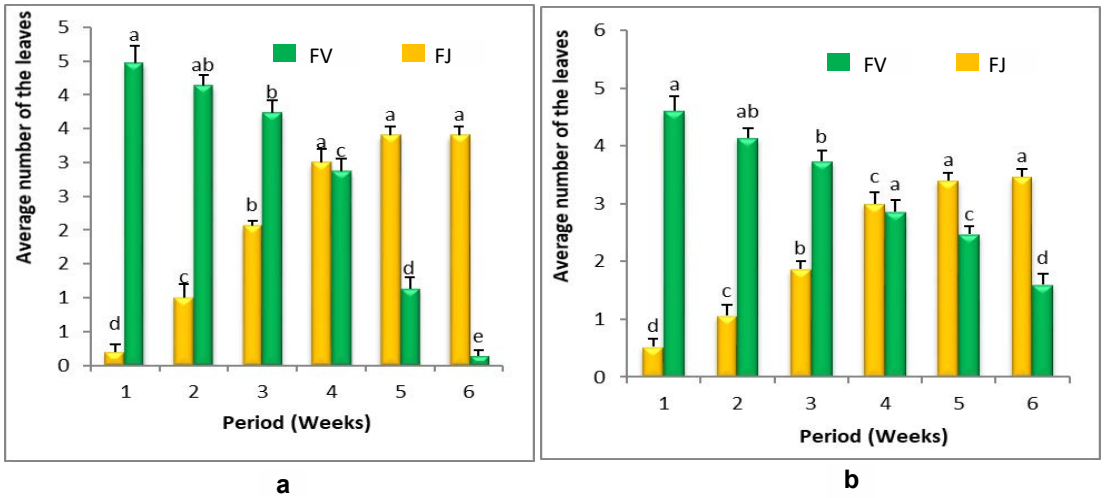


**Fig. 14.** Evolution of the average number of infected leaves as a function of time. **a:**Variety "Avlan"; **b:**Variety "Sotounmon" **FJ :** Yellow leaves ;**FV :** Green leaves

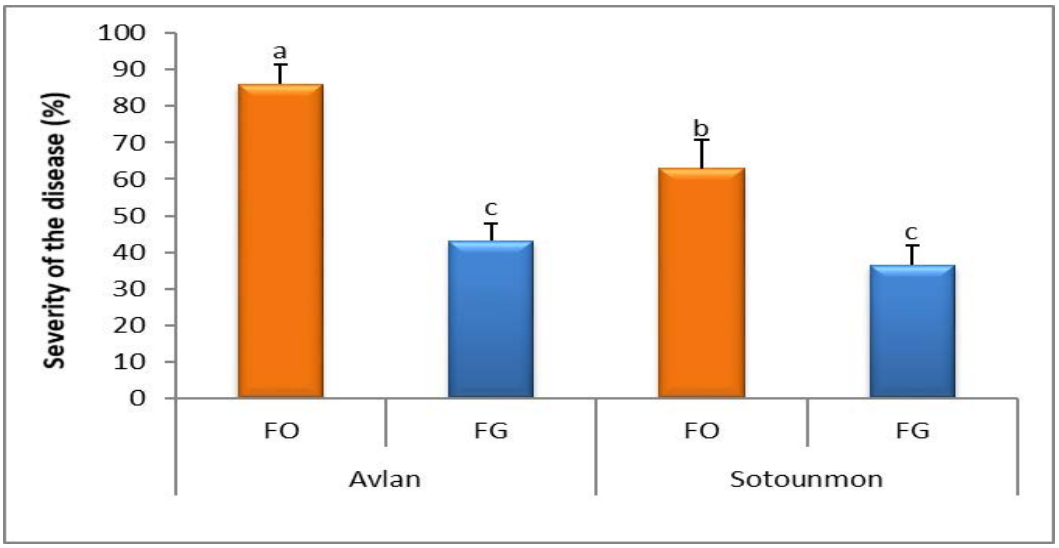


**Fig 15. Disease severity (%) estimated on the leaves six weeks after inoculation of the banana plants with the two Fusarium species tested**

