

# Plant Pathology Journal

ISSN 1812-5387





# **Plant Pathology Journal**

ISSN 1812-5387 DOI: 10.3923/ppj.2017.73.81



# Research Article Distribution and Incidence of Cassava Anthracnose in Côte D'ivoire and Pathogenic Characteristics of *Colletotrichum* gloeosporioides Penz Manihotis Isolates

<sup>1,2</sup>YEO Foungnigué Edwige, <sup>1</sup>EHUI Kouadio Jean Nestor, <sup>1</sup>AMARI Ler-N'Ogn Dadé Georges Elisée, <sup>2</sup>N'Guetta Assanvo Simon-Pierre and <sup>1</sup>KONE Daouda

<sup>1</sup>Laboratory of Plant Physiology, Department of Biosciences, University Félix Houphouët-Boigny of Cocody-Abidjan, 22 B.P. 582, 22 Abidjan, Côte d'Ivoire

<sup>2</sup>Laboratory of Genetics and Species Improvement, Department of Biosciences, University Félix Houphouët-Boigny of Cocody-Abidjan, 22 B.P. 582, 22 Abidjan, Côte d'Ivoire

# Abstract

Background and Objective: Anthracnose caused by Colletotrichum gloeosporioides Penz manihotis is one of the major constraints of cassava production in the world. However, in Côte d'Ivoire, there is little work on the evolution of this disease, which could make it possible to envisage an effective strategy to fight against its spread. This study aims to establish the distribution and the incidence of anthracnose in Côte d'Ivoire on the one hand and to determine the morphological and pathogenic characteristics of Colletotrichum gloeosporioides Penz manihotis isolates on the other hand. Materials and Methods: Assessments of the disease followed through two surveys, in 2014 and 2015, in all the agro-ecological zones on the different varieties of cassava cultivated. The isolates of Colletotrichum gloeosporioides Penz manihotis were analyzed morphologically and their pathogenic activity was evaluated after inoculation to cassava plants under controlled conditions. The data collected were analyzed using Statistica software version 7.1 and then by using ANOVA (one-way). Results: The infection has reached epidemic levels with a national average incidence of 39.44% resulting from an infestation rate of 72% in all the fields visited. The analysis of certain morphological characteristics showed the existence of a phenotypic diversity in the strains of Colletotrichum gloeosporioides Penz manihotis. The pathogenic activity of these isolates, under controlled conditions, also revealed heterogeneity of aggressiveness on cassava varieties. The improved variety (Bocou 1) appeared sensitive as the traditional variety (Yacé) with an average sensitivity score of 27.5. Conclusion: Cassava anthracnose was found in all seven agro-ecological zones of Côte d'Ivoire with variable incidence from one plot to another. In addition, the isolates of C. gleosporioides Penz manihotis were different in appearance, color and growth of their mycelium and in their aggressiveness on cassava varieties. Results of this study required to find effective methods of fight against this disease.

Key words: Cassava, anthracnose, incidence, severity sensitivity, phenotype, pathogenicity

Received: January 17, 2017

Accepted: March 02, 2017

Published: March 15, 2017

Citation: Yeo Foungnigué Edwige, EHUI Kouadio Jean Nestor, AMARI Ler-N'Ogn Dadé Georges Elisée, N'Guetta Assanvo Simon-Pierre and Kone Daouda, 2017. Distribution and incidence of cassava anthracnose in côte D'ivoire and pathogenic characteristics of *Colletotrichum gloeosporioides* Penz *Manihotis* isolates. Plant Pathol. J., 16: 73-81.

Corresponding Author: AMARI Ler-N'Ogn Dadé Georges Elisée, Laboratory of Plant Physiology, Department of Biosciences, University Félix Houphouët-Boigny of Cocody-Abidjan, 22 B.P. 582, 22 Abidjan, Côte d'Ivoire Tel: +225 48 07 58 71, +225 06 08 04 63

Copyright: © 2017 Yeo Foungnigué Edwige *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

