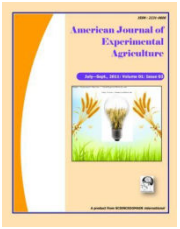

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# Variability of Pathogenicity in *Fusarium xylarioides* Steyaert: the Causal Agent of Coffee Wilt Disease

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## ABSTRACT

Tracheomycosis (or Coffee Wilt Disease) is a vascular disease that causes damage in plantations up to 80% of production in the absence of treatment. The fungus of the disease is *Fusarium xylarioides*. An experiment in micro-plots was put in place to look for 9 strains of this species from different production regions of the Democratic Republic of Congo (Equateur, Nord Kivu and Province Orientale) in terms of their pathogenicity on coffee Robusta (clone L251). The results from this experiment highlight several levels of pathogenicity significantly different ($P \leq 0.05$), and particularly high for aggressive strain Mindembo. The differences do not seem to be related to geographic origins. Mindembo strain, from Equateur, was more aggressive and induces a high mortality (50%). Strains Bunduki and MUCL 45580, originating in Equateur and the Province Orientale, showed high pathogenicity although lower than Mindembo. However, the strain Zobolia (Equateur) multiplies much more slowly and has caused no mortality 3 months after inoculation. This work has important implications for studies on varietal resistance.

*Keywords: Robusta coffee; tracheomycosis; Fusarium xylarioides; pathogenicity; DR congo;*

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## 1. INTRODUCTION

Coffee is one of the first agricultural resources for the Democratic Republic of Congo (DRC). Its operating system is shared between farmers and large plantation agribusiness. The former are about 86% of total coffee acreage (Anonymous, 1996). The second are almost gone if they are not totally abandoned (Kalonji, 2006, personal communication).In the coffee system production, robusta coffee (*Coffea canephora* var Robusta)is about 87.5% of arable acreage, with a production accounting for about 85% of total coffee production. It is the main source of cash income for farmers' growers, and a source of foreign exchange for export (Charrier and Eskes, 1997). The current production of coffee orchard of DRC is limited by the coffee wilt disease, a vascular disease that causes wasting of Robusta coffee. This disease, caused by a fungus, *Gibberella xylarioides* Heim and Saccas, anamorph *Fusarium xylarioides* Steyaert, has been described by various authors since its discovery in DRC (Steyaert, 1948; Fraselle, 1950; Heim and Saccas, 1950) in West and Central Africa (Saccas, 1951) until recently on the occasion of its reappearance in DRC (Katenga, 1989 Tshilenge et al.,1998).

The tracheomycosis is manifested by abrupt vegetation, wilting and yellowing of leaves followed by drying out the leading shoots. The tree is stripped of its foliage quickly and has a skeletal appearance (Heim and Saccas, 1950; Coste, 1989, Steyaert, 1948; Fraselle, 1950; Kalonji, 1975; Tshilenge et al., 1998). Next to the yellowing of leaves, one can also observe the browning and drop, particularly at the stage of seedling nursery (Tshilenge et al., 2004). These different symptoms are signs of the disease in a plantation. Besides these there are signs presumptive typical symptoms are present in all confirmed cases of Coffee Wild Disease. There is a decline, first hemiplegic, then generalized across the crown. The tree dies within a variable period of time, but even shorter than it is young (Coste, 1989; Saccas, 1951; Steyaert, 1948; Fraselle, 1950, Kalonji, 1975; Flood, 1997; Tshilenge et al., 1998; Tshilenge et al., 2004). At the same time there is necrosis of the wood beneath the bark at the base of the stem or trunk. This necrosis, appearance changing to brownish black (Saccas, 1951; Steyaert, 1948; Fraselle et al., 1953; Kalonji, 1975; Flood, 1997; Tshilenge et al., 1998). It appears below, when the bark is peeled-off with a sharp tool, knife or machete, black discoloration along the length cutaway.

After the death of coffee, it can be seen at the base of the trunk dry cracks in which appears a kind of black pad or stroma, bearing globular masses representing black perithecia of the fungus. Many strategies essentially preventive and mechanical nature have been considered as a means of control. They consisted of pulling, cutting and burning on site of affected coffee trees from their location. After several decades of successful application of this form of control, the disease was again reported in the district of Haut-Uele (Eastern Province) to 1982 (Katenga, 1987; Mfwidi, 1994).

From this new home, it has spread in almost all coffee plantations of North-Eastern DRC with average rates of attack estimated at between 19.3% and 100% depending on crop areas (Mukuna et al., 1990; Tshilenge et al., 1998). Several assumptions were made about the possible causes of the reemergence of the disease, we found that there may be either a possible move to alternate hosts, thereby increasing the pressure of inoculum or the emergence of new strains more aggressive (Girma and Hindorf, 2001; Tshilenge et al., 2004, Girma et al., 2005).

