

# Aggressiveness of the Fungi Responsible for Pod Rot in Cropping Systems Based on Cocoa Trees (*Theobroma cacao* L.) in Cameroon

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**Abstract:** To evaluate the Aggressiveness of the fungi responsible for pod rot in cropping systems based on cocoa trees (*Theobroma Cacao* L.), 3 cropping systems having cocoa trees were characterized: Cocoa trees associated with fruits and forest trees (S1), Cocoa trees associated with plantains and palm oil (S2) and associated cocoa trees with coffee trees (S3). 150 pods were collected per year for isolation and *in-situ* pathogenicity tests. Three culture media called V6-agar, V8-agar and Potato - Dextrose - Agar (PDA) were made for the *in-vitro* culture of fungi associated with pods. The test was conducted using a random split split plots arrangement with 9 replicates. As results obtained: of the 8 mushrooms which were isolated from the pods, only 3 are responsible for the burps. It is about *Phytophthora megakarya*, *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides*. Black pods disease caused by *P. megakarya* was the most aggressively from cocoa pods associated with fruits and forest trees at a decay rate of 5% during a dryng season and 55% during a rainy season. The lesion areas developed by *P. megakarya* ranged from 40 cm<sup>2</sup> during a dryng season to 220 cm<sup>2</sup> during a rainy season. Cropping system having cocoa trees associated with fruits and forest trees (S1) is the most vulnerable to the the Aggressiveness of the fungi responsible for pod rot.

**Keywords:** Aggressiveness, Cropping System, Decay Rate, Lesion Area

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## 1. Introduction

Cocoa orchards in the equatorial and Sudano-Guinean zone are under strong pressure from diseases and pests [1]. Brown pod rot caused by *Phytophthora megakarya* is the most common disease causing losses in cocoa production of up to 100% if no phytosanitary control measures are considered [2]. However, other diseases such as black pod rot, anthracnose, black rot and witches' broom are on the rise and present with various symptoms leading to pod loss which can range from 25 to 30% [3].

Numerous trials have already been conducted to assess the mechanisms of action of cocoa pests and control strategies to control the expansion of these microorganisms in cocoa trees.

However, very little work focused on the climatic and environmental factors that drive variability in the level of aggressiveness of the fungi responsible for rotting cocoa pods. The present study therefore proposes to assess the influence of cocoa-based cropping systems and the seasons of the year on the aggressiveness of the fungi responsible for rots.

## 2. Materials and Methods

### 2.1. Location of the Study Area

The study was carried out in three localities located in

three different agroecological zones of Cameroon, in particular:

1. Akonolinga, located in the Center region of Cameroon, at 3° 46'57.95" 'N latitude and 12° 14' 56.65" E longitude. The prevailing climate is equatorial of the Guinean type with bimodal rainfall characterized by two dry seasons alternating with two rainy seasons;
2. Dizangué, located in the Littoral region, between 9° 41 and 9° 50 East longitude and between 4° 42 and 4° 53 North latitude. The climate is of Cameroonian equatorial type characterized by two seasons: an intensive rainy season from March to October and a dry season from November to February;
3. Tonga, located in the western highlands region of Cameroon, at 4° 58'11.13" N latitude and 10° 42 '04.80 E longitude. The climate of this Sudano-Guinean-type locality particularly dominated by a long rainy season from March to November and a dry season of 2 to 4 months.

## 2.2. Equipment

### 2.2.1. Characterization of Plots

The characterization of the plots was carried out according to several criteria, in particular: the geographical coordinates of the plot, the age of the stems, the cultural association, the variety of cocoa tree, the origin of the seed, the area of the plot and the type shading. This characterization made it possible to validate with the 3 cocoa-based cropping systems, in particular: the cocoa system associated with fruit trees and forest species, the cocoa system associated with bananas and oil palms and the cocoa system associated with coffee trees. 9 plots were chosen at random from each system and in each plot two plots of 1000 m<sup>2</sup> each were measured.

### 2.2.2. Sources of Climate Data

The relative humidity as well as the maximum and minimum temperatures were measured in the experimental plots directly above the cocoa trees every day during the dry season (from January to March) and during the dry season (from August to October). over a period of 3 years (from 2017 to 2019).

### 2.2.3. Plant Material

The healthy pods were used to carry out the pathogenicity tests (*in-vitro* and *in-situ*) of the fungi. Rotten pods have been used for the isolation and identification of fungi responsible for pod rot.

### 2.2.4. Laboratory Equipment

V6 - agar, V8 - Agar and Potato - Dextrose - Agar (PDA) culture media were used as substrates for the isolation and growth of cocoa pod fungi.

## 2.3. Methods

### 2.3.1. Collection of Pod Samples

150 pods were collected separately from the village plantations of the 3 cropping systems characterized. These pods were stored in plastic bags bearing the collection coordinates for the isolation of fungi associated with cocoa pods.

