ORIGINAL ARTICLE

INVESTIGATION ABOUT DIEBACK IN COCOA ORCHARDS IN THE BIMODAL HUMID FOREST ZONE OF CAMEROON

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ABSTRACT

Background: Cameroon is the fifth important cocoa production area of the world. Dieback disease is actually one of the major constraints in cocoa orchards in Cameroon. Information about the real causes of that disease is essential to better understanding the epidemiology and, thus to develop integrated control strategies. **Objectives**: This study was done, to get baseline data on dieback, its prevalence and incidence in the bimodal humid forest zone of Cameroon. **Methods**: Survey was carried out in Ndikinimeki, Ntui and Okola, three subdivisions of the Center Region. Thirty-three cocoa plantations in eleven villages were sampled. Data were collected from survey and field observations using the appropriate conventional methods. In each village, twenty five cocoa farmers were interviewed. Laboratory analysis of diseased plant materials were done to identify the causal agents of dieback. **Results**: The results showed that, dieback is present in all the studied sites. According to interviewed farmers, the prevalence of dieback is 32, 92 and 93 % respectively in Okola, Ntui and Ndikinimeki. Disease incidence in cocoa farms varied at 13.9 % in Okola, 22.7 % in Ntui and 24.8 % in Ndikinimeki. However, number of cocoa trees affected per hectare depended of the age of plantation, cocoa variety cultivated, shade trees, and field crop management. Symptoms of disease observed on cocoa trees were similar to those of dieback after conferring with the descriptions of others authors. On the basis of morphological and cultural characters, the isolates were identified as belonging to the species, *Lasiodiplodia theobromae* and other pathogen collected in roots of infected plants was identified as *Fusarium* sp. **Conclusion**: Prevalence and incidence of the dieback disease suggests that, cocoa dieback represents a threat to Cameroonian cocoa orchards productivity and longevity. Control and management strategies should be implemented urgently as means to prevent and / or reduce the incidence of cocoa dieback. Determination of correct role's pathogens associated with dieback disease is essential for the establishment of an adequate control method.

Keywords: *Theobroma cacao, Dieback disease, Prevalence, incidence, causal agents.*

RESUME

Contexte : Le Cameroun est le cinquième producteur mondial du cacao. Le dieback du cacaoyer est actuellement l'une des contraintes majeures dans les plantations cacaoyères du Cameroun. Les informations sur les véritables causes de cette maladie sont indispensables pour mieux comprendre l'épidémiologie et ainsi développer des mesures de lutte. **Objectifs :** Cette étude a été réalisée dans le but de mieux connaître la maladie et ainsi évaluer sa prévalence et son incidence dans la Région du centre Cameroun, zone forestière à pluviométrie bimodale. **Méthodes :** Les travaux ont été menés dans les arrondissements de Ndikinimeki, Ntui et Okola de la Région du centre. Au total, 33 plantations dans 11 villages ont été échantillonnées. Les données ont été collectées à partir des fiches d'enquêtes et des observations de la maladie en champ utilisant les méthodes conventionnelles appropriées. Dans chaque village, 25 planteurs de cacaoyers ont été interviewés. Les analyses au laboratoire des organes de plantes malades ont été menées pour identifier les agents responsables du dieback. **Résultats :** Les résultats ont montré que le dieback est présent dans tous les sites d'étude. Les enquêtes auprès des paysans montrent que la prévalence du dieback est de 32, 92 et 93 % respectivement à Okola, Ntui et Ndikinimeki. L'incidence de la maladie dans les cacaoyères variait de 13,9 % à Okola, 22,7 % à Ntui et 24,8 % à Ndikinimeki. Le nombre de pieds atteints de dieback à l'hectare était cependant fonction de l'âge de la plantation, de la variété de cacaoyer cultivée, de l'ombrage, de l'entretien général des plantations et des traitements utilisés. Les symptômes de la maladie observés sur les plants de cacaoyers étaient similaires à ceux du dieback après une confrontation avec des descriptions de certains auteurs. Sur la base des caractéristiques morphologiques des colonies et les observations microscopiques des échantillons incubés, les isolats étudiés ont été identifiés comme des champignons appartenant à l'espèce *Lasiodiplodia theobromae* et un autre groupe de pathogène collecté dans les racines des plantes infectées étaient identifiés comme des *Fusarium* sp. **Conclusion :** La prévalence et l'incidence du dieback suggèrent que soient proposées, des stratégies de lutte spécifiques et durables contre cette maladie. La détermination du rôle exact des pathogènes associés au dieback du cacaoyer est essentielle pour la mise sur pied d'une méthode de lutte adéquate.

Mots clés : *Theobroma cacao, Dieback, Prévalence, incidence, agents responsables, Cameroun.*

1 INTRODUCTION

Cocoa (*Theobroma cacao* L.) is an important cash crop in Cameroon. The country is the fifth world producer, and nearly 75 % of the populations, mostly peasant farmers derive their livelihood from the crop [1]. In Cameroon, cocoa is mainly cultivated in two agro-ecological zones: the mono-modal humid forest zone and the bimodal humid forest zone. The bimodal humid forest zone produces more than 70 % of Cameroonian exports of cocoa [2]. Being an exotic product in most of its production areas, cocoa has contracted several diseases which are caused by pathogens and ravagers, against which it does not dispose appropriate defense mechanism. It consequently means to considerable production losses and thus explains the small productivity.

The main disease of cocoa in Cameroon is the black pod rot in cocoa causing 80-90 % losses without chemical control with *Phytophthora Megakarya* as causal agent [3]. In additional to black pod rot, cocoa is confronted at several diseases and ravagers already present, thus an emergence of news diseases like dieback. Consequently, the supply does not satisfy the demand. In order to increase economic growth, reduce poverty in rural environment, and ameliorate the benefits and living environment of cocoa producers, the Cameroonian government plans to produce 600 000 tons of cocoa bean on the 2020 horizon [4].