Ineffective disease control strategies, suggests the development of resistant coffee selection as a defense appropriate, less costly for farmers in developing countries (Flood and

Brayford, 1997). Therefore, the study of genetic diversity of the pathogen is a prerequisite for the establishment of a breeding program of Robusta coffee in the DRC (Flood and Brayford, 1997, Girma et al., 2005). The aim in this work is to determine the variability of pathogenic strains of *Fusarium xylarioides* Steyaert. These strains were obtained from contaminated materials, harvested in the Equateur province (DRC). They were compared with those of other geographical origins in order to optimize a program of using varietal resistance to control the disease.

## 2. MATERIALS AND METHODS

### 2.1 Field Trials

This research was conducted at the Experimental Garden of the Department of Biology, Faculty of Sciences of the University of Kinshasa (4° 19'S latitude, 15° 8'E longitude, and 330 m, altitude). According to Koppen's climate is the type $AW_4$ medium, hot and humid climate with two seasons: the rainy season between February and May, respectively, interspersed by a short season from September to December and a short dry season (lull) with inflection rains from December to February and a dry season of almost four months from mid-May to mid-September.

Temperatures accuse slight variations, the average hovers around 24.5 °C. The average annual rainfall of 1500 mm, spread over nine months. Relative humidity is highest in April and May and is minimum in September and October. Evaporation is greatest at the end of the dry season (Makoko et al., 1992). Table 1 show the climatic conditions which prevailed during the trial period.

**Table 1. Temperature, Relative humidity, and rainfalls during the 2005 growing conditions in Kinshasa (DRCongo)**

| Months | Rainfall (mm) | Temperature (°C) | Relative humidity (%) |
|---|---|---|---|
| May | 17.14 | 27.92 | 76.85 |
| June | 0.20 | 27.87 | 76.75 |
| July | 0.19 | 27.50 | 80.00 |
| August | 0.20 | 27.87 | 76.00 |
| September | 17.67 | 28.87 | 76.75 |
| October | 183.40 | 28.70 | 80.60 |
| November | 248.70 | 28.60 | 81.30 |

*Source: Department of Soil physics and hydrology / Regional Nuclear Energy Center.*

These data reveal small amounts of rainfall recorded in June, July and August. Average temperatures during this period are constant; at 27 °C while the high rainfall in October and November.

It should be noted that weather conditions play a significant role on growth and development of the pathogenic fungus *Fusarium xylarioides* particular, because as noted Saccas (1951), dehiscence of the ascus is favored by the alternation of drought and moisture, which may facilitate the spread of contamination bodies.

## 2.2 *Fusarium xylarioides* Strains, Studied

Isolates of *Fusarium* spp. were made from pieces of wood Robusta coffee suffering from CWD collected during surveys conducted from 2002 - 2004 in Equateur province in DRC. We used samples shaped slices of varying dimension (3-5 cm long and 2 - 4.5 cm in diameter), depending on the dimension of the stems. These were surface-disinfected with 70% ethanol and then flamed quickly to evaporate excess alcohol, then split in two, under aseptic conditions using a carpenter's bevel and a rubber mallet, trying to go through areas with subcortical black discoloration. Inside the wood, color dark places, tiny fragments of a few millimeters were taken using a scalpel tip n° 11 and placed on water agar medium Streptomycin (Merck Agar ®: 15 g; H20: 1000 ml Streptomycin: 100 mg). After issue of mycelium, the ends of hyphae were subcultured on medium Synthetic Nutrient Agar (SNA KH2PO4: 1 g; KNO3 1 g; MgSO4.7H2O: 0.5 g, KCl: 0.5 g; Glucose: 0, 2 g, Sucrose, 0.2; Agar Merck ®, 20 g, H20 1000 ml) (Tshilenge *et al*., 2004; Tshilenge *et al*., 2010). A collection consists of different inbred strains is maintained on this medium under paraffin tubes. Reference strains isolated in 1992 have been received from the Mycotheque of the Catholic University of Louvain or "Mycothèque de l'Université Catholique de Louvain" (MUCL) and are also part of the collection study (Table 2).

**Table 2. List of *Fusarium xylarioides* strains, studied**

| Strains | Origin in DRC | Date of harvest | Date of isolation |
|---|---|---|---|
| Bunduki | Equateur | 09/09/2004 | 20/11/2004 |
| Mangbakapale | Equateur | 15/09/2004 | 20/11/2004 |
| Mindembo | Equateur | 17/09/2004 | 20/11/2004 |
| Moboko | Equateur | 09/09/2004 | 20/11/2004 |
| Notre-Dame | Equateur | 15/09/2004 | 20/11/2004 |
| Zobolia | Equateur | 15/09/2004 | 20/11/2004 |
| B10101(2)J | Nord Kivu | 02/12/2002 | 20/11/2004 |
| MUCL35223 | Province Orientale | Nov 1992 | 20/11/2004 |
| MUCL45580 | Province Orientale | 15/12/2002 | 20/11/2004 |