### 2.3.2. Preparation of Culture Media

The culture media were made from vegetables chosen according to the quantities recommended for each type of media. These vegetables were weighed and crushed in 100 ml of distilled water using a Moulinex. Subsequently 150 ml of juice of macedonia, 20 g of Agar and 3 g of CaCO<sub>3</sub> were added to it and 1 ml of vegetable oil (Olio) to promote sporulation [4]. The collected filtrate was seasoned with 20 g of Agar, 40 g of Dextrose (PDA medium) and 1 g of Chloramphenicol (to prevent the growth of bacteria). The volume of the medium was made up to 1000 ml with distilled water [5].

### 2.3.3. Isolation, Purification and Identification of Fungi Associated with Cocoa Pods

The pod surface was pre-washed with tap water, then subjected to a series of disinfections with 95% ethanol for 30 seconds, in 10% sodium hypochlorite, for 2 minutes. The pods were then rinsed three times with sterile distilled water to remove traces of disinfectant [6, 7]. Using a sterile scalpel. The fragments from the growth front of the lesion were taken and cultured on selective medium contained in 90 mm diameter Petri dishes. The identification of the isolated fungal species was made on the basis of the morphological characters of the mycelium (septate or non-septate) and fruiting bodies (conidia) observed under an ordinary microscope.

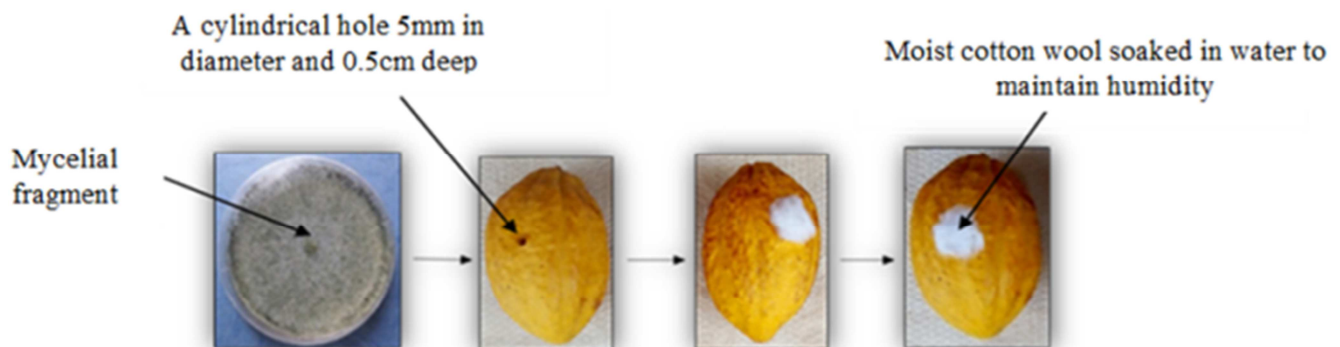


Figure 1. Different stages of inoculation of healthy pods with sporangia of mycelial explants of isolated and identified fungi.

### 2.3.4. Pathogenicity Test of Isolated Fungal Strains for the Identification of Pods Responsible for Pod Rots

Healthy pods of approximately the same age (identical morphology, size and mass) were washed with tap water and soaked in 3% sodium hypochlorite solution for 3 minutes. They were then subjected to three successive rinses of 15, 10, and 5 minutes with sterilized distilled water, and dried on blotting paper. A cylindrical hole 5mm in diameter and 0.5cm deep was created in the middle of each pod using a flambé cookie cutter. A 5mm diameter mycelial fragment from each isolated fungus was inserted into each pod, then covered with moist cotton wool soaked in water to maintain humidity. (Figure 1).

### 2.3.5. Experimental Devices

The study was carried out using a split plot device with 9 repetitions. The studied parameters were observed on a total of 54 samples.

### 2.4. Variables Measured

Frequency of fungi isolated and identified from rotten pods. The frequency of isolation of each fungal species was calculated by the following formula:

$$F(\%) = \frac{\text{NAFS}}{\text{TNAAFS}} \times 100$$

NAFS: Number of appearances of a fungal species;

TNAAFS: Total number of appearances of all fungal species

#### 2.4.1. Pod Rot Rate

The rate of rot (MI) caused by each fungal species tested on pods in-situ consisted of a count of all pods that exhibited at least one specific decay symptom. The count of healthy pods and rotten pods the first day of observation corresponded to the appearance of a lesion characteristic of the type of rot observed. The incidence of each type of decay was calculated using the following formula:

$$ID(\%) = \frac{\text{NICT}}{\text{TNCTO}} \times 100$$

ID (%): Incidence of the disease;