# INTRODUCTION

In Côte d'Ivoire, cassava production ranks second in food crop production after yam, with an estimated tonnage of 2.5 million in 2013 for an average yield per hectare of 6.7 t<sup>1</sup>. According to the FAO<sup>2</sup>, the daily consumption of cassava per capita in 2011 represents an energy value of 331 Kcal. There are a large number of products derived from cassava processing in Côte d'Ivoire. Attiéké is the major derivative from the point of view of human consumption with 5% of food expenditure and 20.5% of calories of the food intake of the Ivorian populations<sup>3</sup>. Industrial processing produces alcoholic beverages, biodegradable paper and flour<sup>4</sup>. Cassava is grown throughout the Ivorian territory. A large variety of local and improved cultivars<sup>4</sup> including Bonoua, Yacé and Bocou1, Bocou2, Bocou3 and TMS4(2) 1425 are encountered in the production plots. Also, crops are threatened by phytoparasites, the most damaging of which are Begomoviruses (ACMV, EACMV and EACMCV), the bacteria Xanthomonas axonopodis<sup>5</sup> and the fungus (Colletotrichum gloeosporioides)<sup>4</sup>. Anthracnose caused by Colletotrichum gloeosporioides Penz manihotis remains the major and dreadful fungal infection as it remains very damaging. It is of high epidemic proportions in high-production areas where it causes significant economic losses<sup>6</sup>. It is an infection of the aerial parts of cassava plants singularly the stem and the petioles<sup>7</sup>. During the crop cycle, these parts show numerous surface cankers, localized lesions on the lignified or non-lignified parts and also strong defoliation as well as apical dryness<sup>8</sup>. For setting in, the infection requires injury or weakening of the tissue that will be the gateway to the pathogen. According to Sharma and Kulshrestha<sup>9</sup>, its evolution occurs at temperatures ranging between 24 and 28°C with a relative humidity level of around 87% and average pH ranging also between 5.8-6.5. In Côte d'Ivoire, the distribution, incidence and specificity of isolates causative agents of cassava anthracnose remain unknown to date. This study aims at studying the distribution and the incidence of cassava anthracnose across the different agro-ecological zones of Côte d'Ivoire on the one hand, characterize the phenotype and evaluate in controlled conditions the pathogenicity of some isolates causative agents of the disease on the other hand.

Table 1: Characteristics of agro-ecological zones (ZAE) of Côte d'Ivoire<sup>10</sup>

This study was needed to identify areas of high prevalence of cassava anthracnose in Côte d'Ivoire with a view to preventing or controlling the spread of this disease.

#### **MATERIALS AND METHODS**

# Material

**Plant material:** The plant material on which the observations were made during the survey was made up of all varieties of cassava grown in the plots of the agro-ecological zones visited. The local varieties encountered were Yacé, Akama, Dankwa and Diarrassouba, while the varieties from the National Center for Agronomic Research (CNRA) and in the process of adoption were Bocou1 and Yavo. The variety of cassava Yacé, unlike other cultivars, is known for its high sensitivity to pests<sup>4</sup>.

**Fungal material:** The isolates of *Colletotrichum gloeosporioides* Penz *manihotis* used in this study were obtained from samples taken in naturally contaminated cassava fields. A total of 10 isolates were used to test the pathogenicity of the fungus and sensitivity of cassava varieties.

**NB:** The values in the column "Annual average temperature (°C)" are those of the annual averages of temperatures followed by those of their Standard Deviations (SD).

# Methods

**Study areas:** Two surveys were conducted from July-August in 2014 and 2015 in the seven agro-ecological zones described to date in Côte d'Ivoire<sup>10</sup>. They tighten agro-climatic specificities with heterogeneity of soils, vegetation cover, temperature and rainfall (Table 1). The first five agro-ecological zones with forest cover are characterized by four seasons, including two rainy seasons and two dry seasons. Agro-ecological zones VI and VII extend over the two-season savanna region, including a long dry season and a short rainy season. Rainy seasons usually occur in two phases, one from April to July and another from September to November. The rest of the year is reserved for the dry season<sup>10</sup>.

| ZAE | Location and vegetation         | Pluviometry (mm) | Annual average temperature (°C) |
|-----|---------------------------------|------------------|---------------------------------|
|     | Southeastern forest zone        | 1400-2500        | 29.00±5.6                       |
| II  | Southwestern forest zone        | 1300-1750        | 23.50±13.4                      |
| III | Western forest zone             | 1300-2300        | 24.50±7.7                       |
| IV  | Eastern and Central forest zone | 1300-1750        | 23.50±1.4                       |
| V   | Transition zone                 | 1300-1750        | 23.51±3.4                       |
| VI  | Humid savannah zone             | 1150-1350        | 26.70±1.8                       |
| VII | Dry savannah zone               | 1150-1350        | 26.70±1.1                       |

Selection of observation sites and sampling: The plots and plants sampled were selected randomly following the major roads that cross the different Agro-Ecological Zones (ZAE) of Côte d'Ivoire. The cassava plots visited were 5 km apart in a sampling area. Observations were made on stems and petioles bearing cankers, deformations, lesions and dryness. In each plot on the 2 diagonals, 10 plants of cassava were randomly selected, observed and scored from 0-5 according to the slightly modified rating scale established by the International Institute of Tropical Agriculture (IITA)<sup>11</sup>. On this scale, depending on the type and extent of the symptoms, an index or degree of infection was assigned to the diseased plants. Thus, the following ratings were defined: 0 = No symptoms; 1 = Less numerous and superficial cankers on old lignified stems, 2 = Numerous and deep cankers on lignified stalks with deformation, 3 = Oval lesions on green stems, 4 = Numerous lesions on young stems and necroses in leaf axils, 5 = Strong defoliation, wilting or dryness of the extremities.