Dieback disease is the progressive death of plants, which may be caused by environment factors such as water stress, physiological and nutritional disorders, insect attacks, pathogens and / or some interactions between those different factors. It affects until 100 % of cocoa trees in some fields [5]. Commonly called "sudden death", dieback is a sanitary constraint which the incidence is the more and more worrying and which origin is little known. However, the main causes of dieback seem to be pathological because, symptoms of dieback disease are typical of fungal infection [6]. In fact, several pathogens have directly associated with cocoa dieback worldwide. It is especially about fungi like *Lasiodiplodia theobromae* [7, 8], *Fusarium* [9, 10] or association of that species and *Lasiodiplodia pseudotheobromae* with main vectors, insects like *Sahbergella singularis* [6-11].

In Cameroon, Mbenoun et al. (2008) have isolated and identified *L. theobromae* as causal agent of cocoa dieback. Begoude et al. (2009) have just isolated *L. theobromae* and *L. pseudotheobromae* from diseased cocoa plant materials [6-12]. Despite interesting results reported by those authors, little is known of the real causes, incidence, epidemiology and control of cocoa dieback in Cameroun. Information about the real causes is crucial for a better understanding disease epidemiology, as well as finding effective management strategies. Therefore, the objectives of this study were to: (i) get baseline data on dieback, (ii) evaluate its prevalence and incidence and (iii) identify pathogens associated with cocoa dieback in the bimodal humid forest zone of Cameroon.

2. MATERIALS AND METHODS

2.1 Sample sites

The study was carried out in the bimodal humid forest zone of Cameroon from August 2015 to September 2016. Characteristics of that agro-ecological zone are: Pluviometry is from to 1500 to 2000 mm / year with two distinct humid seasons. Soils are ferralitic, acid, clayey and have a small retention capacity of nutritive elements [13]. Investigations were done in 11 villages of cocoa producers in three subdivisions of Center Region of Cameroon. The subdivisions were Ndikinimeki (N 07°17′67″; E 05°29′63″; A: 710 m), Ntui (N 07°90′31″; E 05°11′05″; A: 550 m), and Okola (N 07°09′38″; E 05°23′91″; A: 600 m).

Ndikinimeki subdivision presents shrub and herbaceous savannah. Cocoa orchards are generally implanted in the gallery forest or in savannah where cocoa trees are associated with subsistence and fruit crop. Ntui subdivision is a transition zone between savannah and forest. Okola subdivision presents forest vegetation.

2.2 Materials

Cocoa plants aged between 5 to 30 years old were used for observations and sampling for laboratory analysis. Other materials like measuring tape, rope, questionnaire, GPS (Garmin Etrex Legend HCX model), microscope optic (Perfex Sciences), micrometer (Hund), camera (Toup Cam), PDA media, WA media and Petri dishes were used.

2.3 Methods

2.3.1 Collection of baseline data: To gather baseline information around dieback disease, a survey was done with farmers in each village (Biagnimie, Bikong, Endama, Mbianguele, Mbongue in Ntui; Etoundou, Nefante, Nomale in Ndikinimeki and Akok, Nkolnstiba, Voa in Okola). A sample of 25 farmers was interviewed per village, given a total of 275 cocoa farmers. Farmers were interviewed using a sample survey during focus group discussions organized in each village. Information was collected on: age of farmer, origin of plantation, area of plantation, presence or no dieback disease in cocoa orchards, symptoms of cocoa dieback disease, the age of orchard, the cocoa variety cultivated, shade trees, cocoa density, production, agro-diversity, and field crop management.

The number of forest trees per hectare of cocoa plantation is from 35 to 40 [14]. Shade in plantation was characterized thanks to a scale of four parameters. That is: Normal shade (35 to 40 forest trees), thin shade (30 to 35 forest trees), small shade (less than 30 forest trees) and dense shade (more than 40 forest trees).

2.3.2 Evaluation of dieback prevalence and incidence

2.3.2.1 Visual diagnosis: After focus group discussions with farmers, observations were made in the cocoa orchards to confirm or to invalidate presence of dieback. Three orchards were randomly chosen per village. Identification of dieback disease in the fields [8], was done by observation of symptoms and their evolution in the time and space. It was completed with information about circumstances around the date of symptoms apparition, plant development stage and density and distribution of diseased plants in fields.

2.3.2.2 Prevalence: Using responses provided by farmers, the dieback disease prevalence was calculated. It was measure as:

Disease Prevalence (%) = (Number of orchards infected / Total number of orchards recorded) × 100 [15] (1)

2.3.2.3 Disease incidence: In each orchard, a plot of 1 ha was measured out, where 25 subplots of 400 m^2 were used to count the number of infected plants and the total number of plants. Three orchards were selected randomly per village, given a total of 33 orchards. The dieback incidence was calculated using the following formula:

Disease Incidence (%) = (Number of infected plants / Total number of plants) × 100 [15] (3)

2.3.3 Identification of the pathogens:

2.3.3.1 Collection of samples: Samples of roots, fruits, stems, branches and leaves were collected from cocoa trees showing symptoms of disease and sent to the laboratory for isolation of the pathogens. About one hundred and fifty samples of plant tissues were collected from 33 cocoa orchards of 05-30 years old.

2.3.3.2 Isolation and identification: The samples collected were brought in laboratory of Phytopathology of the University of Yaounde I for isolation and identification of the pathogens. Pieces of roots, stems, branches and leaves were cut at the diseased/healthy border region, washed under running tap and surface disinfected in 70 % ethanol for 2 minutes, followed in 0.5 % sodium hypochlorite [6]. The surface of the sterilized tissues was washed three times in sterile distilled water and dried in a sterile Lamina Flow Chamber. The dried pieces were placed on potato dextrose agar (PDA) in Petri dishes, and then they were incubated at 25 °C at room temperature under 12:12 (light: darkness). The best hyphae that grew from the margins of the diseased tissues were sub-cultured on fresh PDA. Two types of fungi cultures were isolated after separation *(Botryosphaeriacea* and *Fusarium*).

