Field Assessment of Cocoa Dieback Due to the Neglected Mosquito True Bug, *Helopeltis* sp. (Hemiptera: Miridae) and Associated Pathogenic Fungi Infections in Southern Cameroon

PIERRE BORIS NSOGA ETAM[1,3], RAYMOND JOSEPH MAHOB[1*], YEDE[1], PAUL SERGE MBENOUN MASSÉ[1], LAURENT BALEBA[2], RICCADO FEUDJIO[1], MOUHAMADOU MOUMBAGNA MBOUTNGAM[1], KOGA MANG'DOBARA[1], STANISLAS RAPHAEL BIKAY[3], CHARLES FÉLIX BILONG BILONG[1]

[1]Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, Yaoundé, CAMEROON
[2]Institute of Agricultural Research for Development (IRAD), Yaoundé, CAMEROON
[3]National Coordination of cocoa program, Ministry of Agriculture and Rural Development, Yaoundé, CAMEROON
raymondmahob@gmail.com, https //www.raymond-joseph.mahob@uy1.uninet.cm

Abstract: Cocoa dieback is an emergence disease in cocoa farms in West Africa, due to the synergistic action of *Sahlbergella singularis* Hagl. and/or *Distantiella theobroma* (Dist.) and opportunist fungi infestations/infections. Data regarding the involvement of others mirid species as *Helopeltis* sp., commonly encountered in plantations in Southern Cameroon, on dieback process of cocoa plants remain unknown. Then, we investigated the effect of *Helopeltis* sp. feeding and associated pathogenic fungi infections on cocoa dieback emergence. Two different infestations (mirids and fine needles) alongside a control, on cocoa branches/twigs of eight genotypes (T79/501×SNK479, UPA143×NA33, T79/501×SNK13, UPA14×SNK64, SNK 7, TIKO 31, Pa 7 and IMC 60), were performed in plantations in order to characterize the cocoa dieback, and identify the associated pathogenic fungi using relevant dichotomous keys. Apart from 20.0% of undetermined species, three pathogenic fungi taxa were inventoried in the study site, namely *Lasiodiplodia* sp. with the highest occurrence (54.3%), followed by *Botryosphaeria* sp. (17.4%), then *Fusarium* sp. (8.3%). Overall, the highest occurrence of pathogenic fungi associated with cocoa dieback disease were obtained on branches infested with mirids (80.0% of the total) compared to those with fine needles (16.0%) and control (4.0%). Our results showed that dieback progression on infested cocoa branches varied amongst cocoa genotypes, mean values ranging from 3.0 ± 1.51 cm for genotype IMC60 (most tolerant) to 10.8 ± 2.16 cm for genotype UPA143×SNK64 (most susceptible). The fungi identified behaved as opportunistic species due to the primary *Helopeltis* sp. infestations of the host plant leading to dieback. Our findings undoubtedly show the synergistic action of *Helopeltis* sp. and fungi in cocoa dieback handing out and should be taken into account in Integrated Pest Management (IPM) programs against the targeted cocoa disease.

Keywords: *Theobroma cacao* genotypes, neglected true bug *Helopeltis* sp., synergistic action, opportunistic fungi, infestations/infections, Dieback, IPM

Received: September 18, 2022. Revised: July 14, 2023. Accepted: August 20, 2023. Published: September 13, 2023.

1 Introduction

Ranked fourth cocoa producing country after Ivory Coast with 33.0% of global production (1,472,313 metric tons), Ghana with 19.2% (858,729 metric tons), Indonesia with 14.7% (656,817 metric tons), Cameroon with 6.5% of the global production (291,512 metric tons) places cacaoculture as one of the highest pecuniary commodities in income-generation after the petroleum sector [1,2,3]. It is known that in Cameroon, the cocoa (*Theobroma cacao* Linnaeus, 1753) sector always generates more than 100 billion francs CFA annually, and contributes close to 28% of the non-petroleum exportation products and 40% of export products from the primary sector [2,4]. Notwithstanding a continuous increase in cocoa beans production within the national cocoa growing area, annual Cameroonian yields remain low compared to those of other African countries such as Ivory Coast. Cocoa yields, per hectare, in Cameroun have been estimated between 300 to 400 kg versus 500 to 600 kg in Ivory Coast [5, 6]. Among the causes of these low yields in cocoa farms are diseases and insect pests [7, 8, 9, 10]. According to many authors [7,8,9,10,11,12,13,14,15], in West and Central Africa in general and in Cameroun in particular, cocoa farms mainly suffer from the black pod disease due to *Phytophthora* spp. and mirids (*Sahlbergella singularis* Haglund, 1895 and *Distantiella theobroma* (Distant, 1909)). Cocoa plantations losses due to these pests and diseases were estimated between 10 to 100% in case of massive attacks and lack of appropriate treatments [7,11,14,15,16,17]. As regards mirids, apart from pods damage, these insect pests also attack branches, leaves and the trunks of cocoa trees, cause damage including dieback which occurs post the feeding puncture of mirids, in synergy with action of some opportunistic fungi species [2,15,18]. Dieback appears as a physiological opportunistic disease that begins at the level of wounds or feeding lesions to the entire host plant by mirid infestations; these insects inject their hemolytic saliva into the plant host tissues during the feeding punctures, which reaches the wood and libber channels of the affected cocoa tree, thus prevents the circulation of the phloem sap for example [19, 20, 21]. The inhibition of phloem sap circulation of the cocoa leads to a progressive drying of the leaves of the parasitized branches/twigs, ultimately causing death of the infected tree known as cocoa dieback [2,10,15,21,22]. So far and to the best of our knowledge, *Sahlbergella singularis* is the only species whose feeding bites are documented as being associated with cocoa dieback in West and Central Africa [2,15,18,23], probably due to its omnipresence/abundance and economic importance in cocoa farms [24,25,26,27]. However, other numerous insect pest species such as *Helopeltis* spp., also belonging to the Miridae family, are commonly encountered in plantations in Southern Cameroun, both during the fruiting and vegetative phases of the host plant [26,28,29]. These species are also piercing-sucking true bug insects for cocoa trees, especially cocoa branches/twigs in the inter-campaign i.e. the period after the pods harvest in the plantations corresponding to the vegetative phase of the host plant (Mahob, com.pers.). However, the involvement of these species in the development of dieback remains unknown. Ecological data regarding the impact of other mirid species such as *Helopeltis* sp. infestations, and the ultimate emergence of dieback to the host plant deserves to be clearly elucidated to understand holistically the interactions between mirid species *sensu lato* infestations and associated opportunistic fungi on the occurrence of cocoa dieback disease under field conditions. Indeed, investigation of the targeted pathology origin can considerably improve the integrated pests' management (IPM) of cocoa-based agrosystems worldwide, especially in Cameroon. Herein, we hypothesized that *Helopeltis* sp.' attacks to cocoa trees also causes dieback such as *S. singualris*, with synergistic action of opportunistic pathogenic fungi infections. The aim of this study was to elucidate the relationship between *Helopeltis* sp. and associated fungal infections on the dieback process of different cocoa genotypes under field conditions.