**Determination of epidemiological parameters:** The epidemiological parameters assessed were: The proportion (diseased incidence) and intensity of symptoms (severity index) of cassava anthracnose.

The Disease Incidence (DI) was determined from the proportion (%) of plants infected with anthracnose on the total number of plants observed in the plot, according to the equation<sup>12</sup>:

Disease Incidece (DI%) =  $\frac{\text{No. of diseased plants}}{\text{Total No. of plants}} \times 100$ 

The Severity Index (SI) was calculated using degrees of infection according to the equation<sup>13</sup>:

Severity Index (SI%) =  $\frac{\sum (\text{Scale} \times \text{No. plants infected})}{(\text{Highest scale} \times \text{Total No. of plants})} \times 100$ 

**Obtaining** *Colletotrichum gloeosporioides* **Penz** *manihotis* **isolates:** The isolation of fungi associated with symptoms of cassava anthracnose was performed according to the method described by Fokunang and Dixon<sup>14</sup>. The samples of symptomatic cassava stems and petioles collected during the surveys were previously cleaned with tap water and then explants were taken from the growth front of the necroses. These explants were disinfected for 3 min in 70% alcohol and 10% sodium hypochlorite (8°C) (both commercial products) and then rinsed 3 times with sterile distilled water (commercial). The explants were finally placed on PDA medium (purchased from Sigma-Aldrich) for 72 h for the culture of *Colletotrichum gloeosporioides* Penz *manihotis* isolates. Two Petri dishes were used for each sample at a rate of 5 explants per Petri dish. The different colonies that appeared were successively transplanted onto a new PDA medium (Sigma-Aldrich) until homogeneous colonies were obtained.

**Phenotypic characterization of isolates:** The phenotypic characterization of the *C. gleosporioides* Penz *manihotis* isolates obtained in the different cities was done at two levels. At the macroscopic level, the different isolates were characterized by the appearance, color and growth rate of the mycelium. The growth rate of the mycelium was calculated from the average mycelial growth in relation to the number of days of culture for each isolate. The growth of the mycelium was measured each day along two perpendicular axes marked out under each Petri dish and passing in the middle of the portion of colony cultured on the PDA medium (Sigma-Aldrich).

At the microscopic level, the characteristics taken into account were the shape, the dimensions and the concentration of spores produced. The number of spores was counted from 1 mL of a 14 day fungus suspension using a Malassez cell.

**Pathogenic characterization of isolates:** A characterization of the pathogenicity of a few isolates from different sources with variable phenotypic characteristics was performed. Ten selected isolates were used for the inoculation of two varieties of cassava (Yace and Bocou1) showing differential behavior to pests.

**Production of plants:** Two varieties of cassava (Bocou1 and Yacé) were used for the pathogenicity and varietal sensitivity test. Apparently healthy cuttings of these varieties were cultured in tanks filled with 2/3 of sterile wet soil. The tanks were placed in greenhouse in accordance with a completely randomized Fisher block experimental design. After 6 weeks of growth, the most vigorous plants were selected for inoculation. For each isolate, 5 plants of each variety of cassava were used for the inoculation.

**Preparation of the inoculum:** The sporulation was carried out from homogeneous colonies from 10 isolates of *Colletotrichum gloeosporioides* Penz *manihotis*, cultured on PDA medium (Sigma-Aldrich) for 14 days. The spores produced were recovered in sterile distilled water and then calibrated with an optical microscope at a concentration of 10<sup>6</sup> spores mL<sup>-1</sup> using a Mallassez cell.

**Inoculation:** The selected plants were artificially injured using a sterile needle. The injuries were made on the stem at internodes, on leaf axils and on petioles. The inoculations were carried out by depositing  $10 \,\mu$ L of the spore suspension on the wounds using a micropipette. The inoculated plants were monitored regularly until symptoms of anthracnose appeared.

**Assessment of symptoms:** The intensity of symptoms induced by the different *Colletotrichum* isolates on each variety of cassava was assessed 4 weeks after inoculation. It expressed the aggressiveness of the isolates on the varieties of cassava tested or the severity index of anthracnose. The rating scale used for symptom assessment included scores ranging between 1 and 5 according to the Wokocha and Nneke scale<sup>15</sup>. Thus, the following ratings were defined: 1 = Lesion length <3 mm with 0% leaf loss, 2 = Lesion length ranging between 3 and 5 mm with 1-10% leaf loss, 3 = Lesion length ranging between 6 and 10 mm with 11-50% leaf loss, 4 = Lesion length ranging between 11 and 25 mm with 51-90% leaf loss, 5 = Lesion length >25 mm with leaf loss >90%. The whole experiment was replicate 3 times.

