

## Post-harvest Evaluation of *Colletotrichum gloeosporioides* f. sp. *manihotis* on Cassava Genotypes

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**Abstract:** The aim of this study was to evaluate the post-harvest survival of *Colletotrichum gloeosporioides* f. sp. *manihotis* on infected cassava materials. Infected stem cuttings from 5 cassava cultivars 88/01084, 88/01983, 91/00395, 91/00317 and 91/00052, incubated at 25±2°C for 8 months, showed a significant decrease in fungal survival amongst the cassava cultivars. At 8 months incubation fungal recovery was lowest below 10% among the infected cultivars. Cassava stakes, stored at humid conditions under tree shades for 16 months, showed a gradual decrease in fungal survival up to the 10th month, after which survival peaked again from the 12th up to the 16th month of storage. Survival of the fungus on soil significantly reduced with incubation time and at the 6th month of sampling, there was no recovery recorded for all the soils from the field plot sites. Burial of infected materials for 150 days below 20-30 cm depth significantly reduced the survival of *C. gloeosporioides* f. sp. *manihotis* on infected materials. Burial at 30 cm depth for 150 days completely eradicated the fungus on the infected cuttings. These studies have shown that *C. gloeosporioides* f. sp. *manihotis* could survive on infected cuttings for more than 8 months, but less likely to survive in soil for up to 4 months. The potential of high cassava anthracnose disease transmission in cassava planting materials during storage is also reported.

**Key words:** Cassava, *Colletotrichum gloeosporioides* f. sp. *manihotis*, post-harvest, anthracnose

### INTRODUCTION

The Cassava Anthracnose Disease (CAD) caused by a host-specific fungus *Colletotrichum gloeosporioides* f. sp. *manihotis*, is considered to be a major economic disease in the tropics due to its potential for causing stem damage in cassava<sup>[1-4]</sup>. Cassava is a starchy root crop, which presents one of the most important food crops in the tropics, constituting a principal carbohydrate source for more than 800 million people in developing countries<sup>[5-9]</sup>. CAD reduces the quality of planting materials, leading to low germination and reduced leaf retention. Total crop failure has been reported in severe infection conditions<sup>[4,10-13]</sup>. CAD on young plants causes stem tip dieback, wilt, defoliation and stem deformation<sup>[14-16]</sup>.

The importance of the stem punctures and damage caused by *Pseudotheraptus devastans* feeding in the spread and development of cassava anthracnose disease has been reported<sup>[1,5-10,14,17,18]</sup>. The CAD fungus penetrates through insect puncture wounds on young cassava stems and extend through the cork layer or the epidermis up to the underside of the sclerenchyma, then immediately extends tangentially under lignified fibres<sup>[14,19,20]</sup>. *C. gloeosporioides* f. sp. *manihotis* and *C. gloeosporioides*, causal agents of

cassava (*Manihot* sp.) and yam (*Dioscorea* sp.) anthracnose diseases produce toxic metabolites which induce symptoms on plants similar to those induced by the pathogens<sup>[2,15]</sup>.

CAD is transmitted through infected plant cuttings during vegetative propagation<sup>[7,12]</sup> and by the insect vector *Pseudotheraptus devastans*<sup>[8,14,17]</sup>. Seed survival *C. gloeosporioides* f. sp. *manihotis* and seed transmission has also been reported<sup>[12,19]</sup>.

Cultural control measure has been described to a lesser extend as a possible control measure for anthracnose diseases of legumes and tropical fruits<sup>[19,22]</sup>. There is however, an information gap on the survival and possible over-seasoning of CAD on cassava post harvest materials. Information on the survival and recovery of CAD fungus on post harvest materials is also important for the adoption of effective post-harvest control measures under an effective farming practice within the intensive cassava cultivation regions, where season to season planting of cassava on the same piece of land is widely practiced. It is possible that by reducing CAD inoculum pressure in the field, the chances of CAD transmission can be controlled.

The aim of this study was to evaluate the post-harvest survival, of cassava anthracnose disease pathogen on infected materials and soil.

Table 1: Soil analysis of experimental plots at the International Institute of Tropical Agriculture Ibadan, Nigeria.

