Spatio-temporal Patterns in Coffee Berry Borer Infestations in Robusta Coffee Plantations Across an Elevation Gradient in Cameroon, Central Africa

RAYMOND JOSEPH MAHOB[1], LAURENT BALEBA[2], PHILIPPE NWANE[1],SERGE ETEME ENAMA[1], KOGA MANG'DOBARA[1], CHARLES FÉLIX BILONG BILONG[1], RACHID HANNA[3,4]

[1]Laboratory of Parasitology and Ecology, Faculty of Sciences, University of Yaoundé I,P.O. Box 812 Yaoundé (CAMEROON)

[2]Institute of Agricultural Research for Development (IRAD), P.O. Box 2067 Nkolbisson-Yaoundé (CAMEROON)

[3]International Institute of Tropical Agriculture (IITA), BP 2008, Yaoundé-Messa (CAMEROON)

[4]Present address: Institute of the Environment and Sustainability, Center for Tropical Research, University of California Los Angeles, Los Angeles, USA

Corresponding author: raymondmahob@gmail.com

Abstract

The coffee berry borer (CBB), *Hypothenemus hampei*(Ferrari), is the most important insect pest of Robusta coffee worldwide. Much of the field knowledge on CBB ecology has been generated from low elevation geographies, but similar knowledge from highland geographies is scarce, particularly from Central Africa which is considered as the native home of CBB. Data from other countries or contrasting growing coffee area conditions and a comparative analysis between them would be useful to a better understanding of CBB bio-ecology. For this reason, we conducted natural field experiments to determine CBB infestation patterns in Robusta coffee plantations across elevation gradient. The experiments were conducted in 6 Robusta coffee plantations (700-1400 m.a.s.l.) in the western highlands of Cameroon; and observations were carried out on residual berries (RB) and new fruits (NF) randomly collected on the branches and/or ground. CBB infestations generally increased per coffee tree with increasing elevations, particularly in RB significantly higher values obtained at highest elevation fields at Banki (56.2 ± 10.47 individuals) compared with lower elevation fields at Mbile (38.6 ± 8.47) and Ntiengue (17.2 ± 6.07);the sex ratio was female-biased in the three study locations. Similarly, monthly relative frequency of infested NF per tree were numerically higher overall at Banki (12.6 ± 3.20%) followed by Mbile (10.6 ± 4.03%) and then Nteingue (8.1 ± 3.59%), but this difference was not statistically significant. In all locations, fruiting season CBB infestations increased with increasing fruits maturation – from young to mature fruits, with a steeply rapid rise during the last 2 months (January and February 2016) of fruits maturation. The findings reported in this study are discussed within the framework of integrated program for the management of CBB in Cameroon and similar agroecologies in Central Africa.

Keywords: Hypothenemus hampei, bio-ecology, *Coffea canephora*, residual berry infestations, new fruit infestations, Western highlands of Cameroon

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1 Introduction

Hypothenemus hampei (Ferrari) (Coleoptera: Curculionidae), known as coffee berry borer (CBB), is one of the major insect pest of coffee plantations due to the important economic losses that it causes in the coffee sector worldwide [1,2,3,4,5]. The annual losses due to this insect pest, in untreated farms and in case of high parasitic pressure, have been estimated to 60% in Colombia, 58-85% in Jamaica, 50-90% in Malaysia, 60% in Mexico, 90% in Tanzania and 80% in Uganda [6]. Presumably native to Central Africa, this insect pest is found nowadays in most coffee growing areas throughout the world [4,7,5]. Feeding exclusively on berries, CBB does not damage the vegetative parts of the host plant,*Coffea canephora* Pierre ex A. Froehner (Rubiaceae) also known as Robusta coffee. Only adult females colonize or infest berries, especially mature/ripe ones, in which offsprings find favourable and growing ecological conditions, such as the presence of fully formed nucleus and dry matter content (>20%) within the endosperm [3,9]. Damon [1] and Jaramillo [3] reported that phenology of fruits, especially mature/ripe ones, and their dry matter content are crucial in determining CBB females' infestation. This infestation is characterized by penetration of coffee berry at the apex of the fruit for ovipositions inside the endocarp galleries. Later, the resulting larvae and adults feed on the seeds in situ, although adult females have a choice to stay or leave a native coffee berry 12 to 16 days post emergence [5,9,10,11]. The feeding activity of CBB involves damage on berries that reduces yields, decreases quality of affected fruits and, in some cases, can result to the abortion of the seeds [4, 5,12, 13, 14].