2 Materials and methods

2.1 Study site and experimental plot description

This study was carried out from July 2022 to February 2023, within three cacao blocks, each measuring ≈2500 m² (100 m × 25 m), situated at the IRAD-Research Station of Nkoemvone (2°40'N and 11°20'E; 630 m a.s.l.) (Fig. 1), in the semideciduous rain forest of Southern Cameroon. Data related to Cultural practices, floristic composition, climate and soil of the study site are documented by Mahob *et al.* [17,30] and Voula *et al.* [2].

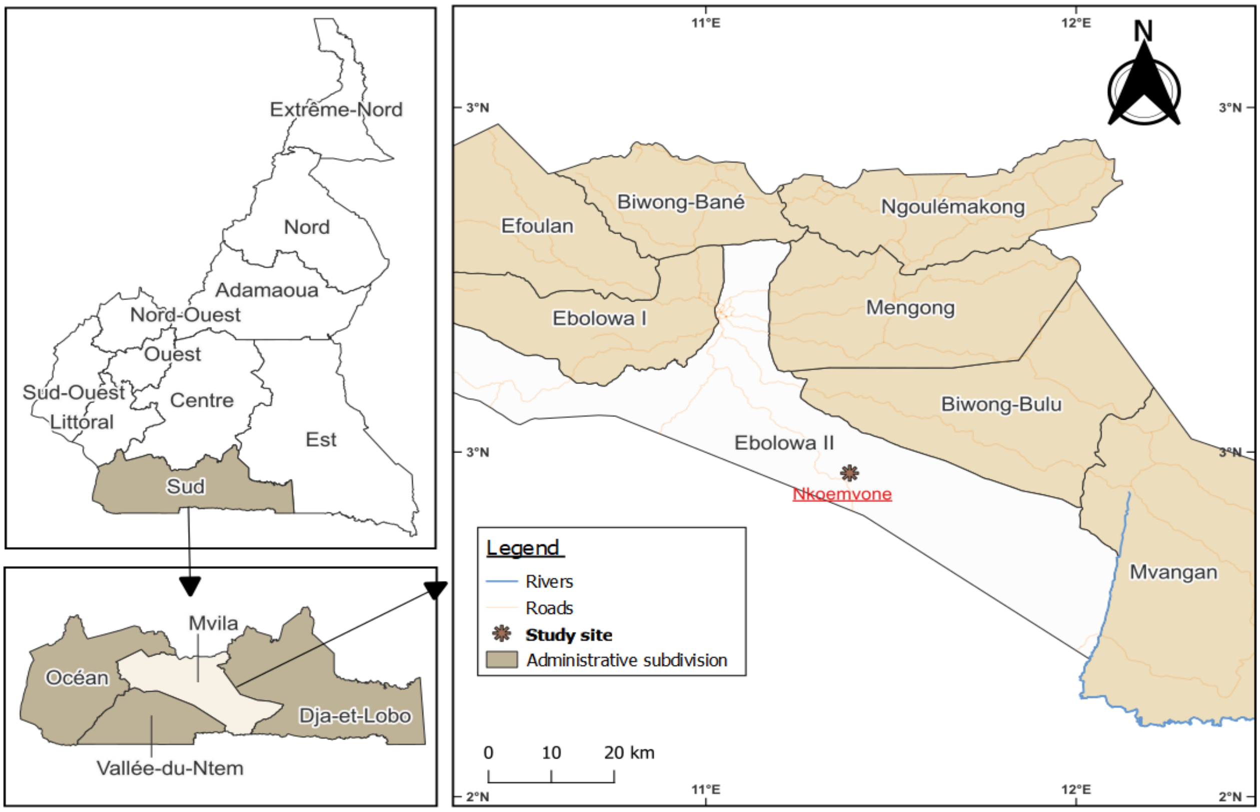

Fig. 1: Geographic location of the study site

Each experimental plot was mainly planted with about 300 cocoa trees; the flora and shade were relatively homogeneous [30]. The distance between two consecutive cocoa trees in a row and between the rows was 3 m in the sampling units. Selected plots were the Fisher's completely randomized blocks containing eight cocoa genotypes for our experiments, including four hybrids (T79/501 × SNK479, UPA143 × NA33, T79/501 × SNK13, UPA14 × SNK64) and four clones (SNK 7, TIKO 31, PA 7 and IMC 60). The tested cocoa genotypes were selected and placed in two genetic groups based on their: (i) numerical abundance compared to other genotypes during the study, and (ii) well-known origin: Upper Amazon = IMC60, PA 7, T60/887, T79/501 and UPA143 and local Trinitario = SNK and TIKO 31 [20,31,32]. This protocol assessed the different genotypes susceptibility/sensitivity or tolerance/resistance towards the feeding punctures of the target mirid and associated synergistic action of opportunistic pathogenic fungi regarding the cocoa dieback [2,15,18,23].