NICT: Number of infected cocoa trees and

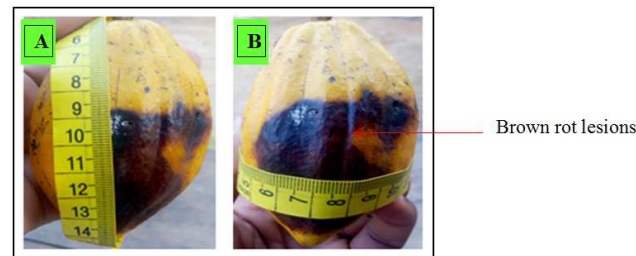
TNCTO: Total number of cocoa trees observed

#### 2.4.2. Surface of Lesions Developed on Pods

Each inoculated pod was placed in a sterile polyethylene bag and then incubated at  $22 \pm 2^\circ\text{C}$ . Three pods (repetition) were considered per plot. The length (L) and width (W) of lesion growth (cm) were measured every second day after inoculation (DAI) using a flexible and transparent graduated ruler. The lesion developed on the infected pods having an elliptical shape, its area (S) was calculated according to the following formula of [8]:

$$S = \frac{\pi \cdot L \cdot l}{4}$$

Figure 2 below shows the measurement of length and width on a pod showing brown rot lesions.



A: Measurement of the length of the lesion L (in cm)

B: Measurement of the width of the lesion l (in cm)

Figure 2. Measurements of lesion length and width.

### 2.5. Data Processing and Statistical Analysis

The graphics were produced with the Excel spreadsheet. Primary data were subjected to analysis of variance (ANOVA). The calculated mean values of the variables were subjected to the T-multiple comparison test ( $p \leq 0.0001$ ).

## 3. Results and Discussion

### 3.1. Results

#### 3.1.1. Prevalence of Fungi Responsible for Cocoa Bean Rots

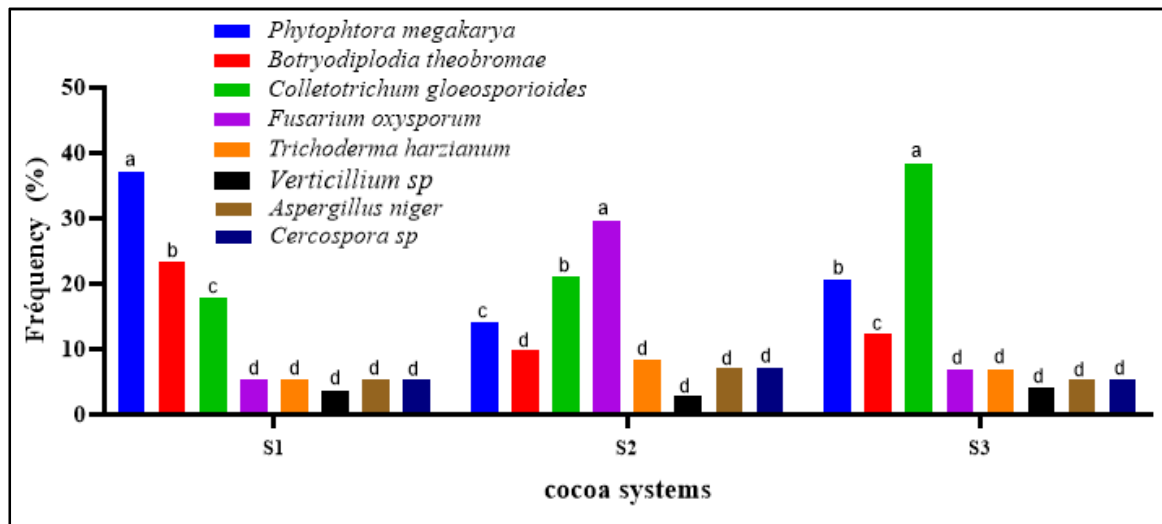
Overall, 8 fungal species were identified as being consistently associated with pods in fields. The frequencies of occurrence of these fungi have varied depending on the cocoa growing systems and the seasons of the year. The diagram in Figure 3 shows the frequency of isolation of each fungus based on cropping systems and seasons of the year. Analysis of this frequency diagram of fungi shows that:

In plots of the cocoa system associated with fruit trees and forest species, *Phytophthora megakarya* was isolated at the frequent average of 39%, while *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* were isolated with frequencies of 24 and 18% respectively.

In plots populated with cocoa trees, fruit trees and forest species, *Colletotrichum gloeosporioides* was isolated at the frequent mean of 39%.

In plots populated with cocoa and coffee, *Botryodiplodia theobromae* was isolated at the frequent mean of 24%.

In plots populated with cocoa trees associated with banana and oil palms, *Fusarium oxysporum* was isolated at the frequent average of 27%.