## 2.3 Characterization of the Pathogenicity

### 2.3.1 Plant material

The pathogenicity of *F. xylarioides* strains was evaluated through artificial inoculations on seedlings of coffee coming from the germination of seed from the research station of "the National Institute for Agronomic Studies and Research" or 'Institut National pour l'Etude et la Recherche Agronomique (INERA) Bongabo (DRC). These seeds were harvested in June 2003 on the clone L251. This clone was chosen for its susceptibility already observed in an earlier study (Kalonji, 1975). Seedlings were transplanted from the deployment of the first true leaves in black polythene bags of 10 cm in diameter filled with soil from the bottom of ponds. This substrate was chosen for its richness in nutrients and water holding capacity. Seedlings were treated with fertilizer every two weeks until 1 month of inoculation by watering the soil with a urea solution (5 g/10 liters of water). In this regard, Corbaz (1990) noted that nitrogen Fertilizers increase the severity of disease and lower the production of phytoalexins.

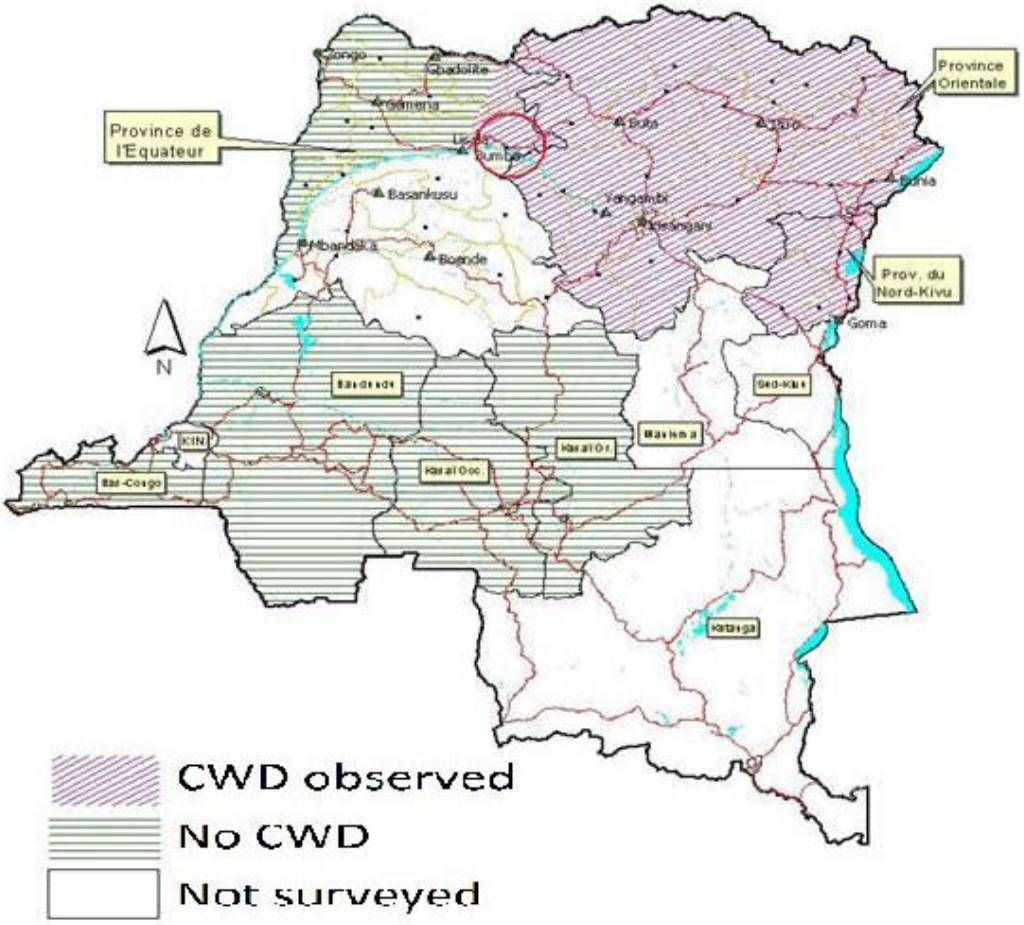


**Fig. 1. Geographical distribution of CWD in DRC between 1991 and 2004 (Tshilenge, 2007)**

## 2.3.2 Inoculation techniques

The inoculation due to an injection with a syringe (Terumo Myjector U-40 insulin, Terumo Europe NV, 3001 Leuven, Belgium) was used. For each strain *F.xylarioides*, inoculum was produced from subcultures on SNA at 27°C under continuous lighting (Tshilenge et al., 2004).