The aggressiveness index of each isolate or severity index of each variety of cassava was calculated using degrees of infection according to the equation<sup>13</sup>:

Severity Index (SI%) = 
$$\frac{\sum (\text{Scale} \times \text{No. plants infected})}{(\text{Highest scale} \times \text{Total No. of plants})} \times 100$$

**Statistical analysis of data and construction of anthracnose distribution maps:** The distribution maps of the disease were established using the Mapinfo Professionnelle Software (7.5) with a map of Côte d'Ivoire on a scale of 1/100000. Data projection was carried out in the system WGS 84, Northern hemisphere zone 29. The zones visited were numbered from the coordinates of points taken using a GPS (Garmin). The colors on the maps were adopted, respecting the different classes that have arisen following the statistical analysis<sup>16</sup>.

The statistical analyzes were carried out using the software Statistical version 7.1. The data collected were subjected to statistical analysis of variance ANOVA (one-way). Separation and aggregation of significantly different averages were done by the Duncan test at 0.05 thresholds<sup>17</sup>.

# RESULTS

**Characteristics of anthracnose symptoms observed on cassava:** The different symptoms characteristic of the disease found during the surveys were in the form of: Numerous lesions on green stems (Fig. 1a) and severe necrosis in leaf

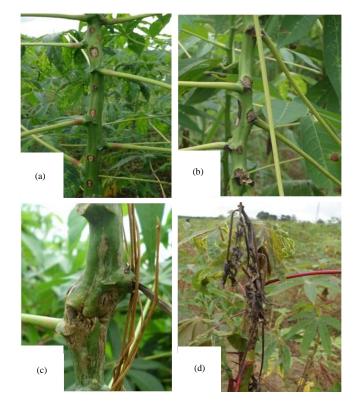
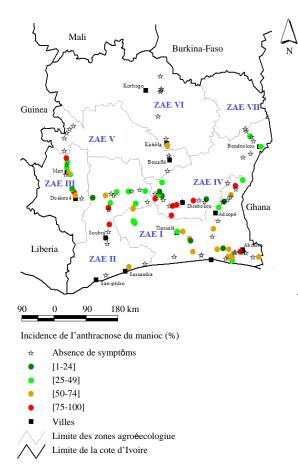
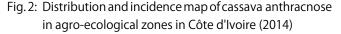


Fig. 1(a-d): Different symptoms of anthracnose observed in cassava fields, (a) Lesions between internodes, (b) Lesions at the insertion level of petioles, (c) Cankers caused by internal lesions and (d) Death of the tops





axils (Fig. 1b), petiole wilt followed by strong defoliation and more or less deep cankers (Fig. 1c), dryness of the tops (Fig. 1d). Some lesions on petioles and lesions with exudates were also found. The incidence of the disease increased from one year to the other and in 2014 was 37.09% and in 2015, 44% of the cassava plants observed, that is, respectively 47.96 and 72% of the plots visited.

Distribution and incidence of cassava anthracnose in Côte

**d'Ivoire:** Cassava anthracnose of caused by *Colletotrichum gloeosporioides* Penz *manihotis* has been found in all the seven agro-ecological zones of Côte d'Ivoire (Fig. 2, 3). The incidence of the disease differs from one plot to another within a single agro-ecological zone and varies according to the agro-ecological zone with a very significant difference (p = 0.00). The incidence of the disease is higher (between 50 and 100) in forest and mountainous zones (ZAE I, III and IV) for both years (2014 and 2015). The presence of the disease has also been observed in savannah zones with an incidence ranging between 25 and 100%, particularly in the

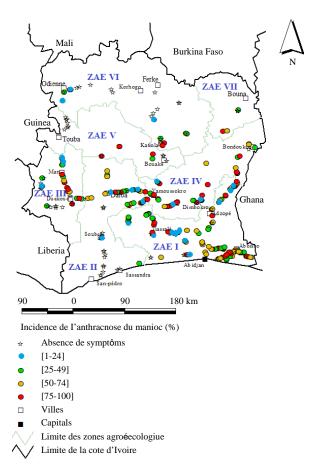


Fig. 3: Distribution and incidence map of cassava anthracnose in agro-ecological zones in Côte d'Ivoire (2015)

Northeastern zone where very few cassava plots were found (ZAE VII). The savanna zone in the Northwestern part (ZAE VI) and the transition zone (ZAE V) had the lowest incidence rates ranging between 1 and 25% (Fig. 2, 3). The national average incidence was 39.44%.

Severity of cassava anthracnose in Côte d'Ivoire: The severity of the disease reflects its seriousness on cassava plants in the fields. The severity of anthracnose was different from one plot to another within an agro-ecological zone. The average index of infection for plants with anthracnose varied from 1-5 in the plots visited. The Severity Index was significantly different p = 0.05 from one agro-ecological zone to another and ranged between 0 and 28.80 in 2014, then between 09.10 and 24.6 in 2015. The forest and mountain agro-ecological zones showed the highest indexes while the transition and savannah zones showed the lowest indexes. The agro-ecological zone VII has distinguished itself from other savanna zones with a fairly high index, but resulting from a very low number of cassava plots (Table 2).