Soils characteristics	Plot locations				
	ES24	C1	BS1	BS17	B3
pH	6.80	6.50	6.70	6.90	6.600
Organic matter (%)	2.60	2.80	2.50	2.40	2.700
Total nitrogen (%)	0.05	0.09	0.06	0.07	0.080
Available phosphorus (ppm)	10.25	10.30	12.71	11.51	12.300
Exchangeable bases (Meq/100 g)	3.03	3.01	2.96	2.86	3.050
Calcium (Ca)	0.70	0.72	1.10	0.91	0.830
Potassium (K)	0.25	0.23	0.30	0.27	0.260
Sodium (Na)	0.26	0.28	0.32	0.30	0.240
Exchangeable acidity (Meq/100 g)	0.23	0.20	0.20	0.20	0.250
Base saturation	95.64	95.61	95.26	95.38	95.570
Texture					
Sand (%)	80.30	80.50	82.40	81.30	82.600
Silt (%)	14.00	14.20	11.50	12.80	13.200
Clay (%)	5.90	5.70	6.10	5.50	6.300

The IITA field plots locations ES24, C1, BS1, BS17 and B3, were used for cassava germplasm multiple pests and disease resistance evaluations screening

## MATERIALS AND METHODS

**Experimental sites:** The study was conducted after cassava annual harvest in the field, greenhouse, nursery and laboratories of the International Institute of Tropical Agriculture (IITA), located to the North of Ibadan at a latitude of 7°30'N and a longitude of 3°45'E. The altitude of the Institute is 210 m above sea level<sup>[23]</sup>. Soil samples were collected from the IITA field plots ES24, C1, BS1, BS17 and B3, used for cassava germplasm multiple pests and disease evaluation screening (Table 1).

**Collection, isolation and identification of the fungus:** Cassava stems (10 cm long) showing CAD symptoms with dark brown lesions and deep cankers were collected from infected field plots. The diseased stems were washed with running tap water and small pieces of infected materials were cut from the edges of the cankers, surface-sterilized for 3 min in 10% sodium hypochlorite solution and rinsed five times in sterile distilled water. The stem pieces were dried on sterilized filter paper and placed on solidified Potato Dextrose Agar (PDA) containing 100 mL<sup>-1</sup> sodium novobiocin as antibiotics to inhibit bacterial growth. The material was incubated at 25±2°C for 7 days. Observation was made daily for any mycelial growth. Identification of the fungus was done under the microscope and confirmation made using the procedure of Barnett and Htunter<sup>[24]</sup>. Pure cultures were obtained by sub-culturing onto fresh plates. Stock cultures were maintained on agar slants in McCartney bottles.

**Preparation of spore suspension:** Spore suspensions were prepared from 8-day-old cultures of the isolated fungus by flooding with sterile distilled water and dislodging the spores with small brush. The suspension was clarified by centrifugation at 2000 rpm for 3 min and the supernatant was filtered through 2 layers of sterile

muslin cheesecloth. The spore concentration was determined with a haemocytometer and adjusted to 2.0×10<sup>6</sup> spores mL<sup>-1</sup> of distilled water.

**Sampling and detection of *C. gloeosporioides* f. sp. *manihotis* on post-harvest materials:** Stem cuttings (10 cm long) from five infected cassava cultivars (91/00396, 89/00033, 88/01084, 91/00684 and 30572) were collected from two sites ES24 and BS1, at the IITA field plots after harvest and incubated at 25±2°C, 80% relative humidity conditions. Thirty infected stem cuttings were collected for each cassava cultivar. Small stem pieces (3 mm) were cut from the canker of infected stems using a knife. The fungal percentage survival for each month sampling was based on the total number of *C. gloeosporioides* grown on infected stem piece on PDA cultured over the total number of PDA-cultured infected stem pieces.

**Investigation of the survival of *C. gloeosporioides* f. sp. *manihotis* on cassava stakes under shade:** Post-harvest cassava stems of 100 cm long, 20-30 mm diameter and each containing at least 5 large CAD cankers (30 mm) sizes, from 5 cassava cultivars mentioned above, were tied into bundles and stored upright under a well-developed tree shade. The oldest ends of the stems were inserted into the soil and watered regularly to keep it moist and humid, thus enhancing twig germination and creating favourable conditions for the fungus to survive for longer periods. The stems were stored for 16 months, up to a stage where most of the old stems collapsed given rise to twig development with large leaf canopies. Sampling for fungal survival was done every two months by examination of infected plant parts for the presence of *C. gloeosporioides* f. sp. *manihotis*.

The percentage survival was calculated as the number of infected PDA cultured materials showing

the presence of *C. gloeosporioides* on the total number of infected stem pieces on the culture plates.