2.2 Field search of mirids and rearing method

Mirids were searched and caught within cocoa farms of the IRAD-Nkoemvone Research Station and in surrounding plantations, early in the morning i.e. between 6: 30 and 9:30 a.m, from July to September 2022 corresponding to the pullulating period of mirids in plantations [21,33,34]. A total of 200 individuals were collected, sexed/identified using relevant dichotomous keys of Lavabre [29] and Delvare and Aberlenc [35]. A total of 65 couples of females and males were constituted for rear. Mirids were reared only in plantations at IRAD-Nkoemvone Research Station due to the high performance of the breeding approach practiced there [2]. Thus, each couple of mirids was reared on cocoa pods covered with an aerated cloth sleeve (20 x 30 cm for immature fruits; and 30 x 40 cm for mature/ripe fruits); this method protects mirids against exogenous aggression [2,17,36,37]. Fourth and fifth instars' larvae were mainly used for experiments due to their being easily manipulated under field working conditions [17,32].

2.3 Assessment of the effect of mirid infestations on dieback

The effect of mirid infestations on dieback was assessed, as previously mentioned, on eight cocoa genotypes present in three completely randomized blocks among others at the IRAD-Nkoemvone Research Station. Two types of infestations were realized under cloth sleeves only during one week post-infestation, and then cloth sleeves were removed to facilitate host plant settlement by pathogen fungi (Fig. 2). The first one called biological infestation consisted of infecting the young shoots of branches/twigs, physiologically and phenotypically exempt of any attack, with mirids. We used one individual, larva stage 3 or 4, which was starved for 48 hours, per cocoa genotype and per replication. The second one called mechanical infestation consisted of infecting young shoots of branches/twigs with fine needles (10 stings at 2 cm depth), per genotype and per replication [2,15]. A negative control i.e. young shoots of branches/twigs without any infestation was also used. A total of 30 replications were done per cocoa genotype, including a negative control. The physiological reaction of the tested organs for each type of infestation per genotype was observed weekly for 3 months (December 2022 to February 2023); the length of dieback progression, when observed, was measured in centimeters with a ruler. Cocoa dieback disease was recorded from December to February in the long dry season because mirids damage on the host plant is easily observed at this period [19,21,22].

Fig. 2: Protocol of branches/twigs infestation with mirid *Helopeltis* sp.: (A) partial opening of cloth sleeve which protecting the arena, (B) complete closure of the cloth sleeve with infested young shoot of branches/twigs.

2.4 Isolation and characterization of parasitic fungi

Isolation and characterization of cocoa plant parasitic fungi was performed at the Yaoundé (Political Capital of Cameroon) Phytopathology Laboratory of IRAD. Collected cocoa twig samples from the three types of infestation were rinsed with tap water for 10 minutes to get rid of impurities and debris on their surface [38,39]; they were subsequently immersed in 70% ethanol for 1 minute, then in a 2.5% sodium hypochlorite (NaOCl) solution for 4 minutes. These samples were put again in 70% ethanol for 30 seconds [40], rinsed thrice with distilled water for 1 minute, then dried on sterile absorbent paper [39,40]. They were later cut into small fragments and placed in Petri dishes containing Potato Dextrose Agar (PDA) previously autoclaved at 121°C for 15 minutes, supplemented with 1 mg/l of chloramphenicol to inhibit bacteria growth; finally, they were incubated at room temperature in order to obtain pure cultures of the fungi (Fig. 3). Fungi identification was done using the morphological characters with the relevant dichotomous keys [40,41,42;43,44]; and the occurrence of each identified fungal species was calculated according to Bush *et al.* [45].

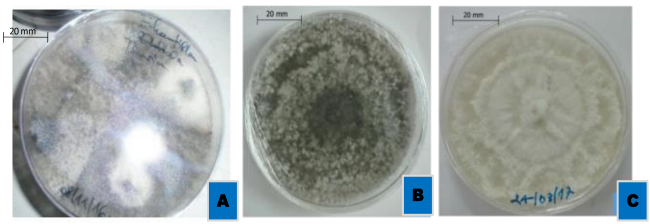

Fig. 3: Petri dishes containing some fungal isolates: A) Negative Control, B) Mirids and C) Fine needles.

2.5 Statistical analysis

The average number of the dieback on young shoot branches, expressed by the decaying length for each tested cocoa genotype per replication, was recorded. The original data were log-transformed for normality before the analysis; then average lengths of dieback of the different cocoa genotypes were compared for by one-way ANOVA using the Generalized Linear Model (GLM). When differences were found between average lengths of dieback, Student-Newman-Keuls (SNK) post hoc was used for pairwise comparisons of the multiple average lengths of dieback of 08 cocoa genotypes. The degree of similarity of the 08 tested genotypes for their susceptibility/sensitivity regarding the dieback cocoa disease, due to mirid infestations was determined using a Cluster analysis, where cocoa genotypes were considered as line individuals and pathogenic fungi species as column individuals. All statistical analyses were performed with STATISTICA (version 10) software and the differences were deemed to be significant at $P<5\%$.

3 Results

3.1 Pathogenic fungi associated to cocoa dieback

Overall, the highest occurrence of pathogenic fungi associated with cocoa dieback disease was obtained on branches infested with mirids (80.0% of the total) and the lowest ones with fine needles (16.0%), and the control (4.0%). Apart from 20.0% of undetermined species, three pathogenic fungi taxa were inventoried in the study site, namely: *Lasiodiplodia* sp., *Botryosphaeria* sp. and *Botryosphaeria* sp.. The occurrence of *Lasiodiplodia* sp. (54.3%), was highest followed by *Botryosphaeria* sp. (17.4%), then *Fusarium* sp. (8.3%) (Fig. 4).