The means assigned the same letter in superscript are not significantly different according to the T-multiple test at  $P < 0.0001$ .

**Figure 3.** Frequency diagram of the different fungi identified according to the typologies of cocoa trees.

Legend:

Factors 1: cocoa cropping systems

S1: Cocoa system under shade of fruit trees and forest species

S2: Cocoa system associated with coffee trees

S3: Cocoa system associated with banana and oil palm trees

Factor 2: the seasons of the year

SS: Dry season

SP: Rainy season

Comparison of the means and standard deviations of the frequencies by the T-multiple comparison test (Table 1) revealed that there is a very highly significant difference ( $P < 0.001$ ) between the *P. megakarya* isolation frequencies and the frequencies. Isolation of *B. theobromae* and *C. gloeosporioides* according to the seasons of the year, the level of relative humidity and the typology of cocoa trees.

**Table 1.** Comparison of the means and standard deviations of the frequencies of fungi by the T-multiple test according to the effect of the seasons of the year, relative humidity and type of association of crops.

Treatments	P-value	Means and standard deviations		
		<i>P. megakarya</i>	<i>B. theobromae</i>	<i>C. gloeosporioides</i>
S1: SS1	0,019442	19,00 ± 3,511 <sup>c</sup>	11,67 ± 0,33 <sup>d</sup>	11,33 ± 3,17 <sup>d</sup>
S1: SS2	0,004605	18,00 ± 9,29 <sup>c</sup>	10,67 ± 10,33 <sup>d</sup>	16,33 ± 9,26 <sup>c</sup>
S1: SS3	0,000993	22,33 ± 4,09 <sup>b</sup>	17,33 ± 6,83 <sup>c</sup>	16,33 ± 3,52 <sup>c</sup>
S2: SS1	0,033501	10,67 ± 12,12 <sup>d</sup>	10,33 ± 10,17 <sup>d</sup>	13,33 ± 0,66 <sup>d</sup>
S2: SS2	0,015527	10,33 ± 11,83 <sup>d</sup>	09,33 ± 2,02 <sup>d</sup>	10,33 ± 15,02 <sup>d</sup>
S2: SS3	0,005811	09,33 ± 9,20 <sup>d</sup>	07,33 ± 13,90 <sup>d</sup>	09,33 ± 5,20 <sup>d</sup>
S3: SS1	0,635851	16,67 ± 3,66 <sup>c</sup>	10,00 ± 12,50 <sup>d</sup>	19,33 ± 9,52 <sup>c</sup>
S3: SS2	0,001444	17,67 ± 3,66 <sup>c</sup>	11,00 ± 4,04 <sup>d</sup>	21,67 ± 1,20 <sup>b</sup>
S3: SS3	0,004213	19,33 ± 14,66 <sup>c</sup>	7,667 ± 3,84 <sup>d</sup>	20,667 ± 3,66 <sup>b</sup>
S1: SP1	0,046560	41,33 ± 12,99 <sup>a</sup>	37,333 ± 3,71 <sup>a</sup>	46,00 ± 6,35 <sup>a</sup>
S1: SP2	0,000723	55,33 ± 15,49 <sup>a</sup>	16,00 ± 8,02 <sup>c</sup>	40,00 ± 3,00 <sup>a</sup>
S1: SP3	0,039658	36,67 ± 3,17 <sup>a</sup>	26,333 ± 4,80 <sup>b</sup>	36,33 ± 3,66 <sup>a</sup>
S2: SP1	0,000115	22,67 ± 6,38 <sup>b</sup>	22,67 ± 17,02 <sup>b</sup>	18,67 ± 6,22 <sup>c</sup>
S2: SP2	0,045802	21,67 ± 18,12 <sup>b</sup>	19,00 ± 14,22 <sup>b</sup>	19,67 ± 5,23 <sup>b</sup>
S2: SP3	0,000276	20,67 ± 11,40 <sup>b</sup>	18,00 ± 15,88 <sup>c</sup>	21,67 ± 5,78 <sup>b</sup>
S3: SP1	0,000283	38,33 ± 6,93 <sup>a</sup>	26,33 ± 5,66 <sup>b</sup>	43,67 ± 10,80 <sup>a</sup>
S3: SP2	0,000506	36,67 ± 16,58 <sup>a</sup>	21,00 ± 10,50 <sup>b</sup>	41,67 ± 5,84 <sup>a</sup>
S3: SP3	0,000173	37,67 ± 13,67 <sup>a</sup>	22,00 ± 10,50 <sup>b</sup>	43,67 ± 8,76 <sup>a</sup>

\*Means assigned the same letter in the same column are not significantly different according to the T-multiple test at  $P \leq 0.001$

### 3.1.2. Pod Rot Rate in Fields

The rates of the various rots in percentages of affected pods were obtained from the pathogenicity test on healthy pods *in-vitro* and *in-situ*. Of the 8 fungi that were isolated from the rotten pods, only 3 including: *P. megakarya*, *B. theobromae* and *C. gloeosporioides* caused different rots based on the primary lesions and symptoms observed over time.