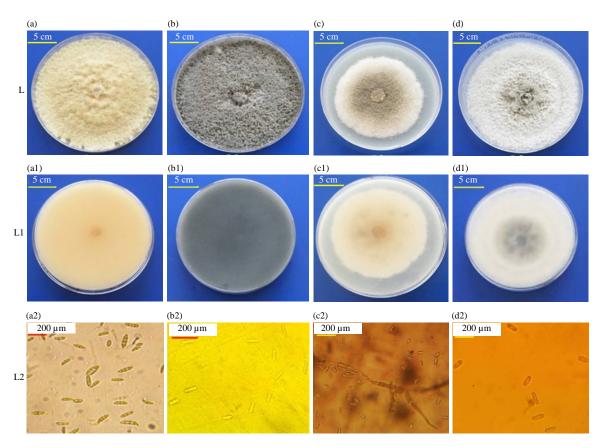


Fig. 4(a-d): Four phenotypic groups of *Colletotrichum gloeosporioides* Penz *manihotis* isolates L: Upper side of the mycelium, (A) Orangey thallus, (B) Gray thallus, (C) Gray-white thallus, (D) White thallus, L1: Lower face of the mycelium, (a1) Orange, (b1) Black, (c1) White-orangey, (d1) Black-white, L2: Conidia, Conidia shape, (a2) Fusiform, (b2) Cylindrical elongated, (c2) Abundant short cylindrical and (d2) Short cylindrical

Table 2: Average severity index of cassava anthracnose per agro-ecological zone in Côte d'Ivoire

|                       | Average severity index |                    |  |
|-----------------------|------------------------|--------------------|--|
| Agro-ecological zones | 2014                   | 2015               |  |
| 1                     | 26.70ª                 | 22.70ª             |  |
|                       | 28.80ª                 | 13.60 <sup>b</sup> |  |
| III                   | 25.30ª                 | 11.60 <sup>c</sup> |  |
| IV                    | 20.00 <sup>b</sup>     | 24.60ª             |  |
| V                     | 7.20 <sup>c</sup>      | 9.10 <sup>c</sup>  |  |
| VI                    | 0.00 <sup>c</sup>      | 9.80 <sup>c</sup>  |  |
| VII                   | 0.00 <sup>c</sup>      | 20.30 <sup>b</sup> |  |

On the same column, the averages followed by the same letter show no significant difference at 5% threshold according to the Duncan test

**Phenotypic characterization of** *Colletotrichum gloesporioides* **isolates:** A total of 102 isolates of *C. gloeosporioides* Penz *manihotis* were obtained. These isolates showed phenotypic diversity in the mycelium (color and appearance of the thallus) as well as in the conidia (age of sporulation, form and abundance of conidia). Indeed, the color and appearance of mycelial colonies varied from one isolate to another. Thus, the upper surfaces of the isolates showed an orange, gray, gray-white and white color while the lower sides were respectively orange, gray, orange and black-white (Fig. 4). Some isolates have borne fruit at very early age, that is, 7 days. In contrast, others sporulated at a later age (2-4 weeks or more). Some isolates obtained have largely borne fruits while others have yielded small amounts of spores. Different forms of conidia have been observed ranging from non-elongated or elongated cylindrical conidia to fusiform conidia (Table 3). Thus, the morphological characteristics made it possible to group these isolates into four strains (Fig. 4).

**Pathogenicity of fungal isolates tested:** All *Colletotrichum gloeosporioides* Penz *manihotis* isolates revealed pathogenic activity on the two varieties of inoculated cassava. These isolates showed significantly different p = 0.05 levels of pathogenicity (Table 4). The isolate A196 was the most aggressive with an average aggressivity index of 33.30. The isolates T012 and M506 were the least aggressive with an average aggressivity index of 19.20. The isolates having an average aggressivity index showed average index values ranging between 20 and 30 (Table 4).

#### Plant Pathol. J., 16 (2): 73-81, 2017

|                 |              |                             | Parameters measured          |                                      |         |              |            |
|-----------------|--------------|-----------------------------|------------------------------|--------------------------------------|---------|--------------|------------|
| Fungal isolates |              | Average spore concentration | Average mycelial growth rate | Average spore<br>dimensions (μm)<br> |         |              | Thallus    |
| Isolate codes   | Origin       | (spore mL <sup>-1</sup> )   | (mm J <sup>-1</sup> )        | Longueur                             | Largeur | Spore shapes | appearance |
| F067            | Foroforo     | 2.10 <sup>5</sup>           | 5.92                         | 14.56                                | 4.59    | Cylindrical  | Spongy     |
| M506            | Man          | 2.73.10⁵                    | 6.70                         | 11.66                                | 4.92    | Fusiform     | Fluffy     |
| Z339            | Zatta        | <b>2.87.10</b> ⁵            | 6.70                         | 19.19                                | 5.71    | Cylindrical  | Fluffy     |
| M505            | Man          | 1.2.10⁵                     | 5.57                         | 10.18                                | 4.36    | Cylindrical  | Fluffy     |
| B472            | Biankouma    | 4.13.10⁵                    | 5.37                         | 14.51                                | 3.71    | Cylindrical  | Fluffly    |
| T012            | Tiébissou    | 4.13.10 <sup>5</sup>        | 5.73                         | 14.56                                | 4.59    | Cylindrical  | Fluffy     |
| Y002            | Yamoussoukro | 3.10⁵                       | 3.95                         | 17.31                                | 4.42    | Cylindrical  | Woolly     |
| A196            | Abengourou   | 1.27.10⁵                    | 5.93                         | 21.8                                 | 5.79    | Cylindrical  | Woolly     |
| G449            | Gbatongouin  | 4.13.10 <sup>5</sup>        | 5.89                         | 19.53                                | 5.44    | Fusiform     | Woolly     |
| A217            | Agnibilekro  | 1.10⁵                       | 5.42                         | 15.8                                 | 5.92    | Fusiform     | Woolly     |