2.3.3.3 Colony and conidial morphology: The pure cultures of *Botryosphaeriaceae* family were identified according to fungal morphological characteristics as described by Barnett and Hunter (1986) et Moubasher (1993) [16, 17]. Then, they were transferred on 2% water agar plates containing sterilized pine (*Pinus morrisonicola*) needles [18], and incubated at 25°C under fluorescent light for 35 days to enhance sporulation. Matured pycnidia produced after 35 days of incubation were transferred into sterile distilled water on a sterilized glass slide and then teased to diffuse spores. In the following, the solution on the slide was observed under an optic microscope (X40 and X100) to confirm the presence and even distribution of spores. Slides with *Botryosphaeriaceae* spores identified were then inverted and pressed onto the surface of solidified water agar (WA) media to obtain their growth. Germinated single spores of each *Botryosphaeriaceae* isolate from the WA media was transferred onto PDA and then incubated at 25 ºC for seven days as recommended by Shah et al. (2010) [19]. Pure cultures which were morphologically similar to species of *Fusarium* were identified and were sub-cultured in Potato dextrose in order to observe conidia under an optic microscope [20]. Growth of Single-spore of *Botryosphaeriaceae* and *Fusarium* cultures was continuously observed and the macroscopic characters (color, sector, border and texture) were recorded [21]. Morphology (size, shape, color, presence of septa and longitudinal striations) of the conidia was examined under a light microscope. To determine the average length and width of conidia, at least 30 conidia from each isolate of *Botryosphaeriaceae* and *Fusarium* were analyzed using light microscope. Pictures were realized with a camera system. The length and width of conidia were measured by using a micrometer (Hung).

2.3.4 Data recording: Data on questionnaires were analyzed using XLSTAT (5.03 versions). Data of prevalence, incidence and measurements of conidia were analyzed using the analysis of variance (ANOVA) procedure of SPSS (20.0 version) and presented as average ± standard deviations. Where ANOVA showed significant difference between means ($P<0.05$), post-hoc mean separation was done using the Duncan test.

3. RESULTS

3.1 Results of baseline data

Results showed that, all sites presented cocoa orchards infected by dieback. However, the number of cocoa trees affected per hectare varies according to age of field, cocoa variety cultivated, density of cocoa, shade trees, and field crop management.

3.1.1 Number of cocoa trees affected by dieback according to age of plantation: Cocoa orchards of Okola were older than those of Ndikinimeki and Ntui subdivisions. In Okola site, 84 % of cocoa orchards were between 24 to 60 years old, while the proportion was 17 % and 10 % at Ndikinimeki and Ntui respectively. Results obtained from cocoa farmers revealed that: orchards aged between [7-10] years old were more attacked (24.72 ± 1.42 trees/ha) by dieback disease. There was no significant difference (p<0.05) between the level of dieback infection in plantation age [3-7] years old when compare with that of orchards aged between [13-18-24-28]. Meanwhile, it was observed that cocoa plantations of 35 years old and above were not affected by dieback disease (Figure 1).

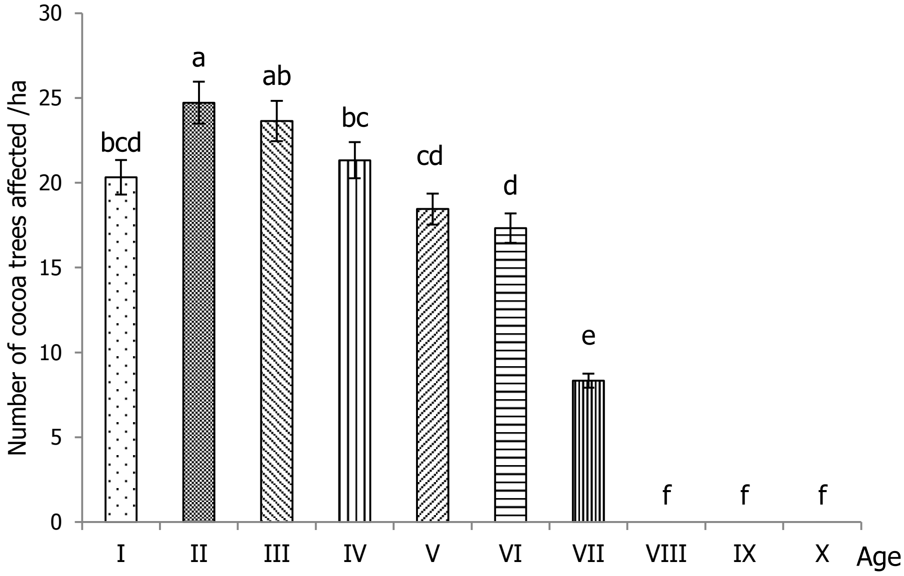

Figure 1: Number of cocoa trees affected by dieback according to age of plantation.
*Values of ages carrying different letter are significantly different when p<0.05. I= [3-7 [; II= [7-10 [;III=[10-13 [; IV=[13-18 [; V=[18-24[; VI=[24-28 [; VII=[28-35 [; VIII=[35-45[; IX=[45-60 [; X=[60-60[.

3.1.2 Number of cocoa trees affected by dieback according to cocoa variety cultivated: Results showed that, two large varietal groups are cultivated in the localities of study: Amelonado variety or "German cocoa" and the hybrid varieties. The German cocoa plants are principally descendants of the first trees introduced by Germans by the end of the XIX century. As for the hybrid cocoa plants, they originate from selection programs of Cameroonian research and distributed by the Cocoa Development Company (SODECAO) or Institute of Agricultural Research for Development (IRAD) since the 70's until now. The Ndikinimeki site showed a higher proportion of hybrid varieties of cocoa plants than the other sites. In this site, 72 % of cocoa orchards were made of hybrid varieties, when only 8 % of the cocoa plantations were made with hybrid varieties in Okola. In Ntui cocoa plantations, 44 % were made with the both varieties. The number of cocoa trees affected by dieback was higher in the fields where hybrid varieties were cultivated (21.61± 1.46 trees/ha), followed by those where the both varieties where cultivated (18.00± 1.66 trees/ha) together. In cocoa orchards where the German variety was cultivated, there was no cocoa tree affected by dieback disease (Figure. 2).