Microscopic observation of pure cultures of these different fungi, 12 days old, revealed that: *Phytophthora megakarya* is characterized by the whitish color of its colony and its filamentous texture. *Botryodiplodia theobromae* exhibited a yellow fibrous mycelium. *Colletotrichum gloeosporioides* showed a whitish cottony mycelium. *Fusarium oxysporum* showed a somewhat purplish appearance.



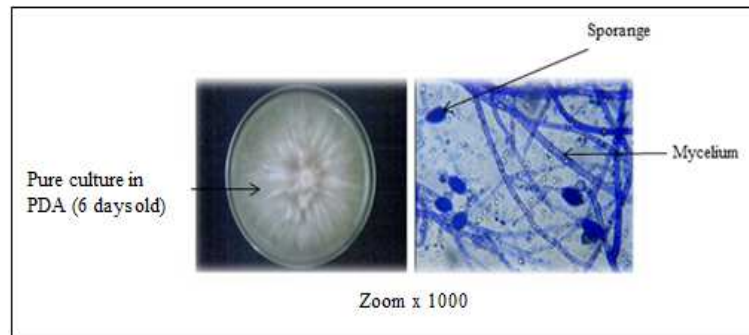


Figure 4. Morphology of *Phytophthora megakarya*.

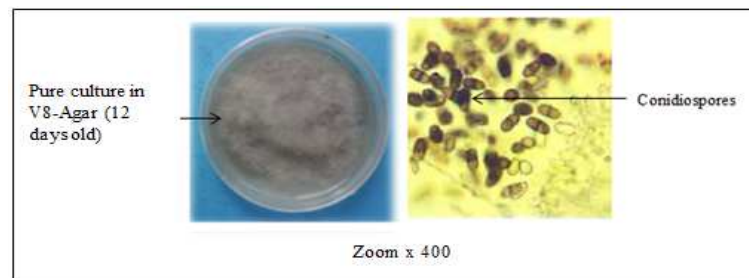


Figure 5. Morphology of *Botryodiplodia theobromae*.

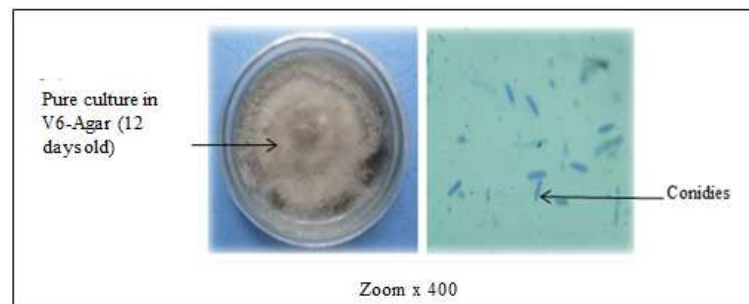


Figure 6. Morphology of *Colletotrichum gloeosporioides*.

In plots of the cocoa system under the shade of fruit trees and forest species, brown rot showed higher rates during the rainy season over a period of 3 years. These rates varied from 26% in 2017, 39% in 2018 and 52% in 2019. As for black pod rot, it evolved in the same direction, but then with relatively lower rates than those of brown rot.

In the plots of the cocoa system associated with bananas and oil palms, it was observed that the 03 types of rots presented very low infection rates (<6%) in the dry season compared to the rates measured during the growing season. rains whose peak oscillates around 20%.

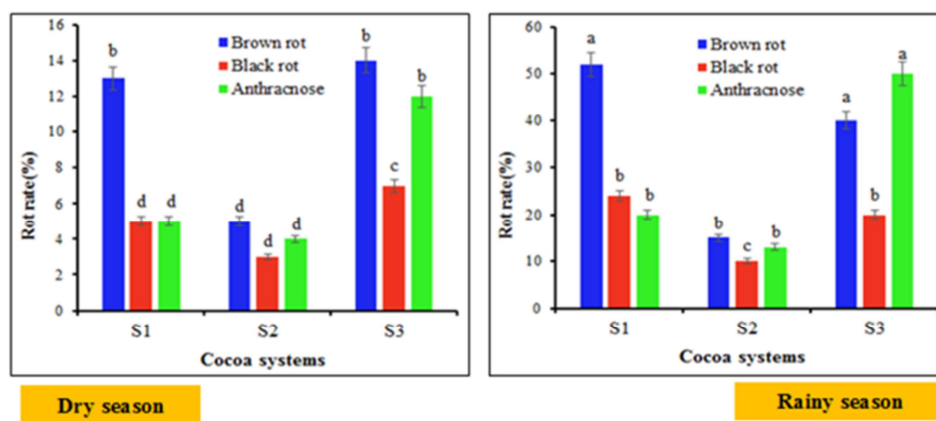


Figure 7. Evolution of pod rot rates in cocoa growing systems according to cocoa growing systems and seasons of the year.