To reduce CBB damage in coffee farms, several control measures are recommended such as the use of (i) synthetic chemical insecticides namely endosulfan and chlorpyriphos [3], (ii) entomopathogenic fungi [1,7,8, 13,15,16, 17,18,19], (iii) an attractive mixture, the Brocap trap®, from methanol and ethanol [20,21], and (iv) the gathering and destroying of residual berries (RB) i.e. the post harvested old berries remaining on the trees and/or fallen to the ground, which shelter CBB during the none fruiting season [13,14]. Despite such endeavours, several factors threaten the success of the integrated pest management (IPM) strategies against CBB. Among these, we have the cryptic life-cycle of *H. hampei* inside the berry, and especially the lack of sufficient updated scientific data and extensive knowledge on the bio-ecology of CBB in contrasting environments with high altitude (≥1000 m a.s.l.). Indeed, it is known that Robusta coffee (*C. canephora*) grows in low altitude areas (<1000 m a.s.l.) contrary to Arabica coffee (*Coffea arabica* L.) which grows in highlands [4]. Moreover, data found and related to the bio-ecology of CBB have been carried out in coffee farms located in low altitude; the results showed that in such an environment, *C. canephora* are severely affected by CBB [12,22,23]. To confirm or refute these previous investigations on CBB bio-ecology, data from contrasting environments such as highland geographies from Central Africa which considered the native home of CBB are needed, because it is known that global changes affect significantly the bio-ecology of Arthropoda fauna including CBB[24,25]. Indeed, a comparative data from contrasting environments would allow a better understanding of the determinism of the berries infestation by CBB in plantations; this would ultimately promote the optimization of the integrated pest management (IPM) against the targeted insect. Jamarillo *et al.* [26] argued, without proving in low elevation geographies conditions which is common habitat of *C. canephora*, that temperature is probably the main factor that determines CBB infestations under field conditions. The lack of field knowledge on, for example, the key factors involved in berries infestation by CBB combined with CBB bio-ecology data under highland conditions shows the need for complementary studies,and thus justifies our workto elucidate the decisive factor(s) mainly involved in the infestation of berries in contrasting coffee growing areas. We hypothesized that altitude and/or climate (temperature and rainfall) substantially affect the infestation of berries in nature. The aim of this study was to assess the level of infestation of *C. canephora* by *H. hampei* under different growing conditions including low and high altitude.

2 Materials and methods

2.1 Study sites

The research reported in this paper was carried out, from May 2015 to February 2016, in 2 Robusta coffee plantations in each of 3 villages distributed across an altitude gradient in the West Regions of Cameroon (Nteingue: 5°16'N and 9°56'E; altitude ≈700 m a.s.l.), Mbile: 5°20'N and 10°01'E, altitude ≈ 800 a.s.l. and Banki: 5°25'N and 10°03E; altitude ≈ 1400 m a.s.l.) (Fig. 1). The pedological and vegetation data of the study area have been widely documented[27,28,29]. Climatic data of each selected village were recorded using a thermometer Task and a rain gauge TFA-Dostmann 47.1001(Littoclime S.A., Caen, France) following Mahob *et al.* [30]. Details on cultural and chemical practices and post-harvest residual berries as well as the main associated trees are provided in table 1.