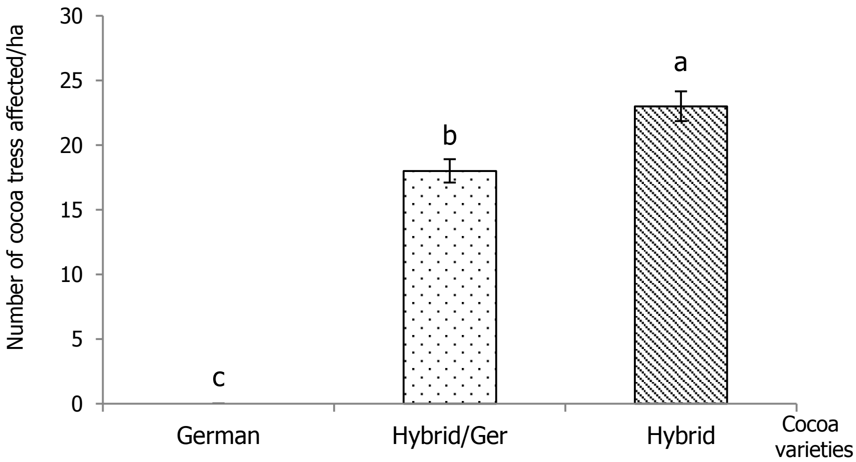

Figure 2: Number of cocoa trees affected by dieback according to cocoa varieties cultivated. * Values of the varieties carrying different letter are significantly different when p<0.05

3.1.3 Number of cocoa trees affected by dieback according to the density of plant in the field: Normal gap between two cocoa trees is 3m x 3m, that is 1111 trees per ha [14].In plantations where the gap between cocoa trees was less than 3 m, they were qualified to thick density plantations and those where the gap between cocoa trees was greater than 3 m were qualified to distant density plantations.

Okola site represented the more important proportion of distant density (44 %) than Ndikinimeki (28 %) and Ntui (27 %). The number of cocoa trees affected by dieback was higher (21.83± 1.79) in the fields with thick density. However, Duncan test did not show significant difference between different densities (Figure 3).

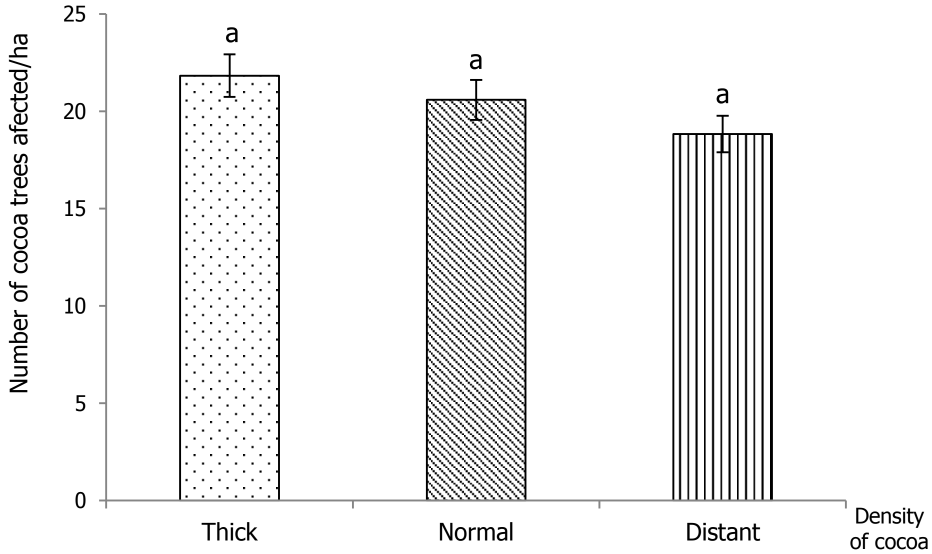

Figure 3: Number of cocoa trees affected by dieback according to the density of cocoa. * Values of densities carrying different letter are significantly different when p<0.05.

3.1.4 Number of cocoa trees affected by dieback according to the shade trees: Okola site presented more important proportion of cocoa farms with dense shade than others. In this locality, 68 % of the area of the farms was at dense shade, when that proportion was 6.66 % of fields in Ndikinimeki.

Results showed that, the number of cocoa trees affected by dieback was higher in small shade fields (28.8 ± 0.2), followed of thin shade fields (21.4 ± 1.5), normal shade fields (17.8 ± 1.1) and dense shade fields (11.8 ± 0.5) (Figure 4).

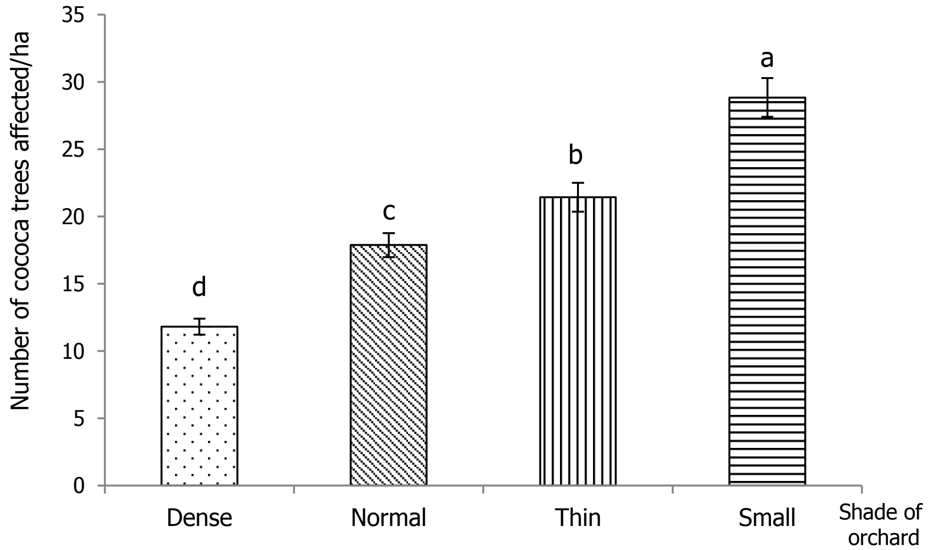

Figure 4: Number cocoa trees affected by dieback according to shade of fields.
* Values of the shade carrying different letter are significantly different when p<0.05

3.1.5 Number of cocoa trees affected by dieback according to field crop managements

Maintenance of plantations mainly consists to cocoa pruning and weed management. Hence, the results showed that, cocoa orchards of Ntui and Ndikinimeki seemed better maintained than those of Okola site.

