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SCREENING *COLLETOTRICHUM GLOEOSPORIOIDES* F.SP *MANIHOTIS* ISOLATES FOR VIRULENCE ON CASSAVA IN AKWA IBOM STATE OF NIGERIA.

Wokocha[1], R. C Nneke[2], N. E. and Umechuruba[1], C. I.
[1]Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike, P. M. B. 7267, Umuahia, Abia State, Nigeria.
[2]Department of Crop Science, University of Uyo, P. M. B. 1017, Uyo, Akwa Ibom State, Nigeria.

ABSTRACT
Six isolates of Colletotrichum gloeosporioides f.sp. manihotis obtained from anthracnose-infected cassava stems in six different cassava-growing locations of Akwa Ibom State were examined in the laboratory for morphological and physiological differences. The isolates were then screened in the greenhouse for virulence on cassava variety NR 8083 widely grown in Akwa Ibom State. Six-week old cassava plants were inoculated with 1ml of the spore suspension of each isolate of the pathogen at a concentration of 10^4 conidia/ml using a sterile hypodermal needle. One injection of inoculum was made at five different internodes of each cassava stem at an ambient temperature of 30^0C and relative humidity of 80%. Results of laboratory examinations showed that mycelial growth on potato dextrose agar (PDA) medium was fastest (10.65mm/day) in isolate CGM 2 from Ikono and slowest (7.29mm/day) in isolate CGM 6 from Uyo. Sporulation of the isolates on PDA medium under alternate light and dark regimes was highest (9.30 x 10^6 spores/ml) in isolate CGM 4 from Nsit Atai and lowest (1.23 x 10^6 spores/ml) in isolate CGM 5 from Oruk Anam. Percentage spore germination and conidia length were highest (85.64% and 23.55μm) respectively in isolates CGM 4 from Nsit Atai and CGM 1 from Essien Udim and lowest (49.86% and 13.75μm) in isolate CGM 3 from Itu and isolate CGM 2 from Ikono, respectively. Results of greenhouse inoculations showed variations in lesion size for the different isolates. The largest (15.30mm) lesion was produced by isolate CGM 4 from Nsit Atai, followed by isolate CGM 1 (12.60mm) from Essien Udim. There were no significant ($p \geq 0.05$) differences in lesion size for isolates CGM 3 (8.63mm) from Itu, CGM 2 (8.45mm) from Ikono and CGM 6 (8.41mm) from Uyo. Lesion size was smallest (5.00mm) in isolate CGM 5 from Oruk Anam. Isolate CGM 4 of C. gloeosporioides f.sp manihotis from Nsit Atai which showed the highest sporulation density and percentage spore germination, one of the longest conidia and largest lesion size on inoculated cassava stems, appeared to be the most virulent of the six isolates of the pathogen in Akwa Ibom State during this investigation.

Key Words: Fungal variability, fungal virulence, *Colletotrichum gloeosporioides* f.sp. *manihotis*, cassava, anthracnose.

INTRODUCTION

The cassava anthracnose disease (CAD) caused by *Colletotrichum glocosporioides* f.sp *manihotis* is one of the most important and widely distributed fungal diseases of cassava, worldwide (Lozano *et al*; 1981; Daudi and Saka, 1995). In Nigeria, incidence of the disease has been reported in Ibadan in Oyo state, described by Owolade *et al*; 2005 as a high infection zone for CAD. The disease has also been reported from Kwara, Ogun, Ondo, Plateau, Osun and Cross River States (Fokunang, 1995). The pathogen establishes in wounds created in young cassava stems by the insect *Pseudotheraptus devastans* (Family: Coreidae). Subsequent dissemination of the disease from plant to plant is by air currents and rain water splash particularly during the wet season at relative humidities of 80% and temperatures of 23 – 24^0C. (Makambila, 1984; Theberge, 1985).

In Africa, the CAD is of major importance because of its ability to cause stem damage in cassava. Severe anthracnose infection could result in loss of planting materials and total crop failure (Fokunang *et al*; 2000). In Nigeria, the disease was reported to have so reduced the amount of healthy plantable stems available to farmers, that a search for resistant cassava varieties was embarked upon (Ikotun and Hahn, 1994).