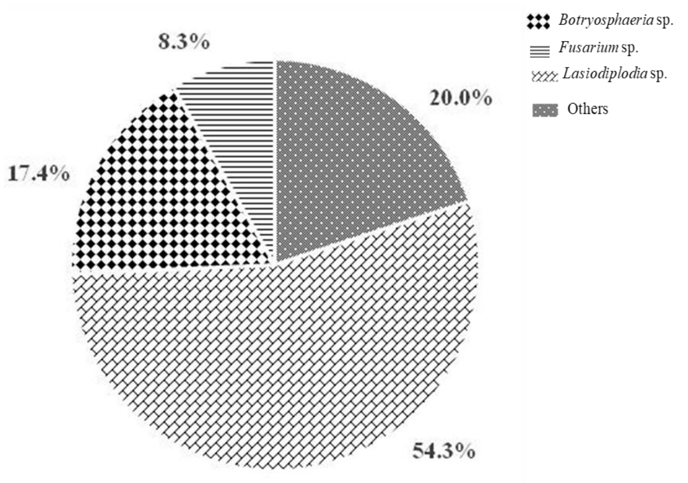

Fig. 4: Occurrence frequency distributions of pathogenic fungi towards the studied cocoa genotypes.

3.2 Dieback length on branches of the studied cocoa genotypes

Cocoa dieback on branches was observed only in cases of mirid infestations; values varied between the studied cocoa genotypes and ranged from o cm for all tested cocoa genotypes to 20 cm for UPA 143 x SNK64 (Fig. 5).

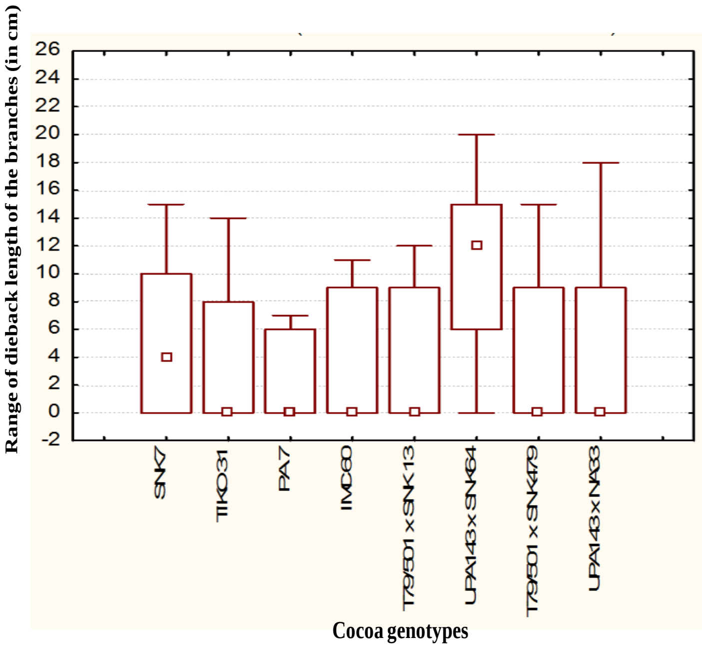

Fig. 5: Frequency distributions of the length (in cm) of twigs dieback in function of tested cocoa genotypes, after the mirid *Helopeltis* sp. infestations.

Average dieback lengths varied significantly ($F_{(7,232)}= 52.6$; $P<0.0001$) between the eight cocoa genotypes tested; obtained values were grouped into three homogeneous subsets, according to ANOVA, and ranged from 3.0 ± 4.9 cm for IMC 60 (most tolerant/resistant) to 10.8 ± 6.8 cm for UPA x SNK 64 (most sensitive) (Table 1).

Table 1: Frequency distributions of the branches dieback length due to *Helopeltis* sp. infestations, and comparison of the obtained average values (± SD) in cm of studied parameter

Cocoa genotypes	Number of samples	Average length (± SD) of dieback
IMC 60	30	3.0 ± 4.9^{b}
PA 7	30	3.8 ± 5.8^{b}
TIKO 31	30	4.0 ± 5.4^{b}
T79/501 x SNK13	30	4.0 ± 5.3^{b}
T79/501 x SNK479	30	4.3 ± 5.9^{b}
UPA141 x NA33	30	4.5 ± 5.8^{b}
SNK 7	30	5.5 ± 6.1^{ab}
UPA x SNK64	30	10.8 ± 6.8^{a}
	Statistics :	$F_{(7,232)}= 52.6$; $P<0.0001$

In column 3 on the right, values with the same letter are not significantly different at 95% of confidence *interval, according to Student-Newman-Keuls (SNK); SD: Standard deviation.*

3.3 Estimation of the degree of cocoa dieback similarity between the tested genotypes, according to their susceptibility to the studied host plant disease

Cluster analysis divided the 08 cocoa genotypes into three homogeneous subsets (A, B and C); within each subset there were also close similarities between the tested cocoa varieties in terms of susceptibility to cocoa dieback disease (Fig. 6).

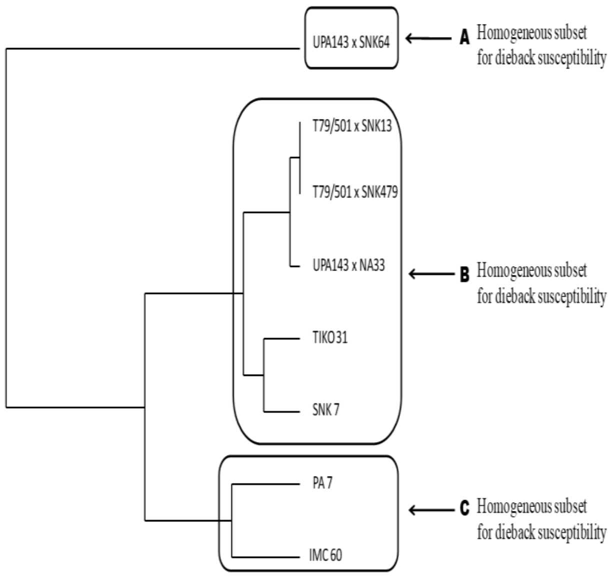

Fig. 6: Similarity of the susceptibility/sensitivity of the tested cocoa genotypes to dieback disease, post mirid infestations, according to cluster analysis. A, B and C: homogeneous subsets.