The number of cocoa trees affected by dieback was higher in the fields weeded third and pruning (23.9 ± 1.3), but it was not significantly different with those weeded twice and pruning (21.2 ± 1.6) (Figure 5-a).

As for phytosanitary treatments, mixed treatments, associating insecticides and fungicides are more frequently applied on the sites. Results showed a variability of practices of the cocoa farmers in terms of phytosanitary treatments. That variability was significant within the sites and for the same site, among plantations. Globally, the use of fungicides is less frequent in Ndikinimeki, as is the case for insecticides in Okola. Mixed treatments associating insecticides and fungicides are more frequently applied in Ntui.

The number of cocoa trees affected by dieback was more important in plantations where people used insecticides (26.34 ± 0.16) than in those where the mixed treatments were applied (20.28 ± 1.36). Cocoa trees were not affected in farms where fungicides were sprayed (Figure 5-b).

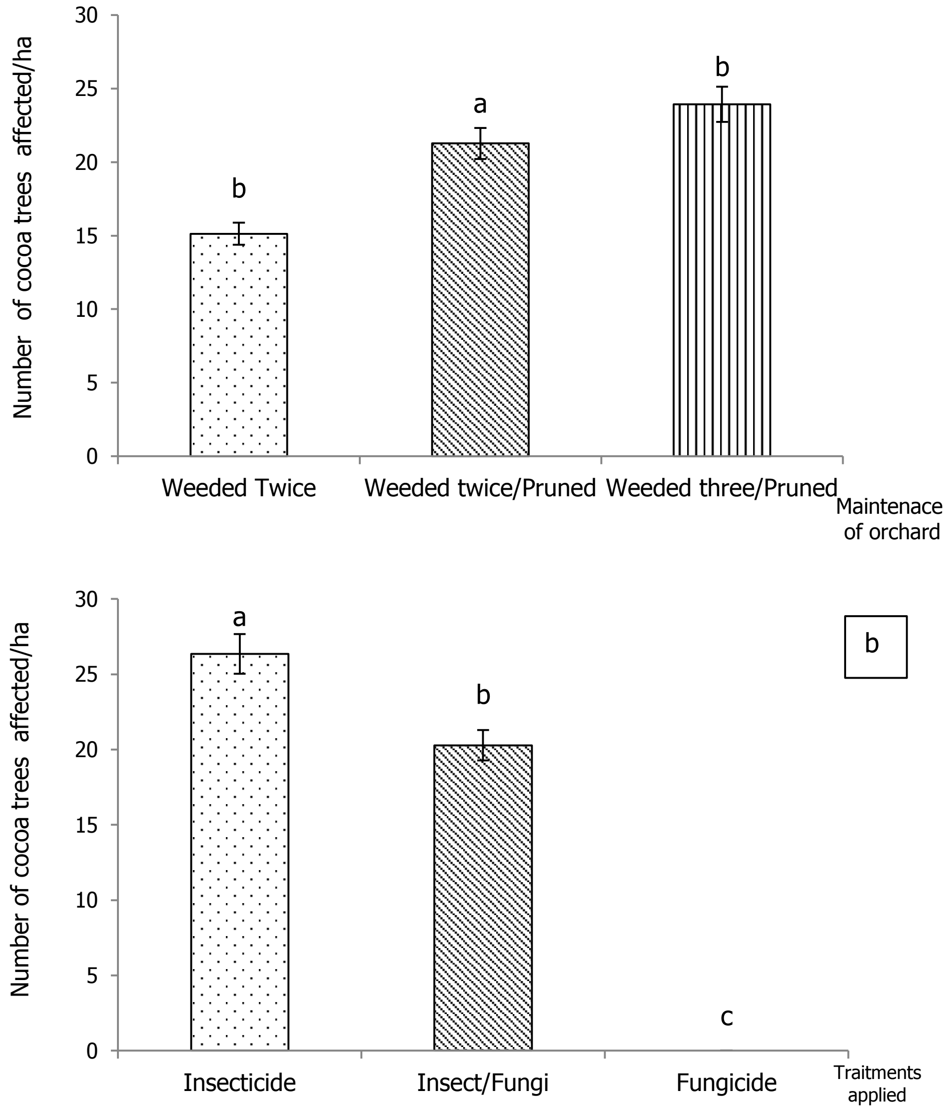

Figure 5: Number of coco trees affected by dieback according to field crop managements. **A:** Maintenance of fields; **b:** Treatments applied; *Values of treatment carrying different letter are significantly different when $p<0.05$.

3.2 Cocoa orchard survey and sampling

3.2.1 Variability of dieback symptoms in cocoa plantations: Visual diagnosis showed that dieback disease was present in all sites of the study. In different cocoa plantations, various symptoms of dieback were observed. Many trees were died (Figure 6-b), someone's had yellowing leaves (Figure 6-a) and the others were apparently healthy. In plantations, the leaves on branches turn to yellow, and then to brown, then the damages stretch throughout the branch, reach the main trunk, thus enhancing the death of the tree. Yellowish streaks were observed in the vascular region.