**Post-harvest soil sampling for fungal survival:** Soil samples were collected shortly after cassava harvest on 5 planting field sites (ES24, C1, BS1, BS17 and B3), at IITA. These plots all have 4 years continuous intensive cassava monocropping cultivation. Freshly collected soil samples from these plots were first analyzed for soil properties. Before investigation of soil for fungal survival, the soils were covered for 2 months with infected debris accumulated from post-harvest leaves and stem fragments serving as fungal baits. The soil mounts were watered regularly to keep the soil moist and humid, a conditions favourable for fungal growth. Bulk soils samples covered with debris were collected on a monthly interval and placed in labeled paper bags and taken to the laboratory. The soils were air-dried then crushed to remove the fungal spores/hyphae from heavier soil particles of the residues. The fine soil particles were mixed thoroughly in distilled water and allowed to sediment for 1 h. The supernatant was filtered through 2 layers of sterile Whatman No. 1 filter papers. The sterile filters trapped spores and were aseptically removed and plated on PDA which was colonized by the spores after germination. PDA was incorporated with 100 mg L<sup>-1</sup> of sodium novobiocin to inhibit bacterial growth and observed for any growth of fungi. From each plot soil 20 PDA plates were cultured each month and the percentage recovery based on the number of plates with fungal recovery on total number of plates. The fungus was identified following the procedure earlier described. Soil screening for fungal survival was terminated at the 6th month when there was no major recovery of fungi from the field plot soils.

**Effect of burial of infected cassava material on the survival of *C. gloeosporioides* f. sp. *manihotis*:** Infected stem cuttings (30cm long) from 5 cassava clones (88/01084, 88/01983, 91/00395, 91/00317, 91/00052), were collected from field plots with at least 3 large cankers (30 mm diameter). Wooden rectangular boxes were filled with steamsterilized soils. Stem cuttings from each cultivar were placed on the surface of the soil or buried at 10, 20 and 30 cm depth, in the wooden boxes. Cuttings were collected from each box treatments at 30, 60, 90, 120 and 150 days of storage. The cuttings were washed with running tap water to remove soil particles, then air dried in filter papers, after which thin sections (3 mm) from the cankered portions were teased out with a sharp table knife and surface-sterilized with 10% sodium hypochlorite for 3 min and rinsed in 5 changes of sterile distilled water. Fungus was isolated and identified.

**Pathogenicity assessment:** To confirm that the fungus surviving from buried CAD infected cassava cuttings was host specific to cassava and not from other soil saprophytic *Colletotrichum*, spore suspension isolated from infected soils and buried materials were used to inoculate 2 months old healthy potted greenhouse cassava plants. Inoculation was carried out using the stem puncture technique<sup>[25]</sup> at the internodal regions, followed by foliar spray of fungal suspension, using a hand sprayer until runoff. The plants were observed for characteristic CAD symptoms of necrotic lesions, wilt and defoliation.

**Statistical analysis:** Data collected for percentage fungal survival on infected stem cuttings, soil plot sites, buried materials and cassava bundles were analyzed according to the general linear model procedures of the Statistical Analysis System<sup>[26]</sup>. Fischer-protected Least Significant Difference test and the Duncan's Multiple Range test of mean separation were performed when the ANOVA showed significance. The means and standard errors of fungal recovery in soil and infected cuttings were pooled for graphic presentations.

## RESULTS

**Survival of *C. gloeosporioides* f.sp. *manihotis* on post-harvest infected stem cuttings from two plot sites:** There was a decrease in fungal viability on infected plant cuttings 91/00396, 89/00033, 88/01084, 91/00684 and 30572, from plots ES 24 and BS1 with increased incubation period (Fig. 1). Fungal survival was below 50% at 6 month of incubation and by the 8th month fungal recovery was at lowest as below 20% in all the cultivars from both plot sites.

**Fungal recovery on post-harvest cassava bundles under shade:** Fungal recovery on cassava bundles from cultivars 91/00396, 89/00033, 88/01084, 91/00684 and 30572, stored under humid conditions to optimize fungal viability, showed a general decrease among the cultivars, up to the 10th month of screening (Fig. 2). At the 12th -16th month fungal survival peaked again. At the 16th month, fungal recovery was at its maximum and the screening was terminated due to invasion of parent plants by the increased leaf canopy population from developing twigs. The twigs at this stage were re-infected by the CAD pathogen from the parent plants, through vector feeding or from rain splash.