Conidia were harvested from cultures 10 days old, adding distilled water containing traces of Tween 20 (polyoxyethylene sorbitan monooleate). The concentration of conidia suspension was adjusted to $10^6$ spores/ ml using a hemacytometer or Thoma cell. Seedlings six months old and with 2-3 pairs of leaves on a stem hardwood were not yet used in this study. Before injection, the upper surface is disinfected with 70% ethanol that was allowed to evaporate for 10 minutes. The injection is given at a point at the first internode below the first leaf. A minimum volume of 0.025 ml was injected until discharge (Tshilenge et al., 2004). A control treatment inoculated only with Tween 20 solution, was included.

### 2.3.3 Experimental design

The test was conducted following a randomized complete block design with three replications. Each block representing a repetition, has 10 treatments corresponding to the strains studied, a total of 30 plots. Each treatment had 12 seedlings.

## 2.4 Data Collection and Analysis

### 2.4.1 Findings of pathogenicity

At the weekly, the recorded observations focused on determining the time interval between the time of inoculation and the appearance of various symptoms on the one hand and the severity of the disease on the other. When they appear, foliar symptoms following were recorded: wilting, browning and drying. Severity was assessed in time and gave rise to the Area Under Disease Progress Curve (AUDPC) (Skajennikoff et Rapilly, 1989; Rapilly, 1991; Tshilenge et al., 2004). This parameter is calculated using the formula:

$$AUDPC = \sum [(x_1 + x_2).0,5][t_2 - t_1] \quad (1)$$

where $x_1$ and $x_2$ represent the severity at time 1 and time 2, $t_2$-$t_1$: time interval between two observations. Mortality is expressed as the percentage of seedlings completely desiccated by the total number of inoculated seedlings. The recorded data were analyzed. The analysis of variance (ANOVA) was performed by general linear model procedure (Girma et *al*., 2005) with the software R (R-2.12.0). Comparison of means was performed by LSD test. At the end of the trial, reisolations were performed in order to verify Koch's postulates on a sample of 36 seedlings at 4 seedlings per strain. This reisolation technique consisted in removal of fragments of wood at three different levels along the stem: (1) 1cm above the collar, (2) 1cm below the cotyledonary leaves, (3) 1cm below the apical bud.

### 2.4.2 Perithecia production

Perithecia production was assessed by their abundance and status of maturation. Topics dead are brought to the laboratory where they proceeded to the observations of perithecia under a microscope.

The abundance of perithecia is evaluated according to the scale proposed by Maraite (2002):

0 = no perithecia;
1 = up to 3 Perithecia single or in groups/$cm^2$;
2 = more than 3 to 10 perithecia;
3 = more than 10 perithecia /$cm^2$.

The status of perithecia is evaluated as follows:

S = dark stromata without perithecia;
G = stromata with ostiolate perithecia;
E = empty degraded perithecia .

## 3. RESULTS AND DISCUSSION

### 3.1 Description and Evolution of Different Symptoms

The different symptoms observed events in this trial focused on changes in appearance and leaf drying of the stem. These symptoms are varied, both in their nature and in their chronological sequence from the time of their appearance. After 16 days of incubation (Table 3), the first signs observed in a seedling, were either wilting leaves or browning leaves. Wilting is characterized by flaccidity of the leaves that look along the shaft due to the loss of turgor (Plate 1; second seedling). The browning is manifested by the change in leaf coloration which changes to brown or dark brown along with a wet look or sometimes more or less oily (Plate 1; first seedling). Following these initial events, symptoms progress to a drying of leaves, their shedding and desiccation of the stem resulting in dieback of seedlings. Drying of leaves is characterized by crumbly to the touch of the sheet (Plate 1; third seedling). The shedding leaves or defoliation is characterized by the detachment at the insertion of the petiole and its freefall, when complete defoliation is followed by drying in "die back" of the stem (Plate 1, 4th seedling).


**Plate 1. Different symptoms on seedlings of coffee after inoculation with *F. xylarioides***

### 3.2 Findings of Pathogenicity

We present in Table 3, the duration of the incubation period and time of onset of various pathological manifestations.

Results of Table 3 reveal that in general, coffee seedlings inoculated with *F. xylarioides* have all the typical symptoms of wilt. However, their nature and chronology differ depending on the strain considered. These symptoms can start with either leaf wilting or browning and progress to dryness, loss and ultimately death of plants. The analysis of the results allowed classifying strains into two groups depending on the nature of symptoms. The first is the strains that cause the first symptom of the disease wilt, which is relevant strain, Zobolia, MUCL 35223, Mindembo and Mangbakapale.