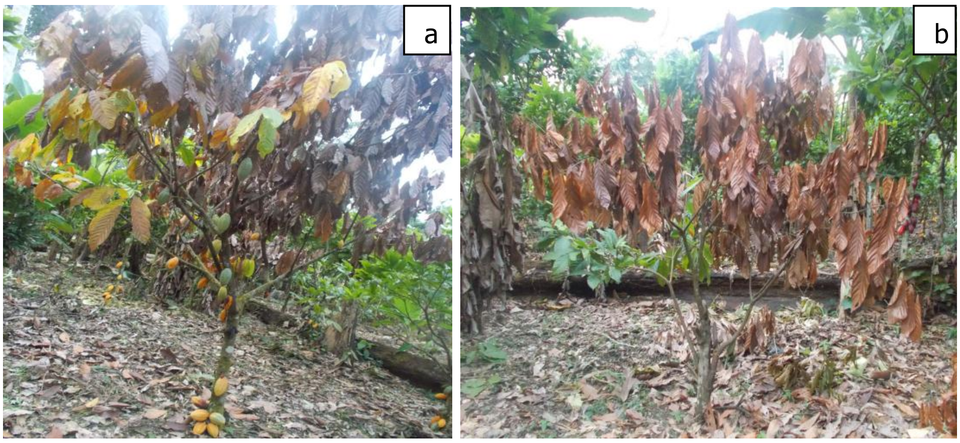

Figure 6. Symptoms of dieback disease in cocoa plantation: **a**: yellowing of leaves; **b**: death of plant.

3.2.2 Prevalence and incidence of dieback disease: Results showed that maximum disease prevalence was registered in plantations of Ndikinimeki site (93 %), followed by Ntui site 92 % showing no significant difference between the two sites. Meanwhile, plantations of Okola site showed the lowest prevalence (32 %) (Figure 7-a). Disease incidence was 24.8 ± 0.1, 22.7 ± 0.4 and 13.9 ± 0.7 % respectively in cocoa plantations of Ndikinimeki, Ntui and Okola sites (Figure 7-b).

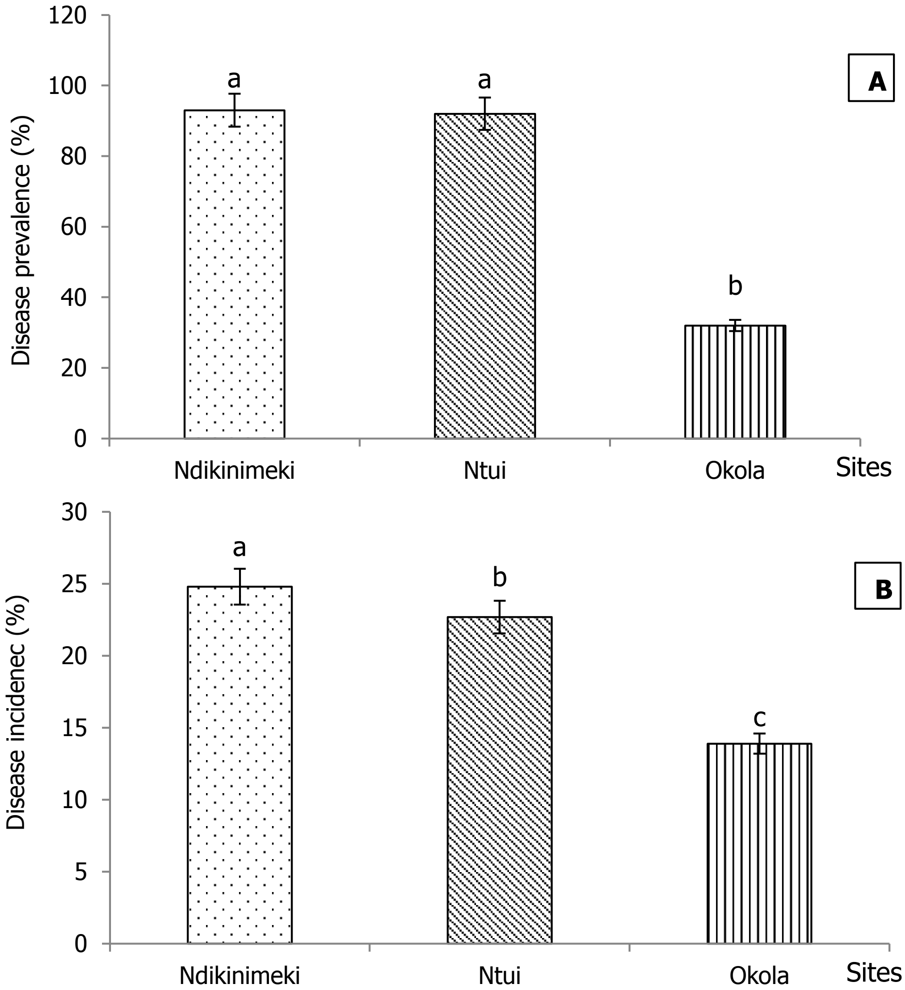

Figure 7: Prevalence and incidence of cocoa dieback; A-Disease prevalence, B-Disease incidence. *Values of site carrying different letter are significantly different when p<0.05.

3.3 Fungi isolates and their morphological characterization

On 150 isolates obtained from the diseased plant materials, 90 % were classified to *Botryospheriaceae* family and 10 % to *Fusarium* genus, based on the morphology of their colonies on PDA plates. After the inoculation of *Botryospheriaceae* fungi on PDA or water agar containing pine needles, most isolates sporulated within five weeks of incubation. Based on the morphology of colonies and conidia, all *Botryosphaeriacea*e isolates were identified as *Lasiodiplodia theobromae.*

Morphological characterization of colonies and conidia of 10 isolates of *L. theobromae* of each site subcultured on PDA, show that *L. theobromae* initially produced white, fluffy aerial mycelium that rapidly covered the surface of Petri dishes within two days of incubation (Figure 7-a). The mycelium then turned gray black or black after 7 to 14 days (Figure 7-b). It produced pycnidia while incubated in water agar or PDA supplemented with sterilized pine needles after 21-35 days only under light (Figure 7-c). Liquid exudates were produced on pines in some cultures (Figure 7-d). Almost, all the isolates sporulated on culture media. The immature conidia were unicellular, hyaline, aseptate, and rounded to ellipsoidal, while the mature conidia were two celled, dark brown with septum (Figure 7-e). Lengths and widths of conidia of the tested isolates were different (Table 1).