Economic losses due to the CAD in Nigeria and elsewhere are difficult to estimate

in view of the fact that the incidence and severity of the disease are weather (rainfall, relative humidity, temperature and wind) dependent, being highest when much rainfall coincides with early plant growth and lowest with scanty rainfall (Lozano and Booth, 1974). Differences in susceptibility of cassava varieties to CAD also influence damage (Van der Bruggen *et al*, 1990). However, considerable stem damage due to the disease has been reported from the Democratic Republic of Congo (DRC), where 90% of local cassava cultivars were rated as severely damaged (Muyolo, 1984).

Cassava (*Manihot esculenta* Crantz) is an important root crop in the developing countries of the humid tropics. Africa is the leading producer of cassava in the world responsible for an estimated 61% of the world total output of 167.7 million tones per annum (SPORE, 2005). Nigeria is the world's largest producer of cassava, accounting for more than 37 million tones per annum (FAO, 2004). Cross River, Akwa Ibom, Rivers and Delta, in that order, dominate state cassava production in Nigeria's South-South (FAO, 2006).

Differences have been observed in inherited morphological and physiological characteristics in isolates of various fungal pathogens (Tjamos *et al*; 1993). Fokunang (1995) reported significant differences in mycelia growth, sporulation density, conidia length and spore germination rate of isolates of the cassava anthracnose pathogen. Such differences including those in conidia size and shape, appresssoria morphology and host specificity have been used in the generic diagnosis of isolates of cassava anthracnose pathogen (Goffart, 1982).

Apart from morphological and physiological differences, there exists among isolates of *C. gloeosporioides* differences in pathogencity. Several isolates of *C. gloeosporioides* have been reported in Colombia in sour sop (*Annona muricata*). As new and more virulent isolates of the pathogen emerged, sour sop cultivars resistant in one year or location were found to have been susceptible in other years or locations (Alvarez *et al*, 2005). Differences in pathogenicity have also been observed in isolates of *C. lindemuthianum*, pathogenic on common bean in Central and South America (Marcial *et al*, 1995). This study was undertaken to determine any differences in the morphological and physiological characteristics and in the pathogenicity of different isolates of the cassava anthracnose pathogen in Akwa Ibom State.

MATERIALS AND METHODS

Sources of infected materials

Cassava stems showing anthracnose symptoms (sunken lesions or cankers) were collected in the wet season, September 2006 from infected fields (15 – 20km apart) in six major cassava-growing areas of Akwa Ibom State (lat. 4^0 - 6^0N; long. 7^0 - 9^0 E; Alt. 106m AMSL). The locations were Essien Udim, Ikono, Itu, Nsit Atai, Oruk Anam and Uyo.

Preparation of potato dextrose agar

Two hundred grammes of peeled and sliced potato was boiled until soft. It was strained through muslin cloth and then made up to one litre with distilled water. Twenty grammes each of agar and glucose were added. The mixture was boiled on a hot plate of a magnetic stirrer to dissolve the agar. It was autoclaved at 121^0C, 1.03 kgcm^2 pressure for 15 minutes. After cooling to 40^0C, 1ml of lactic acid (to inhibit bacterial growth) was incorporated into the molten PDA medium and gently swirled to obtain even distribution of the acid in the medium. Fifteen mililitres of the medium was dispensed into each sterile 9cm Petri dish and allowed to solidify.

Isolation and identification of the pathogen

Infected stems brought back to the laboratory from the six different locations were washed in running water to remove soil particles and other debris. Small pieces (3 -5 mm in size) were cut from the advancing edges of infection, surface-disinfected in 10% sodium hypochlorite solution for 3 minutes and rinsed in three successive changes of sterile distilled water. The stem pieces were dried between layers of sterile filter paper and three stem pieces from each location were plated on the PDA medium. The inoculated dishes were incubated at 28 $\pm$ 2^0C for 7 days and observed daily for fungal growth. Temporary slides of the pathogen was examined under x 40 of the research compound microscope. *Colletotrichum gloeosporioides* was identified according to Barnett and Hunter (1992) and Alexopoulos *et al* (1996).