4 Discussion

The aim of this study was to determine the impact of the feeding mirid (*Helopeltis* sp.) punctures and associated pathogenic fungal agents' infections on the ultimate emergence of cocoa dieback. The susceptibility/sensitivity of different cocoa genotypes was assessed by infecting the young shoots of branches/twigs in the plantations followed by the characterization of the fungi taxa involved on the target host plant pathology under laboratory conditions. Cocoa genotypes susceptibility/sensitivity or tolerance/resistance to dieback disease was determined by measuring the decaying length on the infected twigs in the current work as a step of varietal breeding towards the targeted pathology. Results showed differences between cocoa genotypes in the susceptibility/resistance to dieback disease regarding the decaying length on the twigs of the tested varieties. Concerning the average dieback length values obtained in our investigations, cocoa hybrid UPA x SNK 64 and IMC 60 were most sensitive and tolerant respectively to the fungi involved in the studied plant pathology. This result clearly shows that these cocoa genotypes have a differential sensitivity/tolerance to mirids and associated pathogenic fungi infestations in general, and especially towards dieback disease, although the tolerance/resistance mechanisms used by the host plant remain to be elucidated. The result also supports findings of Voula *et al.* [2], Anikwe and Otuonye [15], Adu-Acheampong *et al.* [18], Crowdy [46], Owen [47], Sounigo *et al.* [48] and N'Geussan *et al.* [49,50], which reported that the sensibility/tolerance of cocoa to mirid infestations and/or dieback disease varies in function of cocoa varieties. Three fungi taxa, namely *Lasiodiplodia* sp. (54.3%), *Botryosphaeria* sp. (13.4%) and *Fusarium* (8.3%) in both biological (mirids), mechanical (fine needles), and in the negative control were identified. However, the cocoa dieback disease has been observed only in cases of mirid infestations involving all the tested genotypes. These fungi species inventoried in both positive and negative (control) trials clearly show their omnipresence in host tissues, and confirm their natural endophyte status on cocoa varieties although not necessarily causing dieback disease [2,15,51,52]. The fact that cocoa dieback disease symptoms were only observed on the branches primarily infested by mirids undoubtedly indicates that mirids infestations create favorable physiological conditions, such as the stress in the host plant, resulting to the emergence of a pathology with the synergistic action of opportunistic fungi [2,15,46]. Indeed, it is known that during their feeding, mirids inject hemolytic saliva into the host tissues, which results in subsequent lesions/wounds in/on the infested cells which are later colonized or invaded by the opportunistic fungi, such as those inventoried in the current study and leading to cocoa dieback [2,15,23,46]. Although the fungi occurrence varies between the taxa (Fig. 4), it is obvious that each fungal taxa played a significant role in the cocoa dieback process. We therefore assume that polyinfection by fungi associated with some ecological factors such as cocoa stress, necrotic lesions, phloem sap disruption, soil quality nutriments, and the catalytic role of mirids are ultimately responsible for the death of the entire affected cocoa tree under field conditions [2,18]. Similar to previous studies on cocoa [2,15] and in other host plants such as *Mangifera indica* Linnaeus, 1753 [53, 54], our results show that *Lasiodiplodia* sp. was the most frequent compared to the other two identified fungi species (Fig.4), and confirms its status as the main opportunistic fungal species associated with cocoa dieback [2,15]. Conversely to our study, cocoa farms in Nigeria, Anikwe and Otuonye [15] observed cocoa dieback on twigs infested with fine needles with decaying mean length values ranging from 7.8 ± 0.16 to 8.7 ± 0.15 mm. This difference could be linked to the difference in the genetic make up of the cocoa varieties and their response to exogenous attacks such as those with fine needles [18,48]. In fact, cocoa varieties in our study showed a great resistance compared to those studied by Anikwe and otuonye [15], which were sensitive to fine needle infestations; the mechanisms underlying the defenses of cocoa trees against mechanical infestations require further investigations. Data on the species richness/diversity and fungal occurrences in this work also differ from those of Anikwe and Otuonye [15] and Voula *et al.* [2]. This could be explained by the fact that frequency distributions of pathogens (including fungi) vary in space and time depending on the heterogeneity (i) in susceptibility or exposure of cocoa genotypes to pathogenic fungi, and (ii) experimental conditions [18,55,56].

In addition to dieback disease, mirids i.e. *Helopeltis* sp., *S. singularis* and other insect species [57] are also involved in the spread of the pod rot cocoa disease, due to *Phythophthora* spp. in fields, known as the main cocoa disease in West and Central Africa [15, 57]. In the current work, *Helopeltis* sp. mimicked the behavior of *S. singularis* regarding the damage towards the host plant; it was also revealed as a major economic insect pest due, among others, to the cocoa field production losses (60 ± 0.11% to 96 ± 0.05% per year) caused by this insect species, due to the absence or inappropriate control measures with insecticides treatments [60]. Therefore, it is crucial to study the interactions between each mirid species present in plantations and cocoa trees, in order to determine its ecological status, then develop a sustainable holistic program for Integrated Pest Management (IPM) against cocoa mirids *sensu lato.*

5 Conclusion

This study shows that the cocoa mosquito mirid true bug *Helopeltis* sp. infestations associated with the synergistic action of opportunistic fungi are also involved in the emergence of dieback disease in plantations. Working in deterministic conditions and in the same study site as Voula *et al.* [2], our study emphasizes the previous findings obtained in West [15,23] and Central [2] Africa with *Sahlbergella singularis,* known as a major economically important insect pest in these cocoa growing area. Indeed, our investigations confirm, once again, the positive relationship between mirid species infestations and the dieback process of the cocoa plants due to the postinfection of pathogenic fungi such as *Lasiodiplodia* sp., *Botryosphaeria* sp. and *Fusarium* sp. inventoried in this work. Compared to both others enumerated fungal species, *Lasiodiplodia* sp. being the most predominant confirmed its status as the main opportunistic fungal species involved in cocoa dieback disease in West and Central Africa in general, and especially in Cameroon at the Nkoemvome IRAD-Research Station. Cocoa trees naturally hosted all the inventoried fungal species but did not cause any dieback disease, while only cocoa branches infested by *Helopeltis* sp. developed dieback disease. This supports a catalytic role of this insect species towards the development of the studied pathology on cocoa varieties. Clone IMC 60 and hybrid UPA x SNK64 were most tolerant/resistant and most sensitive respectively while the six other genotypes (T79/501 × SNK479, UPA143 × NA33, T79/501 × SNK13, SNK 7, TIKO 31 and PA 7) had an intermediate sensitivity/tolerance to cocoa dieback disease. We suggest that the current new data be taken into account in breeding programs of cocoa varieties against the studied pathology for efficiency and sustained IPM against the dieback disease under field conditions.