In plots of the cocoa system associated with coffee trees, brown rot, black rot and anthracnose had such low rates (<15%) compared to the rates measured during the rainy season, the evolution of which was noticed. In particular for brown rot, starting from 22% in 2017 to 39% in 2019; for anthracnose from 28% in 2017 to 47% in 2019.

Caption:

S1: Cocoa system associated with fruit trees and forest species

S2: Cocoa system associated with bananas and oil palms

S3: Cocoa system associated with coffee trees

Comparison of the means and standard deviations of pod rot rates by the T-multiple test (Table 2) showed that brown rot had a higher rate (78.4%). It was observed during the rainy season under a relative humidity of between 80 and 100% in the plots of the cocoa system under the shade of fruit trees and forest species.

**Table 2.** Comparison of means and standard deviations of pod rot rates by the T-multiple test according to the effect of cropping systems and seasons of the year.

Treatments	P-value	Means and standard deviations		
		Brown rot	Black rot	Anthracnose
S1: SS1	0,3828	09,50 ± 3,25 <sup>c</sup>	08,30 ± 0,31 <sup>c</sup>	06 ± 7,63 <sup>c</sup>
S1: SS2	0,8506	09,00 ± 4 <sup>c</sup>	08,27 ± 4,87 <sup>c</sup>	07 ± 1,15 <sup>c</sup>
S1: SS3	<0,0001	08,0 ± 0,57 <sup>c</sup>	09,00 ± 0,00 <sup>c</sup>	07,33 ± 1,33 <sup>c</sup>
S2: SS1	0,4566	03 ± 4,80 <sup>c</sup>	03,33 ± 3,71 <sup>d</sup>	02,66 ± 10,66 <sup>d</sup>
S2: SS2	0,4952	03,3 ± 0,88 <sup>d</sup>	02,00 ± 4,35 <sup>d</sup>	02,66 ± 11,78 <sup>d</sup>
S2: SS3	0,5883	02,0 ± 0,57 <sup>d</sup>	03,23 ± 6,38 <sup>d</sup>	02,66 ± 9,24 <sup>d</sup>
S3: SS1	<0,0001	05,67 ± 0,28 <sup>c</sup>	04,8 ± 5,65 <sup>d</sup>	05,56 ± 3,48 <sup>c</sup>
S3: SS2	<0,0001	05,7 ± 6,35 <sup>c</sup>	05,53 ± 4,22 <sup>c</sup>	0,4 ± 0,26 <sup>d</sup>
S3: SS3	<0,0001	05,3 ± 13,22 <sup>c</sup>	05,33 ± 3,33 <sup>d</sup>	05,33 ± 3,33 <sup>c</sup>
S1: SP1	0,0781	45,3 ± 15,30 <sup>b</sup>	38,00 ± 4,16 <sup>b</sup>	34,66 ± 8,76 <sup>a</sup>
S1: SP2	0,4186	43,0 ± 15,30 <sup>a</sup>	23,3 ± 5,54 <sup>b</sup>	34,33 ± 8,68 <sup>a</sup>
S1: SP3	0,9533	26,3 ± 4,63 <sup>b</sup>	27,0 ± 9,64 <sup>c</sup>	25 ± 2,09 <sup>b</sup>
S2: SP1	0,0344	15,7 ± 6,38 <sup>b</sup>	12,3 ± 0,88 <sup>b</sup>	17,07 ± 5,34 <sup>b</sup>
S2: SP2	0,7548	27,0 ± 13,52 <sup>b</sup>	22,0 ± 6,35 <sup>b</sup>	12,33 ± 6,93 <sup>c</sup>
S2: SP3	0,1855	17,7 ± 19,53 <sup>b</sup>	11,33 ± 5,33 <sup>c</sup>	19,66 ± 10,66 <sup>b</sup>
S3: SP1	0,007	26,7 ± 4,48 <sup>c</sup>	23,0 ± 6,35 <sup>c</sup>	37,66 ± 3,92 <sup>a</sup>
S3: SP2	<0,0001	54,07 ± 9,95 <sup>a</sup>	23,32 ± 0,92 <sup>b</sup>	29 ± 4,93 <sup>b</sup>
S3: SP3	<0,0001	38,4 ± 2,38 <sup>a</sup>	35,07 ± 0,33 <sup>a</sup>	35,33 ± 1,76 <sup>a</sup>

\*Means assigned the same letter in the same column are not significantly different according to the T-multiple test at  $P \leq 0.001$