**Table 3. Average interval (days) between the time of inoculation and early onset of different symptoms recorded on the clone L251 inoculated with different strains of *Fusarium xylarioides***

| Strains | Time (day) of occurrence of various symptoms | | | |
|---|---|---|---|---|
| | Leaf wilt | Browning of leaves | Drying of leaves | Mortality |
| Bunduki | 17.33±1.52 | 16.73±1.10 | 23±2.29 | 23.13±2.12 |
| Mangbakapale | 17.4±0.53 | 18.33±1.62 | 22.83±0.29 | 22.83±0.29 |
| Mindembo | 17.1±0.79 | 17.3±2.10 | 18.3±0.98 | 18.57±0.51 |
| Moboko | 18.07±0.60 | 17.57±1.40 | 21.07±1.10 | 22.80±2.51 |
| N-Dame | 21.83±2.84 | 19.87±1.40 | 23.33±0.58 | 23.07±0.12 |
| Zobolia | 16 ± 00 | 18.5±1.14 | 21.67±0.58 | NO∗ |
| B10101(2)J | 21±5.19 | 18.77±0.80 | 22±0.87 | 23.43±0.87 |
| MUCL35223 | 17±0.86 | 19.33±1.26 | 21±00 | 23±1.73 |
| MUCL45580 | 18±00 | 16.43±2.06 | 21.93±0.90 | 24.43±2.25 |
| Témoin | NO∗ | NO∗ | NO∗ | NO∗ |
| LSD (0,05) | NS | NS | 0.58 | 0.79 |

*NO = Not Observed ∗, ± = SD : Standard Deviation, NS=Not Significant*

The second group comprises those expressed by browning as the first symptom, one finds strains Bunduki, Moboko, N-Dame, B10101 (2) J and MUCL45580. Wilting as the first symptom of infection is early on seedlings inoculated with the strains Zobolia, MUCL 35223 and Mindembo (16-17 days) and is late for strains N-Dame and B10101 (2) J inducing as the first browning symptoms. Comparison of values for periods of desiccation and mortality, showed significant differences for the first symptom ($LSD_{0,05}$ = 0,58) and seedling mortality ($LSD_{0,\ 05}$ = 0,79). The dryness was early in subjects inoculated with strain Mindembo (18 days) followed Moboko, Zobolia, MUCL 35223 (21 days), MUCL 45580, B10101 (2) J, N Dame Mangbakapale and Bunduki (22 - 23 days). The absence of cases of mortality on seedlings inoculated with Zobolia was noted. Mindembo was the unique wich early causes the mortality.

## 3.3 Symptoms severity

Results related to the severity of wilting, browning and drying of leaves expressed in terms of total Area Under Disease Progress Curve (AUDPC) are presented in Figure 2 (a, b, c).

The criterion AUDPC calculated over a period of 3 months revealed significant differences ($LSD_{0,05}$ = 6,1) between strains with regard to wilt the leaves. Three groups have been established. In the first group included strains MUCL 35223, B10101 Zobolia and (2) J with low values. The second group includes strains Moboko, N-Dame and Mangbakapale with intermediate values of AUDPC, and the third, with high values, grouped the strains Mindembo, Bunduki and MUCL 45580. In the case of browning of leaves, the values are significantly different ($LSD_{0,05}$ = 3,1) between strains. The level of AUDPC, induced by the strain Mindembo, Bunduki, Moboko and N-Dame is the same order as B10101 (2) J and MUCL45580, and higher than Mangbakapale and Zobolia. The demarcation between Mindembo and other strains, recorded for the wilting of leaves, is confirmed in the case of drying with clear differences ($LSD_{0,05}$ = 3,7) between the three groups.