Mycelia growth rates

Mycelia discs (5mm in size) were cut from the advancing margins of 7 - day old culture of each of the six isolates of the pathogen using a flame-sterilized cork-borer. One mycelia disc of each isolate was aseptically transferred to the centre of a PDA medium in 9cm Petri dish. There were four replications per isolate. The dishes were incubated at 28 $\pm$ 2^0C for 7 days. Radial

mycelia growth was measured daily along two diameters of the dishes using transparent ruler and the mean obtained for each isolate.

Sporulation density

Spore suspensions were obtained from pure cultures of the isolates on PDA incubated at 28 $\pm$ 2^0C under alternate 12 hours light and 12 hours dark regimes daily for 7 days. One culture plate of each isolate was flooded with 5ml of distilled water and the acervuli were dislodged with a small brush in order to release conidia into the water. The resulting spore suspensions were separately filtered through double-layered muslin cloth into sterile test-tubes. The number of spores (conidia) per suspension of the isolates was estimated using a haemocytometer and tally counter, according to the formula of Duncan and Torrance (1992):

$S = \frac{NV}{v}$, where

S = number of spores/ml
N = mean number of spores counted in 10 large squares
V = 1ml = 1,000mm^3
v = volume of spore suspension under cover glass = 0.0004mm^3

Spore germination

Spore suspensions of the isolates were obtained from 7 - day old cultures of the fungus on PDA. A drop of the spore suspension of each isolate was withdrawn using a sterile 1ml pipette, placed on sterile slides in a moist chamber and incubated at 28 $\pm$ 2^0C for 16 hours. Thereafter, a drop of lactophenol was added to each drop of the suspension on the slide to stop further spore germination (Fokunang, 1995). The slides were examined under the microscope for percentage spore germination based on the formation of germ tubes. One hundred spores were examined per isolate.

Conidia length

Conidia length was obtained by measuring 100 conidia per isolate using a calibrated binocular microscope at x 40 magnification and obtaining the mean.

Planting cassava in the greenhouse

Sandy loam soil was collected from the top soil of the University of Uyo Teaching and Research Farm using a hand trowel. The soil was sterilized in an autoclave at 121^0C for 15mins (1.03$kgcm^2$ pressure) and after cooling to 30^0C, the soil was scooped into 35cm perforated plastic pots. Disease - free cassava variety NR 8083, widely grown in Akwa Ibom State, was collected from the University of Uyo Commercial Farm. Two stem cuttings of the cassava variety, each 15cm long and made to include at least 3 nodes were planted in each pot. The pots were placed in the greenhouse at an ambient temperature of 30^0C and watered daily with 1 litre of distilled water per pot.

Preparation of inoculum

Inoculum was prepared from 7 - day old cultures of isolates by flooding each dish with 5ml of sterile distilled water and dislodging the spores with a small brush. The spore suspension for each isolate was separately filtered through double layers of muslin cloth to remove mycelia fragments and stalling materials and adjusted to 10^4 conidia/ml with the aid of a haemocytometer and tally counter. They were stored in sterile test-tubes in a refrigerator before inoculation.

Inoculation

Six weeks after planting, two cassava plants in each pot were inoculated in the laboratory with 1 ml of each spore suspension at a concentration of 10^4 conidia/ml. The plants were inoculated by the stem injection technique using a sterile hypodermal needle. Inoculations were made at five separate points in different internode regions of each plant. Control plants were similarly inoculated with 1ml of sterile distilled water. After inoculation, plants were covered with moist transparent polyethylene bags (to achieve 80% relative humidity) for 3 days. Thereafter, the polyethylene bags were removed and the inoculated plants were kept in the greenhouse at temperatures of 28 $\pm$ 2^0C and 60% relative humidity for 4 weeks. The design of the experiment was a randomized complete block (RCB) with four replications.

Assessment of pathogenicity

Size of lesions produced on the inoculated cassava stems was measured weekly for 28 days using a transparent ruler. The mean lesion size for each isolate was computed for analysis. Disease severity was assessed on a 5 - point scale similar to that used by Waller *et al.* (1998), where.