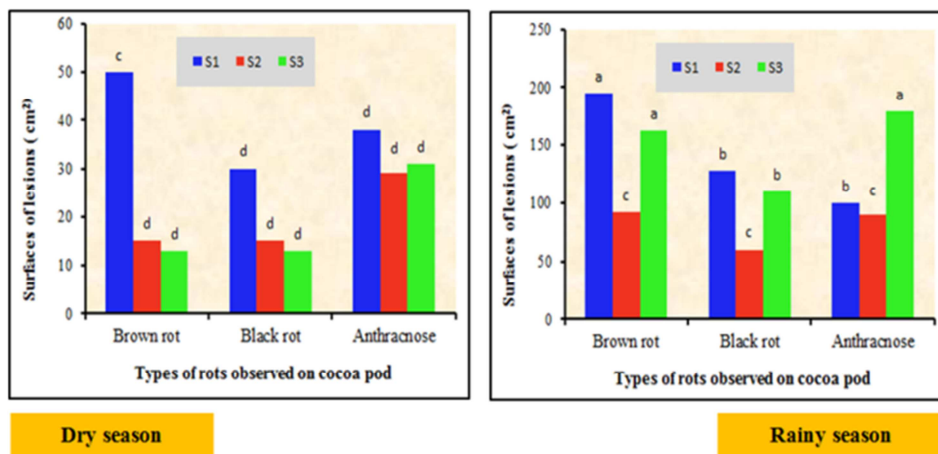
### 3.1.3. Surfaces of Lesions Caused by Pathogenic Fungi on Cocoa Pods

In plots of the cocoa system under the shade of fruit trees and forest species, brown pod rot caused by *P. megakarya* has developed lesion areas of about 40 cm<sup>2</sup> during the dry season and about 220 cm<sup>2</sup> during the rainy season. Black rot caused by *B. theobromae* has developed lesion areas of about 35 cm<sup>2</sup> in the dry season and about 120 cm<sup>2</sup> in the rainy season. Pod anthracnose caused by *C. gloecoporioides* has developed lesion areas of about 32 cm<sup>2</sup> in the dry season and

about 150 cm<sup>2</sup> in the rainy season.

In the plots of the cocoa system associated with bananas and oil palms, the 3 types of rots presented lesion surfaces of less than 25 cm<sup>2</sup> during the dry season and less than 120 cm<sup>2</sup> during the rainy season.

In plots of the cocoa system associated with coffee trees, anthracnose developed lesion areas of about 40 cm<sup>2</sup> during the dry season and about 180 cm<sup>2</sup> during the rainy season. These lesions reflected a higher severity of anthracnose compared to brown rot and black rot.



**Figure 8.** Evolution of lesion surfaces according to the types of rots observed, cocoa-based cropping systems and the seasons of the year.

The comparison of the means of the evolution of surfaces of lesions by the T-multiple test showed that the interaction season of the year: cropping systems has a highly significant effect ( $p < 0.01$ ) on the evolution lesion surfaces.

**Table 3.** Comparison of means and standard deviations of lesion surfaces on pods by the T-multiple test according to the effect of cropping systems and seasons of the year.