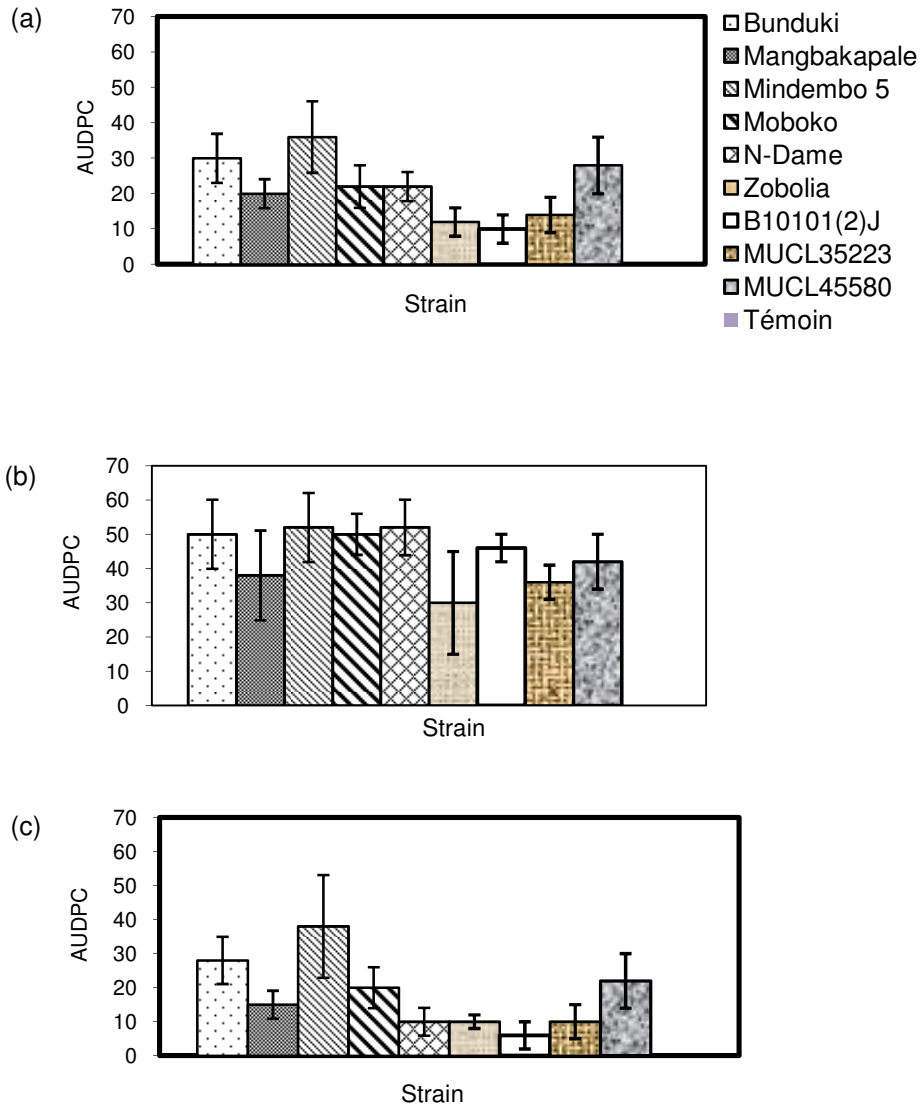


**Fig. 2. Severity (AUDPC) of CWD 3 months after inoculation. (a): wilting of leaves, (b) browning of leaves, (c) drying of the leaves**

## 3.4 Mortality Rate

Table 4 presents the mortality rate caused by different strains. Pathogenicity tests performed with different strains showed differences between the levels of aggressiveness of strains. In reviewing the mortality rate recorded, we find that the results vary (0-50%), with strain Mindembo having the greater aggressiveness (50%). MUCL45580 and Moboko who have the same effect as Bunduki exhibit intermediate aggressive (25-30%). By cons, low aggressiveness is noted on seedlings inoculated with B10101 (2) J, N-Dame, Mangbakapale

and MUCL35223 with average values ranging between 5 and 16%. No mortality was recorded with strain Zobolia.

**Table 4. Mortality rate recorded at 3 months after inoculation with different *Fusarium xylarioides* strain on the clone L251**

| Strains | Origin in DRC | % of mortality |
|---|---|---|
| Bunduki | Equator | 25.0 |
| Mangbakapale | Equator | 13.9 |
| Mindembo | Equator | 50.0 |
| Moboko | Equator | 30.5 |
| N-Dame | Equator | 8.3 |
| Zobolia | Equator | 0.0 |
| B10101(2)J | North Kivu | 5.5 |
| MUCL35223 | Easterne Province | 16.7 |
| MUCL45580 | Easterne Province | 30.5 |
| Témoin | | 0.0 |

## 3.5 Production of Perithecia

Table 5 presents results on the perithecia production. It stands out from table 5 that more than 3 timber perithecia (Grade 1) are produced by majority of strains. Only the strain Mindembo yields 3 and 10 perithecia (Grade 2) per sample observed. When considering the status of maturation of perithecia, we note that 50% of strains (Bunduki, Mangbakapale, Moboko, MUCL35223 and MUCL45580) have dark stromata without perithecia.

**Table 5. Abundance and state of maturity of perithecia**

| Strains | Abundance of perithecia | State of perithecia |
|---|---|---|
| Bunduki | 1 | S |
| Mangbakapale | 1 | S |
| Mindembo | 2 | E |
| Moboko | 1 | S |
| N-Dame | 1 | G |
| Zobolia | - | - |
| B10101(2)J | 1 | E |
| MUCL35223 | 1 | S |
| MUCL45580 | 1 | S |
| Témoin | - | - |

*S = dark stromata without perithecia; G = stromata with ostiolate perithecia; E = empty degraded perithecia.*

Perithecia produced by Mindembo and B10101 (2) J are empty and derelict. N-Dame has *stromata with ostiolate perithecia*. By cons, in Zobolia or abundance, or the ripeness of the perithecia was observed, this could explain the absence of mortality in seedlings inoculated with this strain.

### 3.6 Reisolation of *Fusarium xylarioides*

Table 5 reports the results of reisolation of the subjects performed in patients infected with different strains.