1 = O mm (no lesion)
2 = 1 - 5 mm lesion size
3 = 6 - 10 mm lesion size
4 = 11 - 25 mm lesion size
5 = More than 25 mm lesion size

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) and Fischer's least significant difference (FLSD) test was performed when the ANOVA showed significance

RESULTS

Mycelia growth rates

Three isolates, CGM 2 (Ikono), CGM 3 (Itu) and CGM 5 (Oruk Anam) with mean growth rates of 10.65mm/day, 10.12mm/day and 10.04mm/day respectively exhibited higher growth rates than the other three isolates, CGM 1 (Essien Udim), CGM 4 (Nsit Atai) and CGM 6 (Uyo) with growth rates of 8.20mm/day, 7.84mm/day and 7.29mm/day, respectively. Significant ($p \leq 0.05$) differences were observed between the growth rates of these two groups of isolates but not among them (Table 1). The isolates also showed differences in the colour of mycelium which ranged from white (CGM 1 and CGM 6), grey (CGM 4) to dark grey (CGM 2, CGM 3 and CGM 5) (Fig. 1).

Table 1: Parameters used for screening isolates of *Colletotrichum gloeosporioides* f.sp *manihotis* for virulence

Isolates*	Locations	Mycelia growth rates (mm/day)	Sporulation density (x 10^6 spores/ml)	Spore germination at 16 hours	Conidia length (µm)	Lesion size (mm)	Disease severity (1- 5)
CGM 1	Essien Udim	8.20	6.23	70.66	23.55	12.60	3.72
CGM 2	Ikono	10.65	2.22	64.00	13.75	8.45	3.10
CGM 3	Itu	10.12	2.45	49.86	19.25	8.63	3.23
CGM 4	Nsit Atai	7.84	9.30	85.64	20.90	15.30	4.00
CGM 5	Oruk Anam	10.04	1.23	58.00	16.50	5.00	2.37
CGM 6	Uyo	7.29	3.87	57.33	18.15	8.41	3.00
Mean		9.02	4.21	64.24	18.68	9.73	3.23
LSD (0.05)		1.69	1.13	9.06	2.48	2.01	0.81

* *Colletotrichum gloeosporioides* f.sp *manihotis*

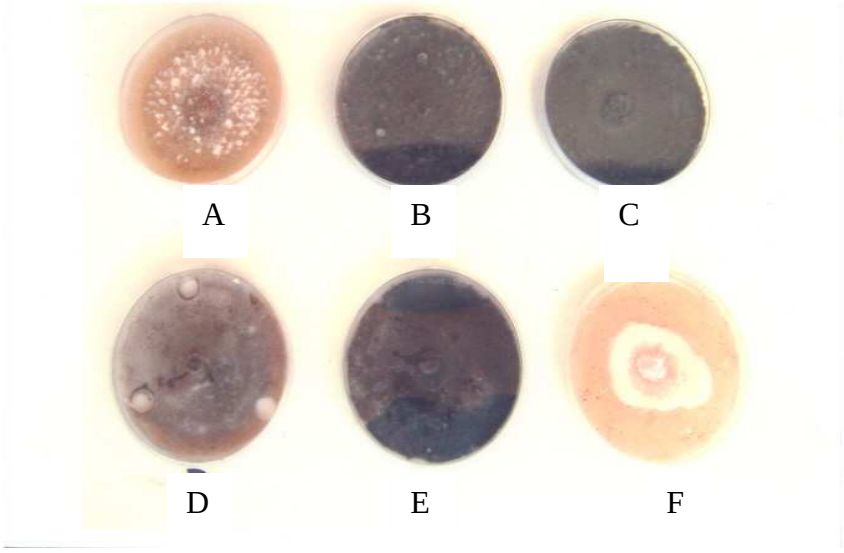

Fig. 1: Pure cultures of isolates of *C. gloeosporioides* f. sp. *manihotis* showing differences in colour of mycelium.

A = CGM 1 (white), B = CGM 2 (dark grey), C = CGM 3 (dark grey), D = CGM 4 (grey), E = CGM 5 (dark grey), F = CGM 6 (white).