## DISCUSSION

Fusarium wilt of bananas is a disease with a very considerable negative impact in Benin. Its degree of attack directly affects the income of populations. Indeed, the external and internal symptoms of Fusarium wilt observed in the banana plantations of the eight localities surveyed were yellowing accompanied by wilting of the leaves and necrosis of the tissues. These symptoms are similar to those observed and described by Ploetz (2006) on banana caused by *Fusarium oxysporum* f. sp. *cubense*. The symptoms observed could be explained by the result of severe water stress due to the occlusion of the perforated plaques of the xylem vessels as well as by the combination of pathogenic activities such as the accumulation of mycelium, the production of toxins and / or the host defense response including the production of tylosis, gum, and vessel narrowing due to growth of parenchymal cells (Beckman, 1990). In addition, when the plant is alive, the pathogens confined to xylem cells and certain complementary cells, but once the plant dies, the pathogen invades the parenchyma and sporulates extensively (Ploetz and Pegg, 2000). Wilting could also result from obstruction of the root vascular system which is the entry route of *Fusarium oxysporum* into the plant, which explains the high rate of isolation of *Fusarium oxysporum* from soil and leaf roots (Li *et al*., 2017).

Results showed that *Fusarium oxysporum* isolated from roots was also found in soil and leaves at variable frequencies. These results corroborate those of Champion (1997) who showed that *Fusarium oxysporum* is widely spread Fusarium species in nature and behaves either as a parasite or as a saprophyte. The high isolation rate of this fungal species in the soils of the banana rhizosphere obtained in our results confirms that observed by Meddah *et al*. (2010) in the soils of banana plantations in Côte d'Ivoire. Indeed, this high incidence of the disease could be explained by the traditional method of banana cultivation, which disseminates the pathogen through the use of suckers from old plantations whose health status is unknown.

Characterization of isolates from each collection area showed a difference in conidial size and shape. Isolates with long or wide macroconidia were fusiform and microconidia were oval with one or two rounded extremities. This difference could be due to genetic diversity of *Fusarium* sp. isolates. Similar results were also obtained by Balali and Iranpoor (2006) who observed variability in the shape of Fusarium species. These authors said that, the difference would be due to genetic variability among Fusarium species. Macro-conidia with three partitions of isolated Fusarium isolates showed an average length which varied from 27 to 48.3 µm. This form is similar to that of *Fusarium oxysporum* f. sp. *cubense* (Foc), the causative agent of Fusarium wilt of banana which ranges from 27 to 55 µm, observed by Ploetz *et al*. (2000). The absence of symptoms on the cultivars of "Gbogui" and "Akpagbo" could be explained by the non-pathogenic nature of Fusarium on these two cultivars. This result was also observed by Nel *et al*. (2005) who demonstrated the presence of non-pathogenic isolates of *F. oxysporum* in the soils of the rhizosphere of banana plantations. However, in the presence of isolates of *F. oxysporum* and *F. graminearum* in the roots, infected bananas developed external and internal symptoms similar to those of Fusarium wilt six weeks after inoculation. These pathogens were also responsible for the development of root rots and the browning of the pseudo stem after inoculation. These results are in line with those of Hadi *et al*. (1987) who showed that the inoculation of banana roots with *F. oxysporum* f. sp. *cubense* had induced lesions after one week and that mechanical injury allowed the pathogen to colonize the cells of the cortex causing red dish brown lesions. David (1997) reported that *Fusarium oxysporum* and *Fusarium solani* are responsible for root rots.

The results obtained also made it possible to show that the number of conidia produced by the pathogen on the host can predict its pathogenicity. Rotem (1978) reported that the most infectious species are those capable of affecting more of the host tissue and allowing the inoculum to multiply. Isolates of *F. oxysporum* are significantly the most aggressive of both varieties compared to isolates of *Fusarium*

*graminearum*. According to Pérez-Vicente and Dita (2014), a susceptible banana plant infected with Fusarium wilt will rarely recover. While recovery can occur, the growth is poor and the mother plant produces many infected suckers before it dies.

## CONCLUSION

The soils of banana plantations infected with the fatal yellowing of banana leaves disease in southern Benin are heavily colonized by *Fusarium oxysporum*. The macroscopic and microscopic characters of isolates from this fungal species are similar to those of *Fusarium oxysporum* f. sp. *cubense*, the causative agent of Fusarium wilt of banana. Isolates of *Fusarium oxysporum* isolated from specimens infected with the disease were able to induce symptoms characteristic of Fusarium wilt in "Avlan", "Planta" and "Sotounmon" bananas. Pathogenicity assessment of the strains under controlled conditions revealed that they were pathogenic on the two varieties "Avlan" and "Sotounmon" and show different degrees of pathogenicity. The disease pressure is linked to cultivars and cropping systems. Cultivar type also influenced the incidence and severity of Fusarium wilt in banana.

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## STATEMENT OF NO-CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this paper.

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