**Table 6. Presence of species of *Fusarium* in dead wood**

| Strains | Level of timber removed | *Fusarium* sp. | | |
|---|---|---|---|---|
| | | ***F. xylarioides*** | ***F. solani*** | ***F.stilboides*** |
| Bunduki | (1) | + | - | - |
| | (2) | + | - | - |
| | (3) | + | - | - |
| Mangbakapale | (1) | + | - | - |
| | (2) | + | - | - |
| | (3) | + | - | - |
| Mindembo | (1) | - | - | + |
| | (2) | + | - | - |
| | (3) | + | - | - |
| Moboko | (1) | + | - | - |
| | (2) | - | + | - |
| | (3) | + | - | - |
| N-Dame | (1) | + | - | - |
| | (2) | + | - | - |
| | (3) | + | - | - |
| Zobolia | (1) | - | - | - |
| | (2) | - | - | - |
| | (3) | - | - | - |
| B10101(2)J | (1) | + | - | - |
| | (2) | + | - | - |
| | (3) | + | - | - |
| MUCL35223 | (1) | + | - | - |
| | (2) | + | - | - |
| | (3) | + | - | - |
| MUCL45580 | (1) | + | - | - |
| | (2) | + | - | - |
| | (3) | + | - | - |
| Témoin | (1) | - | - | - |
| | (2) | - | - | - |
| | (3) | - | - | - |

*+:presence of Fusarium species; - : Not observed*

Seedlings died after inoculation with strains *F. xylarioides* showed positive results in reisolation. The *F. xylarioides* was found at sites at varying distances (from 2 to 4 cm) of the points of inoculation. The presence of *F. solani* and *F. stilboides* has been recorded on dead wood inoculated with Moboko and Mindembo respectively. No cases of reisolation were recorded on samples of seedlings inoculated with Zobolia and the control seedlings. Similar observations have been recorded in previous trials in greenhouse (Tshilenge et al., 2004).

## 4. CONCLUSION

Our study aimed to determine the variability of pathogenic strains of *Fusarium xylarioides*. We used to do the strains of this fungus, collected from the diseased Robusta coffee from the Equator province (DRC) that we compared with other geographic origins on the clone L251.

It stands out from the results obtained after inoculation of seedlings, that alterations in leaves (wilting, browning and drying) were induced by all strains, characteristics of the Coffee Wilt Disease. Regarding the time of onset of these symptoms, wilting and browning are not in chronological order, that is to say, wilting can occur before or after the leaves turn brown and vice versa. But both signs progressing to desiccation and seedling mortality, except where noted on seedlings inoculated with strain Zobolia where seedling mortality was not observed. This reflects a low level of aggressiveness of this strain. The comparison of the onset time of symptoms between different averages of reference strains (B10101 (2) J, MUCL 35223 and MUCL 45580), and those of Equateur, showed that the recorded values in the time length expression of different symptoms on the one hand and their severity expressed as AUDPC on the other have also reported variability between strains. Mindembo is different from others in the early onset of symptoms (Table 3) and higher values of AUDPC (Fig. 2). Our results confirm those previously obtained by Tshilenge et al. (2004) who worked on the characterization of *Fusarium* spp. associated with dieback of Robusta coffee in the DRC; their study was conducted on plant material originating from Costa Rica. Regarding the mortality rate of seedlings induced by different strains, Mindembo was more aggressive and induced a high mortality. As for the other strains, they are considered less aggressive. Zobolia induced no deaths (Table 4). Almost all strains produced fewer perithecia which for the most part without perithecial stromata are dark (state S). Reisolation of strains confirmed the presence of the *Fusarium xylarioides* which was found at all levels of timber harvest parasitized (Table 5). However, the presence at certain levels, other *Fusarium* species has been reported, including *Fusarium solani* and *Fusarium stilboides*. These results are consistent with the literature (Tshilenge *et al*., 2004). It appears in light of the results obtained great variable among strains of *Fusarium xylarioides*, variability suggesting that some level of genetic diversity among strains of *Fusarium xylarioides* responsible for the wilt of coffee robusta. However, this diversity, seen in relation to phenotypic criteria (wilting, browning and drying of leaves), has not yet been confirmed by molecular analysis. This variability of pathogenicity seems to be related to geographic origin and age of strains (Moboko from Equateur and isolated in 2004) and MUCL 35223 (from the Province Orientale and isolated in 1992) induce dryness in the same period (21 days). In the case of death, we find that strains B10101 (2) J (from Nord Kivu and isolated in 2003), N-Dame and Bunduki (from the Equateur province and isolated in 2004) causing the death of seedlings about 23 days after inoculation. To better understand the reasons for the resurgence of this disease in the Democratic Republic of Congo, it would be important for further research of the genetic diversity of *Fusarium xylarioides.*

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## REFERENCES


Anonyme. (1996). Etude sur la relance de la production caféière et la lutte contre la trachéomycose. Document Officiel Zaïrois de café (OZACAF), réalisé par le Bureau d'Etudes et d'Ingénieurs Conseils (BETIC), p.85.

Charier, A., Eskers, A.B. (1997). Amélioration des plantes tropicales. Service des éditions du CIRAD, 4ème trimester, p.171.