Fig. 2: Large lesions (Arrowed) produced by isolate CGM 4

Sporulation density

Results (Table 1) show that isolate CGM 4 (Nsit Atai) had significantly ($p \leq 0.05$) the highest sporulation density (9.30×10^6 spores/ml). This was followed by isolates CGM 1 (Essien Udim) and CGM 6 (Uyo) with sporulation densities of 6.23×10^6 spores/ml and 3.87×10^6 spores/ml, respectively. The lowest sporulation densities of 2.45×10^6 spores/ml, 2.22×10^6 spores/ml and 1.23×10^6 spores/ml were recorded in isolates CGM 3 (Itu), CGM 2 (Ikono) and CGM 5 (Oruk Anam), respectively. Sporulation density and mycelia growth rate were not positively correlated ($r = 0.71$) at 5% probability level.

Spore germination

The highest (85.64%) significant percentage spore germination was recorded in isolate CGM 4 (Nsit Atai). This was followed by isolates CGM 1 (Essien Udim) and CGM 2 (Ikono) with percentage spore germination values of 70.66% and 64.00% respectively. The lowest spore germination was obtained in

isolates CGM 5 (Oruk Anam), CGM 6 (Uyo) and CGM 3 (Itu) with mean values of 58.00%, 57.33% and 49.86%, respectively. The spore germination values for these isolates were not significantly ($p \geq 0.05$) different from each other but were significantly ($p \leq 0.05$) lower than the other three isolates (Table 1). Spore germination and sporulation density were positively correlated ($r = 0.88$) at 5% probability level.

Conidia length.

Results in Table 1 further show that the longest significant ($P \leq 0.05$) conidia were observed in isolate CGM 1 (Essien Udim), followed by isolate CGM 4 (Nsit Atai), CGM 3 (Itu), CGM 6 (Uyo), and CGM 5 (Oruk Anam) with mean conidia lengths of 20.90µm, 19.25µm, 18.15µm and 16.50µm, respectively. However, no significant differences were observed in length of conidia between isolates CGM 1 (Essien Udim) and CGM 3 (Itu) and between isolates CGM 6 (Uyo) and CGM 5 (Oruk Anam). The shortest (13.75µm) conidia was recorded in isolate CGM 2 (Ikono). Conidia length and spore germination were not positively correlated ($r = 0.04$) at 5% probability level.

Assessment of pathogenicity

The six isolates of *C. gloeosporioides* f.sp *manihotis* were found to be pathogenic on cassava variety NR 8083 which showed necrotic lesions at the points of inoculation on cassava stems. Lesion damage was also observed on control plants inoculated with sterile distilled water. However, lesions produced on these plants were thin (0.50mm) and did not expand further throughout the duration of the experiment, as compared to the infected lesions that were very distinct, slightly depressed and expanded spots of various sizes. It is possible that the limited lesions observed in control plants were due to needle injury during inoculation.

There was a gradual increase in the size of necrotic lesions on infected plants at 7, 14, 21, and 28 days after inoculation. Results show that there was significant ($p \leq 0.05$) differences in lesion size produced by the six isolates tested. The largest (15.30mm) lesion was produced by isolate CGM 4 (Nsit Atai), 28 days after inoculation (Fig. 2). This was significantly ($p \leq 0.05$) larger than lesions produced by all other isolates. It was followed by isolate CGM 1 (Essien Udim) with a mean lesion size of 12.60mm. No significant ($p \geq 0.05$) differences were observed in isolates CGM 3 (Itu), CGM 2 (Ikono) and CGM 6 (Uyo) with mean lesion sizes of 8.63mm, 8.45mm and 8.41mm, respectively. Necrotic lesion size recorded in isolate CGM 5(Oruk Anam) was significantly ($p \leq 0.05$) smaller (5.00mm) than those of other isolates of the pathogen (Table 1).

Assessment of disease severity on infected plants on a 5 – point scale showed that isolate CGM 4 (Nsit Atai) recorded the highest (4.00) disease severity score and isolate CGM 5 (Oruk Anam) the lowest (2. 37). Disease severity scores for other isolates varied from 3.00 to 3.72 (Table 1).

DISCUSSION

This study shows that isolate CGM 4 (Nsit Atai) was the most pathogenic (severity score 4.00) on cassava variety NR 8083 among the six isolates tested. This isolate was found to have low (7.84mm/day) mycelia growth rate. Alvarez *et al* (2005) in their studies on soursop anthracnose disease caused by *C. gloeosporioides*, reported that slow growing isolates of the pathogen were more pathogenic than isolates with higher mycelia growth rates.