Table 4: Average aggressivity index of *Colletotrichum gloeosporioides* Penz *manihotis* isolates on cassava varieties Yacé and Bocou 1

| Fungal isolates | Average agressivity index |  |
|-----------------|---------------------------|--|
| M506            | 19.20 <sup>c</sup>        |  |
| T012            | 19.20 <sup>c</sup>        |  |
| M505            | 20.00 <sup>b</sup>        |  |
| B472            | 20.80 <sup>b</sup>        |  |
| Z339            | 20.80 <sup>b</sup>        |  |
| F067            | 22.50 <sup>b</sup>        |  |
| A217            | 25.80 <sup>ab</sup>       |  |
| Y002            | 26.70 <sup>ab</sup>       |  |
| G449            | 28.30 <sup>ab</sup>       |  |
| A196            | 33.30ª                    |  |

| Table 5: Sensitivity of cassava varieties to anthrachnose (Bocou 1 and Yacé) |
|--|
| 30 days after inoculation of <i>Colletotrichum gloeosporioides</i> isolates  |

| Colletotrichum  | Severity index per cassava variety |                    |  |
|-----------------|------------------------------------|--------------------|--|
| gloeosporioides |                                    |                    |  |
| Isolates        | Bocou 1                            | Yacé               |  |
| F067            | 30.00ª                             | 30.00ª             |  |
| M506            | 21.70ª                             | 21.70ª             |  |
| Z339            | 26.70ª                             | 26.70ª             |  |
| M505            | 26.70ª                             | 26.70ª             |  |
| B472            | 28.30ª                             | 28.30ª             |  |
| T012            | 16.70ª                             | 16.70ª             |  |
| Y002            | 28.30ª                             | 28.30ª             |  |
| A196            | 35.00ª                             | 35.00 <sup>a</sup> |  |
| G449            | 31.70ª                             | 31.70ª             |  |
| A217            | 30.00ª                             | 30.00ª             |  |

On the same line, the averages followed by the same letter show no significant difference at 5% threshold according to the Duncan test

Sensitivity of cassava varieties to anthracnose under controlled conditions: The two varieties tested Yacé and Bocou1 showed symptoms characteristic of anthracnose 30 days after the inoculation of the fungus. These symptoms had the appearance of longitudinal slits and rounded necroses on the stems of the improved variety Bocou1. However, on the local variety Yacé, they occurred in the form of dryness and strong defoliation. All symptoms developed around the inoculation zone. For a same isolate, the varieties of cassava Bocou1 and Yacé presented a level of sensitivity identical to the fungus. The severity indexes of the varieties Bocou1 and Yacé varied between 16.70 with isolate T012 and 31.70 with isolate G449 (Table 5).

On the same column, the averages followed by the same letter show no significant difference at 5% threshold according to the Duncan test.

## DISCUSSION

The parasite pressure of anthracnose was observed on all cassava cultivars found in the different agro-ecological zones of Côte d'Ivoire. The disease was expressed by various symptoms appearing along the stems and on the petioles. Indeed, the diversity of the symptomatic parts of the diseased plant and their different aspects might derive from the variable level of agressivity of the strains and the hypersensitivity reaction of certain varieties to the saliva of the vector<sup>10</sup>. Thus, ligneous stems of cassava had superficial cankers, the surface of which showed a regeneration of the cuticle. When the cankers became deep, it was followed by a deformation which might be linked to mycelial fungal proliferation in subepidermal cells9. The numerous oval lesions on the young stems and at the base of the petioles as well as the dryness of the tops might demonstrate the radial growth of the pathogen Colletotrichum gloeosporioides Penz manihotis. Similar symptoms have been described in several African countries, including Congo (RDC)<sup>8</sup>, Nigeria<sup>7</sup>.