Acknowledgements

This work was funded by the special research allowances from the Cameroon Ministry of Higher Education and the complementary use of internal allowances from the University of Yaoundé I. Thanks to the Institute of Agricultural Research for Development (IRAD) for logistic and material support, and to Dr NJUA Clarisse, Senior Lecturer at the Faculty of Science of the University of Yaoundé I, for proofreading of the manuscript. We also thank Dr Mbenoun Michael, Researcher at Forest Pathology, Natural Resources, Edmonton, Alberta, Canada, for help in the fungi identification.

References:

[1] Beg M.S., Ahmad S., Jan K. and Bashir K., Status, supply chain and processing of cocoa- A review, *Trends Food Science Technology*, 66, 2017, pp. 108–116.

[2] Voula V.A., Manga Essouma F., Messi Ambassa L.M., Mahob R.J. and Begoude B.D., Impact of mirids and fungal infestation on dieback of cocoa in Cameroon, *Journal of Entomology and Zoology Studies*, 6, 2018, pp. 240–245.

[3] Suh N.N. and Molua E.L., Cocoa production under climate variability and farm management challenges: Some farmers' perspective, Journal of Agriculture and Food Research 8, 100282, 2022,https://doi.org/10.1016/j.jafr.2022.100282.

[4] Ndah N.R., Nanje Ekole P., Agwa M.H., Taku J., Fonyikeh-Bomboh Lucha C. and Agbor D.T., Crop diversification and sustainability in a cocoa agroforestry system in Meme Division South West Region, Cameroon, *Asian journal of Research in Agriculture and Forestry*, 9, 2, 2023, pp. 1-15.

[5] Wessel M. and Quist-Wessel P.M.F., Cocoa production in West Africa, a review and analysis of recent developments, *NJAS - Wageningen Journal of Life Sciences*, 74–75, 2015, pp.1–7.

[6] Bomdzele E.Jr. and Molua E.L., Assessment of the impact of climate and non-climatic parameters on cocoa production: a contextual analysis for Cameroon, *Frontier in Climate,* 5,1069514, 2023, https://doi: 10.3389/fclim.2023.1069514.

[7] Mahob R.J., Ndoumbè-Nkeng M., Ten Hoopen G.M., Dibog L., Nyassé S., Rutherford M., Mbenoun M., Babin R., Amang A Mbang J., Yede and Bilong Bilong, C. F., Pesticides use in cocoa sector in Cameroon: characterization of supply source, nature of actives ingredients, fashion and reasons for their utilization, *International Journal of Biological and Chemical Sciences*, 8, 2014, pp. 1976–1989.

[8] Babin R., Pest management in organic farming. In: Vacante, V. & Kreiter, S. (Eds) *Handbook of Pest Management in Organic Farming*, CAB International, Wallingford, U.K., 2018.

[9] Cilas C., Sounigo O., Mousseni Efombagn B., Nyassé S., Tahi M. and Bharath S.M., Advances in pest- and disease-resistant cocoa varieties. University of the West Indies, Trinidad and Tobago. In: Pathmanathan, U. (Ed.) *Burleigh Dodds Series in Agricultural Science*, 2018, pp. 1–19.

[10] Cilas C. and Bastide P., Challenges to Cocoa Production in the Face of Climate Change and the Spread of Pests and Diseases, *Agronomy*, 10, 2020, pp. 1232-1238.

[11] Armengot L., Ferrari L., Milz J., Velásquez F., Hohmann P. and Schneider M., Cacao agroforestry systems do not increase pest and disease incidence compared with monocultures under good cultural management practices, *Crop protection*, 130, 105047, 2020, https://doi.org/j.cropro.2019.105047.

[12] Riedel J., Kägi N., Armengot L. and Schneider M., Effect of rehabilitation pruning and agroforestry on cacao tree development and yield in an older full-sun plantation, *Experimental Agriculture*, 0, 2019, pp. 1-17.

[13] Sonwa D.J., Coulibaly O., Weise S.F., Akinwumi Adesina A. and Janssens M.J.J., Management of cocoa: Constraints during acquisition and application of pesticides in the humid forest zones of southern Cameroon, *Crop Protection,* 27, 2008, pp. 1159-1164.

[14] Asitoakor B.K., Asare R., Raebild A., Ravn H.P., Eziah Yao V., Owusu K., Mensah Opoku E. and Vaast P., Influences of climate variability on cocoa health and productivity in agroforestry systems in Ghana, 327, 109199, 2022,https//doi.org/10.1016/j.agrformet.2022.109199.

[15] Anikwe J.C. and Otuonye H.A., Dieback of cocoa (*Theobroma cacao* L.) plant tissues caused by the brown cocoa mirid *Sahlbergella*

singularis Haglund (Hemiptera: Miridae) and associated pathogenic fungi, *International Journal of Tropical Insect Science*, 35, 2015, pp. 193–200.

[16] Yede, Babin R., Djieto-Lordon C., Cilas C., Dibog L., Mahob R. and Bilong Bilong C.F., True bug (Heteroptera) impact on cocoa fruit mortality and productivity. *Journal of Economic Entomology*, 105, 2012, pp. 1285–1292.

[17] Mahob R.J., Feudjio Thiomela R., Dibog L., Babin R., Fotso Toguem Y.G., Mahot H.,·Baleba L.,·Owona Dongo P.A. and·Bilong Bilong C.F., Field evaluation of the impact of *Sahlbergella singularis* Haglund infestations on the productivity of different Theobroma cacao L. genotypes in the Southern Cameroon, *Journal Plant Disease and Protection*, 126, 2019, pp. 203–210.

[18] Adu-Acheampong R., Archer S. and Leather S. Resistance to dieback disease caused by *Fusarium*and *Lasiodiplodia* species in cacao (*Theobroma cacao* L.) genotypes. *Experimental Agriculture*, 48, 1, 2012, pp. 85–98.