**Fungal survival in post-harvest field plot soils:** Survival gradually reduced in all the soils from field plots C1, BS1, BS17 and B3 with increased sampling duration (Fig. 3). At the 4th months of soil sampling, fungal recovery of below

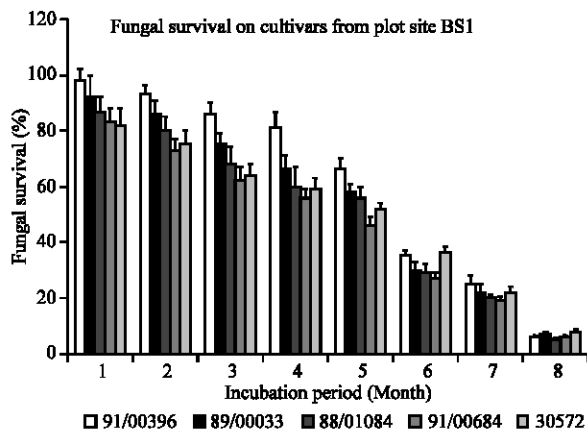
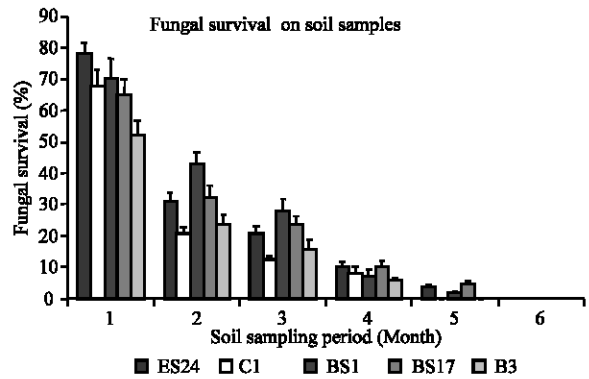
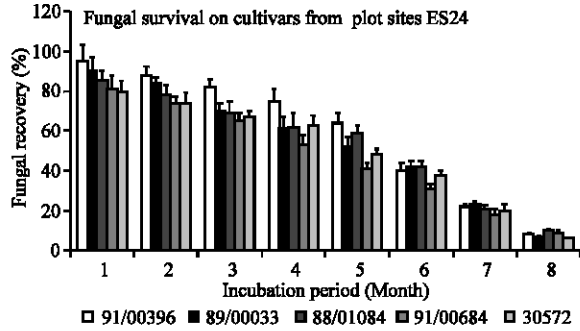


Fig. 3: Survival of *C. gloeosporioides* f. sp. *manihootis* on field plot soils

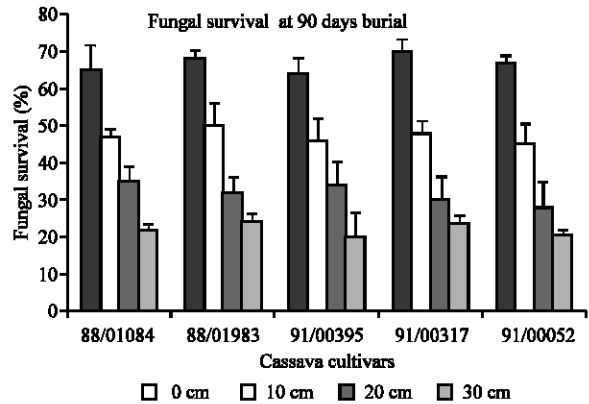


Fig. 1: Fungal survival of *C. gloeosporioides* f. sp. *manihootis* on cassava stems from plot sites ES24 and BS1

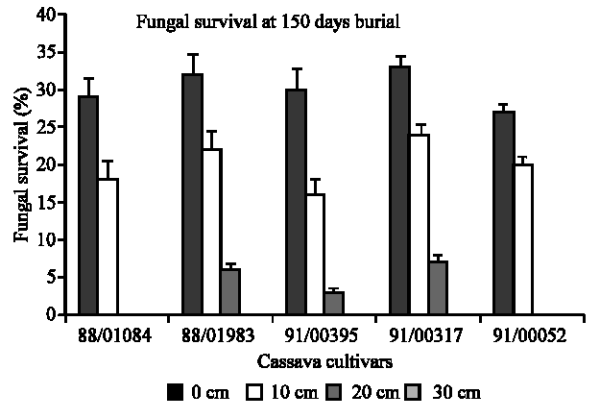
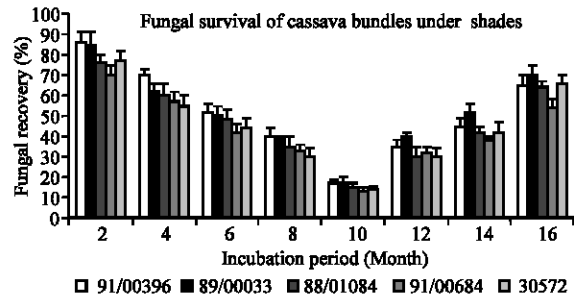


Fig. 2: Fungal recovery on post harvest cassava stakes under shades

Fig. 4: Fungal recovery on cassava infected materials buried in soils

20% was recorded in all the soil plot sites. At the 6th months of sampling, there was no evidence of fungal survival recorded in the soils for all the plot sites.