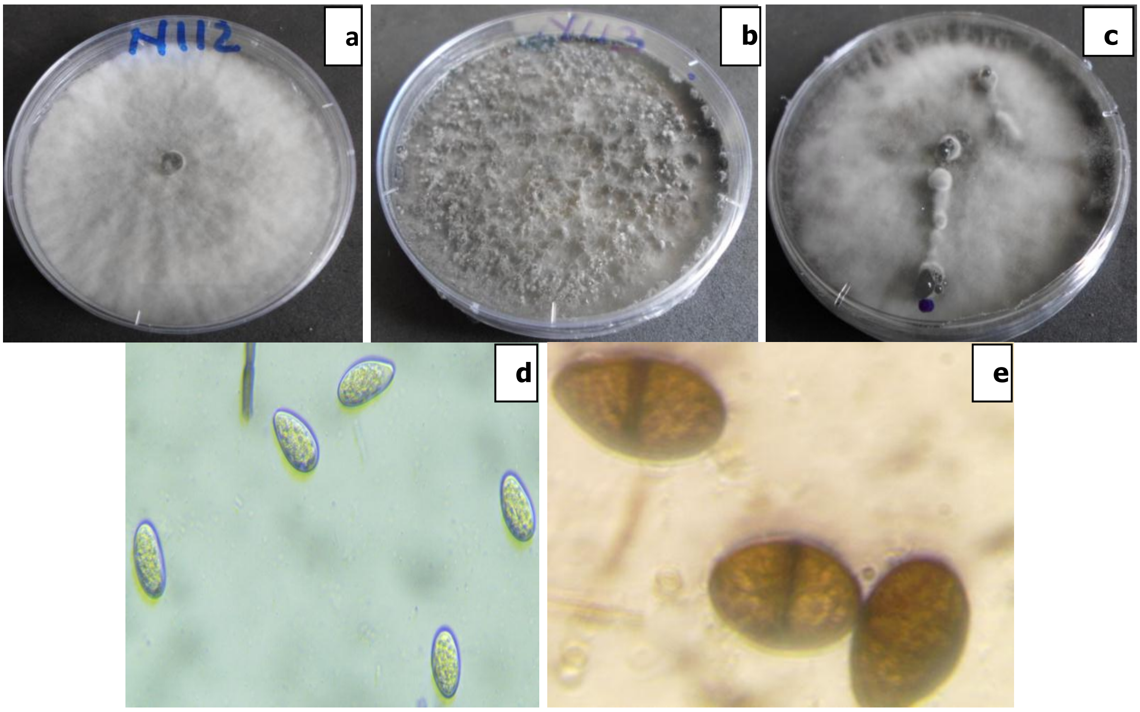

Figure 8: Isolate of *Lasiodiplodia theobromae*. **A**: After 48h; **b:** after 14 days; **c:** Pigment on pines needles; **d:** Immature conidia (microscopic view x 400); **e:** Mature conidia (microscopic view x 1000).

Table 1: The table shows morphological variations existed among conidia of *L. theobromae* isolates.

	Length (µm)	**Width (µm)**
Isolates of Ndikinimeki	20.66 ± 0.95 to 24 .83 ± 0.91	10.26 ± 0.44 to 12.25 ± 0.50
Isolates of Ntui	22.30 ± 0.46 to 26.00 ± 0.00	10.35 ± 0.48 to 14.35 ± 0.35
Isolates of Okola	20.26 ± 0.44 to 26.00 ± 0.00	10.35 ± 0.48 to 14.36 ± 0.49

Growth of *Fusarium* isolates was slow, the surfaces of Petri dishes were covered after 6 days. Its colonies were initially white (Figure 9-a) and later became creamy (Figure 9-b). Conidia were produced on Potato Dextrose (Figure 9-c). Length of conidia varied from 35.90 ± 0.30 to 41.92 ± 0.26 µm, and width was from 3.30 ± 0.29 to 3.70 ± 0.30 µm.

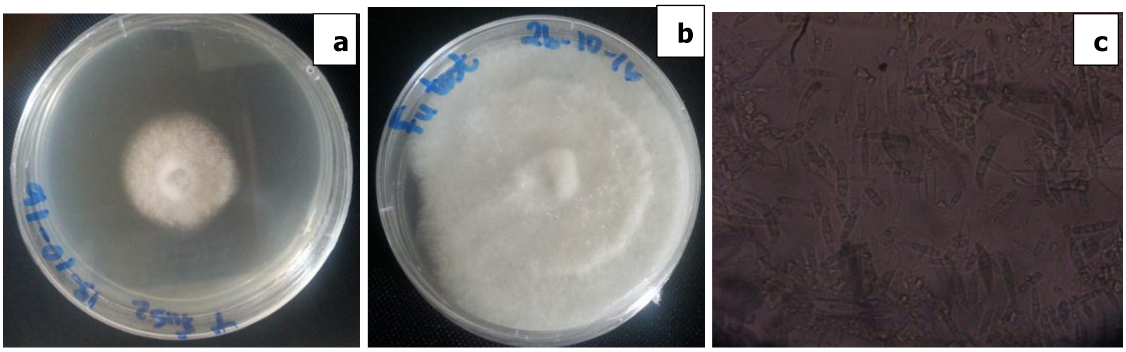

Figure 9: Isolates of *Fusarium*; **a:** one day after incubation; **b:** five days after incubation; **c:** onidia (microscopic view x 200).