[19] Entwistle P.F., *Pests of cocoa*, Longman Group Ltd, London, UK, 1972.

[20] Mbondji P.M., *Le cacaoyer au Cameroun*, Presse de l'Université Catholique d'Afrique Centrale, Yaoundé, Cameroun, 2010.

[21] Mahob R.J., Dibog L., Ndoumbè-Nkeng M., Begoude Boyogueno A.D., FotsoToguem Y.G., Nyassé S. and Bilong Bilong C.F., Field assessment of the impact of farmers' practices and cacao growing environment on mirid abundance and their damage under unshaded conditions in the southern Cameroon, *International Journal of Tropical Insect Science*, 40(2), 2020, pp. 449-460.

[22] Mahob R.J., Baleba L., Yede D.L., Cilas C., Bilong Bilong C.F. and Babin R., Spatial distribution of *Sahlbergella singularis* Hagl. (Hemiptera: Miridae) populations and their damage in unshaded young cacao-based agroforestry systems, *International Journal of Plant Animal Environmental Sciences*, 5, 2015, pp. 121–131.

[23] Adu-Acheampong R. and Archer S., Diversity of fungi associated with mirid (Hemiptera: Miridae) feeding lesions and dieback disease of cocoa in Ghana, *International Journal of Agricultural Research*, 6, 2011, pp. 660–672.

[24] Anikwe J.C., Evaluation of Field Damage and Chemical Control of Outbreak of *Sahlbergella Singularis* Haglund in a Cocoa Plantation in Ibadan, Nigeria, *American Eurasian Journal Sustainable Agriculture*, 3, 2009, pp. 19–23.

[25] Mahob R.J., Pesticides utilisés en cacaoculture et Essai de lutte intégrée contre *Sahlbergella singularis* Haglund 1985 (Hemiptera : Miridae), principal bio-agresseur du cacao (*Threobroma cacao* Linné, 1753) au Cameroun, Thèse de Doctorat Ph.D, Université de Yaoundé I, Cameroun, 2013, pp.1-179.

[26] Yede. Diversité des peuplements des hémiptères dans les cacaoyères de la Région du Centre Cameroun: impact économique et essai de lute biologique, Thèse de Doctorat PhD, Université de Yaoundé I, Yaoundé, Cameroun, 2016, pp. 1-174.

[27] Bagny Beilhe L., Babin R. and Ten Hoppen M., Insect pests affecting cacao. In: Umahara, P. (Ed.), *Achieving Sustainable Cultivation of Cocoa,* Burleigh Dodds Science Publishing, Cambridge, UK, 2018.

[28] Lavabre E.M., *Insectes nuisibles des cultures tropicales (cacaoyer, caféier, colatier, poivrier, théier)*, Edition G.P. Maisonneuve et Larose, Paris, France, 1970.

[29] Lavabre E.M., *Les mirides du cacaoyer*, Edition G.P. Maisonneuve et Larose, Paris, France, 1977.

[30] Mahob R.J., Babin R., Ten Hoopen G.M., Dibog L., Yede, Hall D.R. and Bilong Bilong C.F., Field evaluation of synthetic sex pheromone traps for the cocoa mirid

Sahlbergella singularis (Hemiptera: Miridae), *Pest Management Science*, 67, 2011, pp. 672–676.

[31] Dibog L., Babin R., Mbang J.A.A., Decazy B., Nyassé S., Cilas C. and Eskes A.B., Effect of genotype of cocoa (*Theobroma cacao*) on attractiveness to the mirid *Sahlbergella singularis* (Hemiptera: Miridae) in the laboratory, *Pest Management Science,* 64, 2008, pp. 977-980.

[32] Mahob R. J., ·Mama Ngah I.,· Dibanda Feumba R., Mahot H. C., Bakwo Bassogog C B. Bilong Bilong C.F., Edoun Ebouel F., Nsoga Etam P. B., Taliedje D.M., Hanna, R. and Babin·R., Secondary metabolite effects of different cocoa genotypes on feeding preference of the mirid *Sahlbergella singularis* Hagl., *Arthropod-Plant Interactions*, 2021, https://doi.org/10.1007/s11829-021-09857-x.

[33] Babin R., Ten Hoopen G.M., Cilas C., Enjalric F., Yede, Gendre P. and Lumaret J.P., Impact of shade on the spatial distribution of *Sahlbergella singularis* in traditional cocoa agroforests. *Agricultural and Forest Entomology*, 12, 2010, pp. 69–79.

[34] Bisseleua H.B.D., Yede and Vidal S., Dispersion models and sampling of cacao mirid bug *Sahlbergella singularis* (Hemiptera: Miridae) on Theobroma cacao in southern Cameroon, *Environmental Entomology*, 40, 2011, pp. 111–119.

[35] Delvare G. and Aberlenc H.P., *Les Insectes d'Afrique et d'Amérique Tropicale* : *Clés pour la Reconnaissance des Familles*, CIRAD/PRIFAS, Montpellier, France, 1989.

[36] Babin R., Bisseleua D.H.B., Dibog L. and Lumaret J.-P., Rearing method and life-table data for the cocoa mirid bug *Sahlbergella singularis* Haglund (Hemiptera: Miridae), *Journal of Applied Entomology*, 132, 2008, pp. 366–374.

[37] Babin R., Anikwe J.C., Dibog L. and Lumaret J.-P., Effects of cocoa tree phenology and canopy microclimate on the performance of the mirid bug *Sahlbergella singularis*, *Entomologia Experimentalis et Applicata,* 141, 2011, pp. 25–34.

[38] Hazalin N.A., Ramasamy K., Lim S.M., Wahab I.A., Cole A.L. and Abdul Majeed A.B., Cytotoxic and antibacterial activities of endophytic fungi isolated from plants at the National Park, Pahang, Malaysia. *BMC Complementary and Alternative Medicine*, 9, 2009, pp. 46-50.