**Effect of burial of infected cassava material on fungal survival :** *C. gloeosporioides* f. sp. *manihootis* survival generally reduced with increased depth and duration of burial of infected plant materials (Fig. 4). In all the five

infected cultivars (88/01084, 88/01983, 91/00395, 91/00317, 91/00052), burial below 20-30 cm and incubated for 90 days and 150 days showed maximum reduction of fungal survival. Increased duration of burial for 150 days showed an overall recovery of below 10% for all the cultivars at 20 cm burial and complete eradication of fungal survival at 30 cm burial.

## DISCUSSION

There was a general decrease in fungal survival with increased incubation on post-harvest materials and on the soils at field plot sites. *C. gloeosporioides* f. sp. *manihotis* is an important disease of cassava, causing significant crop failure and economic losses to cassava growers, who depend on this crop as their main source of income<sup>[2-4,22,26,27]</sup>. The potential of high cassava anthracnose disease transmission in cassava planting materials during storage is also reported. Despite the devastating effect of CAD, there have been no studies to assess the survival potential of the fungus on post harvest infected materials. The study indicates that the CAD pathogen could possibly survive on infected stem cuttings for at least 8 months and in soil for up to 4 months.

Cassava is mostly propagated by cuttings and after harvest are stored upright in bundles under shades, partly tips buried in the soil for maintaining freshness and humidity. *C. gloeosporioides* f. sp. *manihotis* is host specific for cassava, this limits invasion and survival of the fungus on alternative host and thus creates a potential for control under good sanitation and cultural control practices. The development of twigs and leaf canopy at a later stage of the storage of cassava bundles creates a potential post-harvest field-field CAD transmission<sup>[27,28]</sup>.

The most successful control strategy for CAD has been through breeding and the use of disease resistant varieties for propagation<sup>[9,29]</sup>. Other control strategies using growth inhibition agents which are less dangerous than chemical reagents have been reported<sup>[28,30-32]</sup>.

This study has shown that deep burial of infected materials significantly reduced *C. gloeosporioides* f. sp. *manihotis* inoculum under field conditions. Planting of cassava and other tuber crops is commonly done on constructed ridges or mounts. The ridges are usually separated from each other by furrows which can attain depth of about 70 cm depending on the height of the ridges. This cultivation system makes it easier for farmers to effectively adapt deep burial systems of infected material for CAD control under the crop rotation system. After cassava harvest, the infected materials could be laid in the furrows and new ridges are constructed on these furrows thus burying the infected materials. The burial of infected materials in furrows after harvest within a crop rotation system could break the life cycle of *C. gloeosporioides* f. sp. *manihotis* and serve as a potential cultural control option by reducing the inoculum pressure under field conditions.

Pre-and post-harvest survival of anthracnose diseases on avocado and other tropical fruits has been reported<sup>[20,33,34]</sup>. The CAD fungus survives in warm wet conditions<sup>[14,16-35]</sup>. Although there was a decline of the CAD pathogen with increased incubation time during burial it is not known, whether the decline in survival was related to weather conditions, host resistance or antagonistic activity of other soil invading fungi. There is the need for further investigation of the host-pathogen interactions of CAD development in order to make a conclusive statement about the factor influencing the decline in fungal population.

The adoption of host resistance breeding and cultural control measures for cassava improvement, within the integrated pest and disease control strategies at the Tuber and Root Crop Improvement Program, at the International Institute of Tropical Agriculture, has led to increased productivity within the low-income subsistence farmers in cassava growing regions of Nigeria<sup>[14,25]</sup>. The increased production level now ranks Nigeria as the leading world producers of cassava and the prospect of achieving a sustainable food crop production within the region to meet the basic food supply to the over 100 million population has been enhanced<sup>[1,2,5,10,18]</sup>.

These control options have significantly reduced the use of fungicides that are very expensive for the local poor farmers to purchase. The lack of training and poor management on the use of pesticides by the local farmers had led to deleterious effects on the farmers as well as their crops and environments.

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