Sporulation density was highest in isolate CGM 4 (Nsit Atai) with a mean value of 9.30×10^6 spores/ml. It is possible that heavy sporulation in *C. gloeosporioides* f.sp. *manihotis* may be necessary for high levels of pathogenicity and virulence in the occurrence of the CAD. Muimba (1982) reported that isolates of *C. gloeosporioides* f.sp. *manihotis* that consistently sporulated heavily were able to produce abundant scecondary and tertiary inoculum for subsequent cycles of CAD infection. However, Alvarez *et al.* (2005) reported that isolates of *C. gloeosporioides* presenting limited sporulation *in vitro* were highly pathogenic on soursop in Colombia.

Spore germination is a necessary step in the sequence of events leading to development of disease among fungal pathogens. The high percentage of spore germination (85.64%) recorded in isolate CGM 4 (Nsit Atai) may be responsible for the high pathogenicity of the isolate observed in this study. The relationship between conidia length and pathogenicity in isolates of *C. gloeosporioides* f.sp. *manihotis* have not been previously reported. Results in this study show that isolate CGM 4 (Nsit Atai) and CGM 1 (Essien Udim) which have been shown to be more pathogenic than the other isolates in the study, had the longest conidia (20.90µm and 23.55µm), respectively. It is possible that conidia length and pathogenicity are positively correlated in isolates of the pathogen. The plausibility of a positive correlation between conidia length and pathogenicity in isolates of *C. gloeosporioides* f.sp. *manihotis* is being

reported for the first time. Differences in pathogenicity among isolates of *C. gloeosporioides* f.sp. *manihotis* observed in this study have been previously reported (Goffart, 1982; Jefferies *et al.* 1990; Fokunang, 1995). Besides, differential virulence has been reported among isolates of *C. lindemuthianum* pathogenic on common bean in Central and South America (Marcial *et al*, 1995).

Results of this study further show significant differences in isolates of *C. gloeosporioides* f.sp. *manihotis* in terms of mycelia growth rates, sporulation density, spore germination and conidia length. Fokunang (1995) reported that variations existed among isolates of *C. gloeosporioides* f.sp. *manihotis* in mycelia growth, spore germination and conidial length. Goffart (1982) adopted such parameters as radial growth, conidia size and shape, colony characterizations, appressoria morphology and host specificity in his generic diagnosis of isolates of *C. gloeosporioides* f.sp. *manihotis.* In a similar experiment, Ruppel (1972) reported a great variation in linear growth and sporulation of *Cercospora beticola* pathogenic on sugar beet, in his studies on isolates.

Despite its susceptibility to the CAD, cassava variety NR 8083 is still widely cultivated by farmers in Akwa Ibom State. This may be due to the fact that the variety was one of the earliest to have been introduced in the state. Results of this investigation, indicate that the variety was susceptible to the six isolates of the anthracnose pathogen. The high pathogenicity of isolate CGM 4 (Nsit Atai) to this cassava variety shown in this study should encourage screening of other cassava varieties grown in Akwa Ibom State for resistance to this isolate.

REFERENCES

Alexopoulos, C. J; Mims, C. W. and Blackwell, M (1996). Introductory Mycology (4th Ed.) John Wiley and Sons Inc. New York.338p.

Alvarez, E. Mejia, J. F; Llano, G. and Loke, J. (2005). Characterization of *Colletotrichum gloeosporioides*, causal agent of anthracnose in soursop in Valle del Cauca, Colombia. CIAT.

Barnett, H. L. and Hunter, B. B. (1992). Illustrated Genera of Imperfect Fungi (4th Edition) Burgess Publishing Company, Minneapolis, 241p.

Daudi, A. T. and Saka, V. W. (1995). Diseases and Nematodes of Cassava and Sweet Potato in Malawi. Proc. 6th Symp. ISTRC – AB pp. 44 – 47.

Duncan, C. and Torrance, L. (1992). Techniques for the Rapid Detection of Plant Pathogens. Blackwell Scientific Publishers, London. 234p.