[39] Khan R., Shahzad S., Choudhary M.I., Khan S.A. and Ahmad A., Communities of endophytic fungi in medicinal plant *Withania somnifera*, *Pakistan Journal of Botany*. 42, 2010, pp. 1281-1287.

[40] Pimentel I.C., Glienke-Blanco C., Gabardo J., Stuart R.M. and Azevedo J.L., Identification and colonization of endophytic fungi from soybean (*Glycine max* (L.) Merril) under different environmental conditions, *Brazilian Archives of Biology and Technology*, 49, 2006, pp. 705-711.

[41] Botton B., Breton A., Fevra M., Gauthier S., Guy P., Larpent J.P., Reymond P., Sanglier J.J., Vayssier Y. and Veau P., *Moisissures utiles et nuisibles. Importance industrielle*, Edition Masson, Paris, France, 1990.

[42] Pitt J.I. And Hocking A.D., *Fungi and food spoilage*. Academic Press Inc, Sydney, Orlando, San Diego, New York, London, Toronto, Montréal, Tokyo, 1985.

[43] Champion R. *Identifier les champignons transmis par les semences*. Ist Edition INRA, Paris, France, 1997.

[44] Barnett H. L. and Hunter B., *Illustrated Genera of Imperfect Fungi*. Edited By H. L. Barnett and B. B. Hunter, 4th Edition, APS Press, St Paul, Minnesota. USA, 1998.

[45] Bush A.O., Lafferty K.D., Lotz J.M. and Shostak A.W., Parasitology meets ecology on its own terms: Margolis et al revisited, *Journal of Parasitology*, 83(4), 1997, pp. 575-583.

[46] Crowdy S. H., Observations on the pathogenicity of *Calonectria rigidiuscula* (Berk and Br.) Sacc. On *Theobroma cacao* L., *Annals of Applied Biology,* 34,1947, pp. 45–49.

[47] Owen H., Further observations on the pathogenicity of *Calonectria rigidiuscula* (Berk and Br.) Sacc. To *Theobroma cacao* L., *Annals of Applied Biology,* 44, 2, 1956, pp. 307–321.

[48] Sounigo O., Coulibaly N., Brun L., N'Goran J.A.K., Cilas C., Eskes A.B., Evaluation of resistance of *Theobroma cacao* L. to mirids in Côte d'Ivoire: results of comparative progeny trials, *Crop Protection,* 22, 2003, pp. 615–621.

[49] N'Guessan K.E., N'Goran J.A.K. and Eskes A.B., Resistance of cacao (*Theobroma cacao* L.) to *Sahlbergella singularis* (Hemiptera: Miridae): investigation of antixenosis, antibiosis and tolerance, *International Journal of Tropical Insect Science*, 28, 2008, pp. 201–210.

[50] N'Guessan K.F., Lachenaud Ph. and Eskes A.B., Antixenosis as a mechanism of cocoa resistance to the cocoa mirid, *Sahlbergella singularis* (Hemiptera: Miridae), *Journal of Applied Biosciences*, 36, 2010, pp. 2333–2339.

[51] Arnold A. E. and Herre E. A., Canopy cover and leaf age affect colonization by tropical endophytes: ecological pattern and process in Theobroma cacao (Malvaceae), *Mycologia*, 95, 2003, pp. 388–398.

[52] Arnold A. E., Mejı´a L. C., Kyllo D., Rojas E. I., Maynard Z., Robbins N. and Herre E. A., Fungal endophytes limit pathogen damage in a tropical tree, *Proceedings of the National Academy of Sciences of the United States of America*, 100, 2003, pp. 15649–15654.

[53] Ismail A.M., Cirvilleri G., Polizzi G. and Crous P.W. *et al*., *Lasiodiplodia* species associated with dieback disease of mango (*Mangifera indica*) in Egypt, *Australan Plant Pathology*, 41, 2012, pp. 649-660.

[54] Mbenoun M., Wingfield Michael J., Begoude Boyogueno A.D., Nsouga Amougou F., Petchayo Tigang S., Ten Hoopen G.M. *et al*., Diversity and pathogenicity of the Ceratocystidaceae associated with cacao agroforests in Cameroon. *Plant Pathology*. 65(1), 2016, pp. 64-78.

[55] Combes C., *Interactions durables. Ecologie et évolution du parasitisme*, Edition Masson, Paris, France, 1995.

[56] Thomas F., Guégan J.F. and Renaud F., *Ecologie et évolution des systèmes parasites*, Groupe De Boeck SA., Brussel, Belgium, 2012.

[57] Taylor B. and Griffin M. J., The role and relative importance of different ant species in the dissemination of black pod disease of cocoa, In Epidemiology of Phytophthora on cocoa in Nigeria. Commonwealth Mycological Institute, Kew., 1981, pp. 114–131.

[58] Varlet F. and Berry D. Réhabilitation de la protection phytosanitaire des cacaoyers et caféiers du Cameroun. Tome I : rapport principal. Cirad/Conseil interprofessionnel du cocoa et du café : Douala, Cameroun, 1997, p 204.

[59] Adu-Acheampong R., Jiggins J., Van huis A., Cudjoe A.R., Johnson V., Sakyi-Dawson O., Ofori-Frimpong K., Osei-Fosu P., Teiquartey E., Jonfia-Essien W., Owusumanu M., Nana Karikariaddo M.S., Afari-Mintah C., Amuzu M., Nyarkoeku-X N. and Quarshie E.T.N., The cocoa mirid (Hemiptera: Miridae) problem: evidence to support new recommendations on the timing of insecticide application on cocoa in Ghana, *International Journal of Tropical Insect Science* **34**, 2014, pp. 58–71.

[60] Mahob R.J., Nsoga Etam P.B., Dibog L., Babin R., Voula A.V., Begoude D., FotsoToguem Y. G., Baleba L., Owona Ndongo P. A. and Bilong Bilong C.F., Assessment of the effect of cocoa mosquito mirid true bug, *Helopeltis*sp. (Hemiptera: Miridae) on the cocoa (*Theobroma cacao* L.) production in Cameroon (Central Africa), *International Journal of Biological and Chemical Sciences*, 12,4, 2018, pp.1865-1875.