The presence of anthrancnose on cassava was detected in all seven agro-ecological zones of Côte d'Ivoire, from the forest zones in the south to the savanna zones in the North. These observations reveal a rapid spread of this disease in the agro-ecological zones of Côte d'Ivoire. Indeed, revealed in western Africa in 1956 by Chevaugeon, the distribution of cassava anthracnose was confirmed in forest and transition zones<sup>18</sup>. Similarly, the eastern and southeastern forest zones covering the agro-ecological zones I, IV, in Côte d'Ivoire, had the highest indexes. Moisture factors associated with heavy rainfall and temperature changes might be the causes of this prolific activity of the pathogen. These conditions are exacerbated in zone I by rainfall heights and high temperatures and then in zone IV by loss of vegetation density and low temperatures. Indeed, Owolade et al.18 showed that there is an affinity between the variation in the severity of anthracnose and environmental conditions. However, according to Wokocha and Nneke<sup>15</sup> the intensity of the disease might specifically be related to the intrinsic abilities of the varieties. This argument seems more plausible to justify differences in the health status of plots within the same agro-ecological zone as shown in this study. The savannah zones of Northeastern Côte d'Ivoire are undergoing expansion and intensification of anthracnose damage. This observation could be explained by the adoption of a bad cropping systems due to the reuse of the already infected cultivation equipment and also to the voracious activity of the vector in this zone where it seems more mobile<sup>8</sup>.

The phenotypic identification revealed a great diversity in the isolates of *C. gloeosporioides* Penz *manihotis* obtained. This diversity might be the consequence of an existing genotypic variability within the populations of this fungus. Previous works on this pathogenic fungus in yam have shown its great spatial, temporal<sup>19</sup> and genetic variability<sup>20</sup>. Indeed, several genotypes have been found in the same lesion in Nigeria. This large variability may be due, in part, to the existence of a sexed form of the fungus called *Glomerella cingulata* (Ascomycete), also encountered in lesions at advanced stages of the disease. This suggests the potential for recombination that causes significant genetic shuffling<sup>21</sup>.

The pathogenic activity of *Colletotrichum gloeosporioides* Penz *manihotis* isolates revealed a total infection of the inoculated plants after injuries. These results confirm, on the one hand, the effectiveness of this method of inoculation as already noted on mangoes<sup>22,23</sup> and then on the other hand, the pathogenic efficiency of the isolates<sup>15,6</sup>. The mycelium of the fungus proliferating in intercellular and intracellular interstices is the cause of stem swelling and deformation. Similarly, the colonization of dead cells conditions the formation of necrotic plaque having various colors. This cumulative pathogenic activity on the selected varieties has helped detect a heterogeneity in the aggressivity of the different isolates tested. This difference in aggresivity has already been described by Wokocha *et al.*<sup>6</sup>.

## CONCLUSION

This study reveals that anthracnose has reached quite high epidemic levels in some agro-ecological zones of Côte d'Ivoire. It is found in all cassava cultivation areas of the country. Its incidence and severity are distributed heterogeneously according to plots and agro-ecological zones. These two epidemiological parameters were modulated by the great diversity of varieties domesticated and cultivated throughout the country. Thus the anthracnose epidemic in Côte d'Ivoire was expressed by various symptoms on both stems and leaf petioles in close interaction with environmental factors including temperature, humidity, type of vegetation and wind. At the phenotypic level, the strains were characterized by changable thallus colors. The high pathogenicity of strains circulating in cassava production areas impacts the distribution and intensity of the disease. The variable aggressivity of Colletotrichum gloeosporioides Penz manihotis isolates is a reliable pathway for their biological characterization in contrast to morphological characters.

# SIGNIFICANCE STATEMENTS

This study makes it possible to establish the distribution of anthracnose of cassava according to its intensity in Côte d'Ivoire on one hand and the morphological and pathogenic diversity of the isolates of *Colletotrichum gloeosporioides* Penz *manihotis* on the other hand. It was able to demonstrate a precise relationship between the agro-ecosystems where cassava is grown, the severity of the disease and the *Colletotrichum gloeosporioides* Penz *manihotis* strains present. This study will help the researcher to define the best management strategies for cassava anthracnose in the different zones of its culture in Côte d'Ivoire.

#### ACKNOWLEDGMENT

The authors wish to thank the Plant Physiology Laboratory, Department of Biosciences and of Félix Houphouêt-Boigny University (Cocody-Abidjan / Côte d'Ivoire) for providing the laboratory facilities.