FAO (2004). Food and Agriculture Organization. FAO Food Production Statistics. FAO, Rome.

FAO (2006). Food and Agriculture Organization. A Cassava Industrial Revolution in Nigeria. FAO Corporate Document Repository.

Fokunang, C. N. (1995). Evaluation of Cassava Genotypes for Resistance to Anthracnose, Bacterial Blight and Mosaic Diseases through Integrated Control Strategies. PhD Thesis, Univ. of Ibadan, Ibadan, Nigeria. 246p.

Fokunang C. N; Akem, C. N; Ikotun, T, Dixon, A G. O. and Tembe, E. A. (2000). Role of the insect vector, *Pseudotheraptus devastans* in cassava anthracnose disease development. *European Journal of Plant Pathology* 106:319 – 327.

Goffart, J. P. (1982). Variability of *Colletotrichum gloeosporioides* f.sp. *manihotis* MSc Thesis, Catholic Univ. Louvain, Belgium. 210p.

Ikotun, T and Hahn, S. K. (1994). Screening cassava cultivars for resistance to the cassava anthracnose (CAD). IITA, Ibadan.

Jefferies, P; Dodd J. C; Jeger M. J. and Plumblay, R. A. (1990). The biology and control of *Colletotrichum* spp in tropical fruit crops. *Plant Pathology* 39:343-366.

Lozano, J. C. and Booth, R. H. (1974). Diseases of Cassava (*Manihot esculenta* (Crantz). *Pest Article and News Summary* (PANS) 20: 30-54.

Lozano, J. C; Bellotti, A. C., Reyes, J. A., Howland, R. H., Leihner, D and Doll, J. (1981). Field Problems in Cassava. CIAT, Colombia 125p.

Makambila, C. (1984). Epidemiology of anthracnose in cassava. In: Root Crops: Production and Uses in Africa. Terry, E. R; Doku, E. V; Arene, O. B. and Mahungu, N. M. (Eds.) ISTRC, Canada. pp. 73-78.

Marcial, A. P., Maria, M. O. and Albeiro, M. I. (1995). Resistance to *Colletotrichum lindemuthianum* isolates in different common bean races. *Plant Diseases* 74(1):63-67.

Muimba, K. A. (1982). Predisposition of Cassava Plants to Infection by *Colletotrichum manihotis* Henn and some Factors Involved in the

Initiation of Anthracnose Disease. M. Phil. Thesis, Univ. of Ibadan, Ibadan, Nigeria, 242p.

Muyolo, G. (1984). Studies on the Interaction Between *Xanthomonas campestris* pv *manihotis* and *Colletotrichum gloeosporioides* f.sp. *manihotis* on Cassava and its Effects on Yield. M. Phil. Thesis, Univ. Ibadan, Nigeria 255p.

Owolade, O. F; Dixon, A. G.O; Adeoti, A. A. and Osunlaja, S. O. (2005). Sources of resistance to cassava anthracnose disease. *African Journal of Biotechnology* 4 (6): 570 – 572.

Ruppel, E. G. (1972). Variation among isolates of *Cercospora beticola* from sugar beet. *Phytopathology* 62:134-136.

SPORE (2005). Cassava-Booming Outputs meet Flagging Inlets. Issue 120, Dec. 2005. CTS – ACP-FC, Cotonou, pp. 4 -5.

Theberge, R. C. (1985). Common African Pests and Disease of Cassava, Yam, Sweet Potato and Cocoyam. IITA, Ibadan, Nigeria. 107p.

Tjamos, E.C; Papavisas, G. C. and Cook, R. J. (1993). Biological Control of Plant Diseases and Challenges for the Future. Plenum Press, New York . 462p.

Van der Bruggen, P; Gregoire P. D. and Maraite, H. (1990). Temperature-induced alteration in the expression of susceptibility to cassava to *Colletotrichum gloeosporioides* f.sp. *manihotis*. *Journal Phytopathoology* 130:46-58.

Waller, J. M; Ritchie, B. J. and Holderness, M. (1998). Plant Clinic Handbook. IMI Technical Handbooks No. 3, CAB International, Egham. 94p.