Treatments	P-value	Mean and standard deviation		
		Brown rot	Black rot	Anthracnose
S1: SS1	0,0186607	50,3 ± 20,85 <sup>c</sup>	25,00 ± 7,51 <sup>d</sup>	32,33 ± 22,06d
S1: SS2	0,0188734	51,0 ± 26,10 <sup>c</sup>	29,3 ± 6,96 <sup>c</sup>	25,66 ± 29,78 <sup>c</sup>
S1: SS3	0,040554	15,33 ± 8,68 <sup>d</sup>	14,33 ± 10,01 <sup>d</sup>	20,43 ± 0,66 <sup>d</sup>
S2: SS1	0,85951	16,3 ± 22,67 <sup>d</sup>	15,33 ± 26 <sup>d</sup>	22 ± 35,82 <sup>d</sup>
S2: SS2	0,9873	14,00 ± 14 <sup>d</sup>	16,67 ± 12,57 <sup>d</sup>	23,60 ± 13,83 <sup>d</sup>
S2: SS3	0,96829	16,67 ± 10,72 <sup>d</sup>	16,00 ± 22,52 <sup>d</sup>	15,66 ± 11,54 <sup>d</sup>
S3: SS1	0,0388173	35,7 ± 41,01 <sup>d</sup>	28,67 ± 11,28 <sup>d</sup>	36,60 ± 17,47 <sup>d</sup>
S3: SS2	0,0047085	31,7 ± 5,66 <sup>d</sup>	27,0 ± 6,66 <sup>d</sup>	40,33 ± 15,13 <sup>d</sup>
S3: SS3	0,043418	29,7 ± 26,59 <sup>d</sup>	200,0 ± 12 <sup>d</sup>	39,42 ± 27,15 <sup>d</sup>
S1: SP1	0,0404959	233,0 ± 17,78 <sup>a</sup>	105,3 ± 14,52 <sup>b</sup>	201,33 ± 23,84 <sup>a</sup>
S1: SP2	0,0699416	214,7 ± 16,66 <sup>a</sup>	106,7 ± 6,11 <sup>b</sup>	208 ± 9,68 <sup>a</sup>
S1: SP3	0,0242609	224,3 ± 46,23 <sup>d</sup>	84,33 ± 15,02 <sup>c</sup>	122,66 ± 8,41 <sup>b</sup>
S2: SP1	0,789771	56,7 ± 40,08 <sup>c</sup>	54,7 ± 20,03 <sup>c</sup>	56 ± 28,38 <sup>c</sup>
S2: SP2	0,0464514	73,67 ± 9,20 <sup>c</sup>	65,00 ± 7,33 <sup>c</sup>	65,33 ± 5,50 <sup>c</sup>
S2: SP3	0,0473976	62,33 ± 3,28 <sup>c</sup>	63,67 ± 25,45 <sup>c</sup>	59,30 ± 7,17 <sup>c</sup>
S3: SP1	0,002128	152,7 ± 9,26 <sup>a</sup>	93,00 ± 49,26 <sup>c</sup>	108,66 ± 15,87d
S3: SP2	0,000037	109,3 ± 12,34 <sup>b</sup>	165,3 ± 26,43 <sup>b</sup>	175,66 ± 38,43 <sup>a</sup>
S3: SP3	0,000076	139,0 ± 4 <sup>b</sup>	135,0 ± 22,33 <sup>b</sup>	120,66 ± 31,74 <sup>b</sup>

\* Means assigned the same letter in the same column are not significantly different according to the T-multiple test at  $P \leq 0.000$

## 3.2. Discussion

### 3.2.1. Prevalence of Fungi Responsible for Cocoa Bean Rots

The variations observed in the development of fungi associated with cocoa pods revealed that the prevalence of fungal species depends on the seasons of the year, the relative humidity prevailing in the cocoa tree and the nature of the plants associated with the cocoa trees. These results corroborate those obtained by previous studies [1, 2], who explained that long-term changes in rainfall, relative humidity and temperatures have direct consequences on the aggressiveness of pathogens and insect pests cultures [9]. *Phytophthora megakarya* appeared to be the most aggressive fungus on cocoa pods associated with fruit trees *Fusarium oxysporum* was identified as the most frequent species on pods of cocoa trees associated with plantain and oil palm. Banana and oil palm are crops that are quite vulnerable to *Fusarium* wilt; the spores of *F. oxysporum* are generally disseminated in the plots by the action of strong winds in the equatorial zone. *C. gloeosporioides* has been isolated with a higher frequency from cocoa pods associated with coffee trees; this fungus has long been identified with *C. kahawae* as exerting a pathogenic action in the co-infection of coffee berries for the development of anthracnose. The prevalence of anthracnose on cocoa pods associated with coffee trees in the locality of Tonga can be explained by the association of the cocoa tree with the coffee tree. These results corroborate those obtained by previous studies [10, 11, 14], who asserted that disease is quite common in a field when the previous crop and the combination crops are all susceptible.

### 3.2.2. Rotate of Pods and Surfaces of Lesions Caused by Pathogenic Fungi

All pods inoculated with isolates of *P. megakarya*, *B. theobromae*, and *C. gloeosporioides* developed rots compared to negative controls. These fungi are therefore responsible for rotting cocoa pods. These results are contrary to those obtained by previous studies [12], who observed that cocoa pod rots in Cameroon are caused by 8 strains of *Phytophthora megakarya* which can be collected in different cocoa production areas. The infection rates of brown rot, black rot, anthracnose as well as lesion areas on cocoa pods were highest during the rainy season i.e. from August to October. This result corroborates those obtained by previous studies [13], who showed that rain is a factor in the dispersion of the disease.

## 4. Conclusion and Recommendations

This study revealed a diversity of fungi constantly associated with cocoa pods in fields. However, it has been observed that pod rots in cocoa-based cropping systems are usually caused by *Phytophthora megakarya*, *Botryodiplodia theobromae*, and *Colletotrichum gloeosporioides*. These rots have a high incidence during the rainy season. In addition, the prevalence of fungi varies from one cocoa plantation to another depending not only on the seasons of the year, but also on the plant species associated with the cocoa trees.

Producers are therefore advised to considerably reduce the number of fruit trees and forest species which would promote the rise in relative humidity, which is a factor that favors the development of pathogenic fungi in cocoa pods.

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