Flood, J. (1996). A study of the Tracheomycosis or vascular wilt disease of coffee in Zaire. – International Mycological Institute (IMI), UK.

Flood, J., Brayford, D. (1997). Re-emergence of *Fusarium* wilt of coffee in Africa. In: Proceedings of 17th International Scientific Colloquium on Coffee (ASIC), 20–25 July 1997. Nairobi, Kenya, pp. 621– 627.

Fraselle, J. (1950). Observations préliminaires sur une trachéomycose de Coffea robusta. Bull. agricole du Congo belge., Vol. XLI, n°2 , pp. 361-372.

Fraselle, J., Valleays, H., Deknop, O. (1953). La lutte contre la trachéomycose du caféier à Yangambi et le problème que pose actuellement cette maladie au Congo belge. Bulletin d'information de l'INEAC., volume 2, n°6, pp. 373-394.

Girma, A., Hindorf, H. (2001).Investigation on coffee tracheomycosis, Gibberella xylarioides (*Fusarium xylarioides*) in Ethiopia.19th International Scientific Colloquium on coffee (ASIC)., 14-18 may, Trieste, Italy.

Girma, A., Hindorf, H., Steiner, U., Nirenberg, H. I., Dehne, H.W., Schellander, K. (2005). Genetic diversity in the coffee wilt pathogen (Gibberella xylarioides) populations: Differentiation by host specialization and RAPD analysis. Journal of Plant Diseases and Protection, 112 (2), 134–145.

Kalonji, M. (1975). La trachéomycose fusarienne (carbuncularioise) du caféier robusta (*Coffea canephora* Pierre), Mémoire de fin d'études, Faculté des Sciences Agronomiques, UNAZA, inédit., 41pp.

Katenga, M. (1987). Identification de la trachéomycose du caféier et évaluation de son ampleur dans le Haut-Uelé et dans la zone de Poko. Document OZACAF, Inédit.

Makoko, M., Ndembo, L., Nsimba, M. (1992). Les sols du Mont Amba, caractéristiques pédologiques, mécaniques et stock d'eau de sol. Révue Zaïroise des Sciences Nucléaires, CREN-Kinshasa., volume 2.

Maraite, H. (2002).The life of *Gibberella xylarioides* Heim and Saccas in developpent of a long term strategy based on wilt disease in Africa. 1st intermediate report, international scientific, cooperation projet (INCO), pp 25-40.

Mfwidi-Nitu, P. (1994). The recrudescence of tracheomycosis (Gibberella xylarioides) of Robusta coffee in Zaire. Afr. Coffee Bull., 40, 9–12.

Mukuna, K., Isungu, N., Masozera, R., Kalonji, M. (1990). Relance de la caféiculture et lutte contre la trachéomycose dans la région pilote du Haut-Zaïre. Rapport présenté à l'Office Zaïrois de Café (OZACAF). p63 , Inédit.

Rapilly, F. (1991). L'épidémiologie en pathologie végétale: mycoses aériennes, service des Editions, route de Saint Cyr, 78026 Versailles Cedex, France.

Saccas, A. (1951). Recherches préliminaires sur la trachéomycose du caféier exelsa Chev. Bull. Stat. Centre Boukoko, pp, 2110-2115.

Skajennikoff, M., Rapilly, F. (1989). Variabilité du pouvoir pathogène chez Septoria nodorum Berk. (*Leptophaeria nodorum* Müll.). Agronomie, 9, 693 – 702.

Tshilenge-Djim, P., Kalonji, M., Onyembe, P.M.L., Mukuna, K., Dibwe, M., Oripale, M. (1998). Caractéristique et évolution spatio-temporelle de la trachéomycose fusarienne du caféier robusta en République Démocratique du Congo (RDC). Rev. Cong. Sci., 14, 132-140.

Tshilenge-Djim, P., Munaut, F., Kalonji, M., Maraite, H. (2004). Caractérisation des *Fusarium* spp. associées au dépérissement du caféier Robusta en République Démocratique du Congo. Parasitica, 60(3-4), 67 - 82.

Tshilenge-Djim, P. (2007). Maladie du dépérissement du caféier (Coffea canephora Pierre) Robusta en République Démocratique du Congo: Analyse de la diversité dans la population pathogène (Gibberella xylarioides Heim et Saccas, anamorphe *Fusarium xylarioides* Steyaert) et dans les accessions locales du caféier. Thèse de doctorat, Université de Kinshasa, Kinshasa.

Tshilenge, L., Kalonji, M., Tshilenge-Djim P. (2010). Determination of cultural and biometrical characters of *Fusarium* species isolated from plant material harvested from coffee (*Coffea canephora* Pierre.) infected with CWD in Democratic Republic of Congo. Afr. J. Agric. Res., 5(22), 3145-3150.

Vanderplanck, J.E. (1984). Disease Resistance in plants, second Ed, plant protection research Institute Pretoria, Republic of South Africa, 125, 57-80.