#### REFERENCES

1. FAO., 2017. FAOSTAT database. Food and Agriculture Organization of the United Nations, Geneva. http://faostat.fao.org/site/339/default.aspx

- 2. FAO., 2013. FAOSTAT database. Food and agriculture organization of the United Nations, Rome, Italy.
- 3. Edith, K.K.N., K.K. Felix, K.Y. Francis, G. Theophile and T. Kablan, 2016. Characterization of waste from Attieke factory: Case of Azito village (Abidjan, Cote d'Ivoire). Eur. Scient. J., 12: 73-81.
- 4. N'zue, B., G.P. Zohouri and V. Yapi-Gnaore, 2013. Cultivating cassava in Cote d'Ivoire. CNRA (Direction of Research Programs and Development Support-Direction of Information Systems), Cote d'Ivoire, pp: 1-4.
- Kone, D., S. Dao, C. Tekete, I. Doumbia and O. Koita *et al.*, 2015. Confirmation of *Xanthomonas axonopodis* pv. *manihotis* causing cassava bacterial blight in Ivory Coast. Plant Dis., 99: 1445-1445.
- Wokocha, R.C., N.E. Nneke and C.I. Umechuruba, 2010. Screening *Colletotrichum gloeosporioides* F. sp *Manihotis* isolates for virulence on cassava in Akwa Ibom State of Nigeria. Agro-Science, 9: 56-63.
- 7. Obilo, O.P. and B. Ikotun, 2009. The effect of cassava anthracnose disease on the yield of some cassava cultivars in Eastern Nigeria. J. Applied Biosci., 14: 761-767.
- Mahungu, N.M. and K.W. Tata Hangy, 2014. Multiplication of cassava planting material and management of diseases and pests. Training Manual for Field Workers 1, International Institute of Tropical Agriculture (IITA) (CGIAR), Module 2: The Main Diseases of Cassava in the Democratic Republic of Congo and their Means of Fighting, pp: 14-23.
- 9. Sharma, M. and S. Kulshrestha, 2015. *Colletotrichum gloeosporioides*: An anthracnose causing pathogen of fruits and vegetables. Biosci. Biotechnol. Res. Asia, 12: 1233-1246.
- Halle, B. and V. Bruzon, 2006. Environmental profile of Côte d'Ivoire. Final Report Consortium AFC, AGRIFOR Consult, pp: 133.
- 11. IITA., 2005. Growing Cassava Commercially in Nigeria: Cassava Illustration Guide Book. International Institute of Tropical Agriculture, Ibadan, Nigeria, pp: 21-22.
- Martin, A.A., A. Kouabenan, T. o Seydou, N. Boni and K. Daouda, 2017. Geographical distribution and incidence of cassava bacterial blight (*Manihot esculenta* Crantz) caused by *Xanthomonas axonopodis* pv. *manihotis* in two agro-ecological zones of Cote d'Ivoire. Plant Pathol. J., 16: 1-11.

- Song, W., L. Zhou, C. Yang, X. Cao, L. Zhang and X. Liu, 2004. Tomato Fusarium wilt and its chemical control strategies in a hydroponic system. Crop Protect., 23: 243-247.
- 14. Fokunang, C.N. and A.G.O. Dixon, 2006. Post-harvest evaluation of *Colletotrichum gloeosporioides*f. sp. *manihotis* on cassava genotypes. Plant Pathol. J., 5: 60-66.
- 15. Wokocha, R.C. and N.E. Nneke, 2011. Cassava anthracnose disease and varietal screening for resistance in Akwa Ibom State of Nigeria. J. Agric. Sci. Technol., 1: 889-895.
- 16. StatSoft, 2010. STATISTICA: Prise en main. StatSoft, Pages: 336. http://www.statsoft.fr/pdf/STATISTICA-prise-enmain.pdf
- 17. Winer, B.J., D.R. Brown and K.M. Michels, 1991. Statistical Principles in Experimental Design. 3rd Edn., McGraw-Hill Inc., Boston, USA., pp: 1057.
- Owolade, O.F., A.G.O. Dixon and A.Y.A. Adeoti, 2006. Diallel analysis of cassava genotypes to anthracnose disease. World J. Agric. Sci., 2: 98-104.
- Onyeka, T.J., D. Petro, S. Etienne, G. Jacqua and G. Ano, 2006. Optimizing controlled environment assessment of levels of resistance to yam anthracnose disease using tissue culture derived whole plants. J. Phytopathol., 154: 286-292.
- 20. Barriere, V., 2009. Genetic analysis of resistance to anthracnose in yam *Dioscorea alata* L. Master Thesis, Avignon University, France.
- Abang, M.M., R. Asiedu, P. Hoffmann, G.A. Wolf, H.D. Mignouna and S. Winter, 2006. Pathogenic and genetic variability among *Colletotrichum gloeosporioides* isolates from different yam hosts in the agroecological zones in Nigeria. J. Phytopathol., 154: 51-61.
- Kouame, G.K., F. Sorho, D. Kone, L.E. Bomisso, S. Ake and J. Yatty, 2011. [A comparison of pathological activities of two *Colletotrichum gloeosporioides* (Penz.) isolats from two varieties of mangoes (*Mangifera indica* L.)]. Agronomie Africaine, 23: 33-42.
- N'Guettia M.Y., H.A. Diallo, N. Kouassi and F. Coulibaly, 2013. [Morphological and pathogenic diversity of strains of *Colletotrichum* spp. responsible for the anthracnose of mangoes in Cote d'Ivoire]. J. Anim. Plant Sci., 18: 2775-2784.