4. DISCUSSION

Results obtained from surveys showed that, all cocoa plantations were affected by dieback in the studied sites. The most affected site was Ndikinimeki. Agro-diversity and field crop management notably showed a great variability of dieback infection among plantations of the same site. These results seemed to indicate that, whether dieback is known or not by cocoa farmers, the geographical location of plantations is not a determining factor of its attack level. The variability of dieback attacks would be linked at the variability of the conditions in the cocoa farming parcels. In this study, it was noticed that the disease can occur at any time during the year, but it is more visible and severe in dry season. The symptoms are more serious in areas under hydric stress as compared to regularly watered areas. In addition, plantation plots with low shade are the most affected by dieback. Similar results were obtained others authors [5-9]. As well, presence of dieback in all sites could be probably attributed to climatic conditions which are favorable for the development of the disease. Furthermore, most farmers lacked knowledge on disease epidemiology and identification, and had limited resources for disease management. Consequently, they do not use any appropriate strategies to control disease. The presence of many host plants of *Lasiodiplodia theobromae* and *Fusarium* fungi (*Terminalia superba, Mangifera indica, Percea Americana, Manihot escuenta, Elaeis guineensis …*) in studied cocoa plantations could equally justify these results [22, 23-9].

Disease incidence was higher in Ndikinimeki, showing a significant difference between those recorded in Ntui and Okola. The high incidence in Ndikinimeki may be due to climatic conditions which are more favorable for the development of the disease in that site. Indeed, Ndikinimeki is a savannah area; consequently, the majority of fields were devoid of shade forest, thus creating favorable conditions for the development of the dieback disease. An argument supporting that hypothesis is that, dieback attacks are more severe on shade-deprived plots [5-9]. In addition, the most cultivated cocoa variety in that site is the hybrid cocoa (72 %) which is more sensitive to dieback disease than Amelonado cocoa, according to cocoa farmers whose were interviewed. Furthermore, plantations of this site were seriously infested by mirids which are the main vectors of dieback. These results are similar at those of Adu-Acheampong and Archer (2011) and at those of Anikwe and Otuonye (2015) who qualified mirids as being the main vectors of cocoa dieback [6-11]. However, Adu-Acheampong et al. (2012) have showed that, Amelonado variety is sensitive to dieback [24]. Finally, incidence should be higher in Ndikinimeki because most of the cocoa plantations are successors of cassava cultivation, and trees like oil palm, mango, pear and plum (which are hosts able to shelter *L. theobromae*) used as substitutes of forest trees. These results corroborate those of Blancard (2013) for whom cassava, oil palm, mango and pear are all susceptible to shelter and conserve the fungi, *L. theobromae* [23].

Results concerning visual diagnosis of the disease showed that, dieback affecting cocoa begins with a progressive yellowing of leaves, which then stretches along the branch, reaches the main trunk and then leads to the death of the plant. Brownish streaks are observed in the vascular tissue of infected trees. This progression of dieback affecting cocoa was also reported by Mbenoun et al. (2008), Kannan et al. (2009) and by Rosmana et al. (2013) [5, 8, 9].

Based on the cultural characteristics and conidial morphology of the fungi isolated from the diseased cocoa plant materials in different studied sites in Cameroon, the pathogens were identified as *Lasiodiplodia theobromae* and *Fusarium* sp.

In culture, isolates of *L. theobromae* grew much faster than the other fungal species, able to fully colonize a 90-mm Petri dish within 48 h. Furthermore, they produced mature conidia were dark brown and oval shaped with a sizes equivalent to $20.26 \pm 0.44 – 26.00 \pm 0.0$ µm (length) and 10.26 ± 0.48 -14.36 ± 0.50 µm (width). Sizes of conidia of this study were similar to that reported by Magd et al. (2012) [25]. Conidial shape, color and the presence of septa and longitudinal striations were robust characteristics for identification and separation of *L. theobromae* from the other *Lasiodiplodia*. These morphological distinctions were also described in previous literature [18-6-26]. *L. theobromae* was previously reported as being the pathogen agent responsible for dieback affecting cocoa in Hawaï [7], and in India [8]. As an important pathogen of woody hosts, it has been reported to cause cankers, dieback, fruit rot, and root rots on over 500 different hosts, including perennial fruits, nut trees, vegetable crops, and ornamental plants [27, 28, 29-18]. Nevertheless, in Cameroon, *L. theobromae* is also known as being responsible for dieback affecting *Terminalia catappa* [12].

Fusarium isolates were obtained from diseased roots and twigs. It was isolated with *L. theobromae* in some diseased plant materials of Ndikinimeki. Only *L. theobromae* was isolated from diseased plants materials of Ntui and Okola. Colony color of *Fusarium* sp. on PDA medium and conidia size in this study were similar to that reported by Adu-Achaempong and Archer (2011); and Rosmana and al. (2013) [6-9]. Its growth rate was low and the color of mycelium was white creamy*. Fusarium* sp. was identified as being responsible of cocoa dieback in Indonesia [9, 10]. *Fusarium* species are ubiquitous fungi found in the temperate and tropical areas of the world and cause a wide range of plant diseases that affect many crops, often with devastating socio-economic impact [9].

The both fungus and *L. pseudotheobromae* were identified to be responsible of cocoa dieback in Ghana and Nigeria [6-11]. It became so essential to show the real role of each species in the apparition of symptoms of cocoa dieback in Cameroon.

5. CONCLUSION

Observations made in cocoa orchards and the communications with cocoa farmers during this work indicate that, the dieback greatly devastates cocoa trees, reason why some plantations have totally been abandoned by farmers in Ndikinimeki site. Therefore, dieback becomes a major constraint to production of cocoa in Cameroon. Data obtained during survey indicated that, cultivated cocoa varieties varied across the sites. Amelonado varieties are more tolerant to dieback disease than hybrid varieties. Furthermore, farmers should avoid using the same machetes to cut diseased cocoa trees and pruning healthy cocoa trees. *L. theobromae* and *Fusarium* spp. isolated from diseased plant materials most be considered as the main agents of dieback disease in Cameroon. Others studies should be done to establish the real role of each species associated to cocoa dieback in order to determine appropriate strategies to control this disease.

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