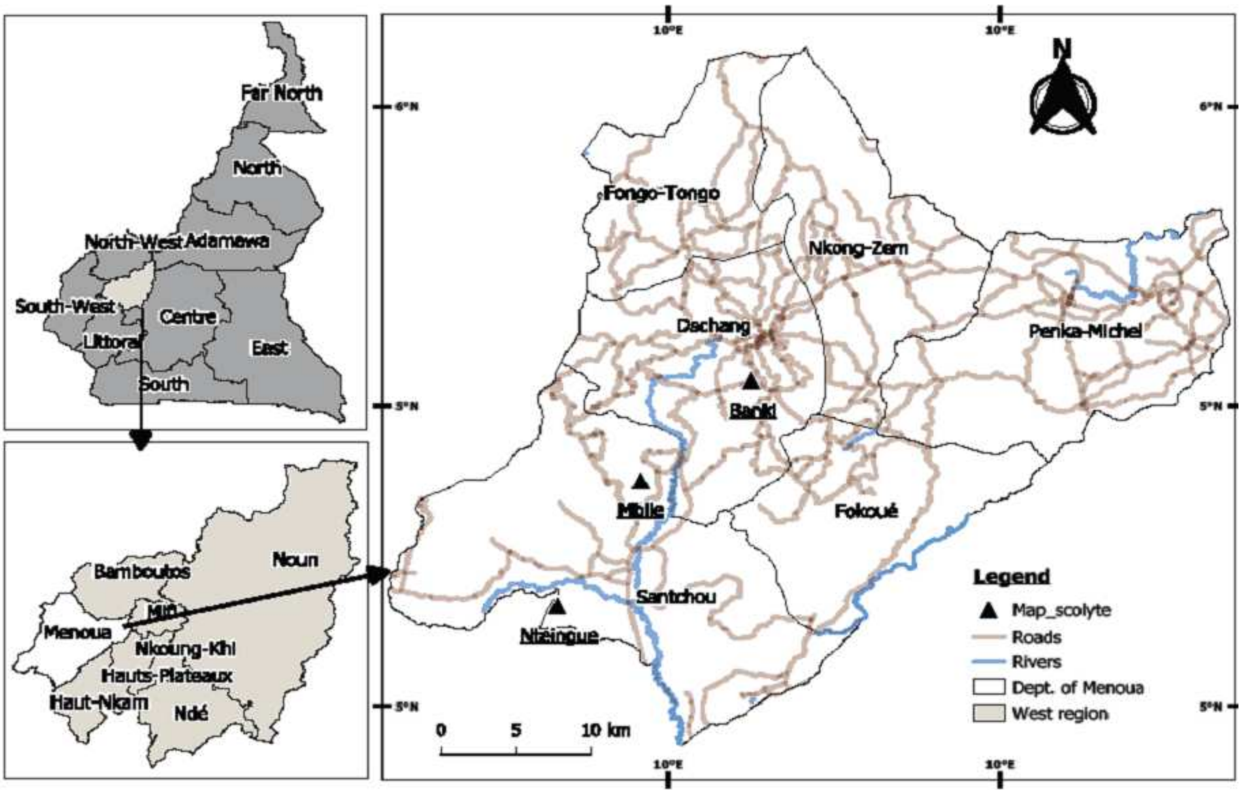

Fig. 1: Geographic localization of the study sites

2.1 Plot selection

A productive coffee plot (60 x 60 m) was delimited within each of the 6 plantations used in our study. Five sub-plots, each with 100 coffee trees (≈ 30 x 30 m), were selected in each plantation following a randomized and balanced checkerboard experimental design. Within each sub-plot, 5 producing coffee trees (≈ 3 m within and between row spacing), for a total of 25 observation trees per plantation, were randomly selected and marked for subsequent monitoring of CBB dynamics. The distance between marked coffee trees was approximately 12 x 9-15 m. Geographical position of the selected plots was provided in Table 1.

2.2 Sampling and inspection of residual berries

Residual berries (i.e. left-over ones from the previous season) were sampled from April to May 2015. In each of the 6 selected plots, all RB on 30 randomly-selected coffee trees and the ground below the trees throughout the plantations were collected once during the stated period following Remond [31] and Remond *et al.* [32]. The RB were placed in polyethylene plastic bags (28 x 19 cm) and transferred to the laboratory at the University of Yaoundé I (Cameroon). RB were dissected with scalpel blade to check for CBB presence. CBB abundance and prevalence were calculated per plantation (6 total) and sampling level (tree branches or ground) after Bush *et al.* [33]. CBB was identified using the dichotomous keys [34] and the sex ratio (SR) of adults was calculated by dividing the number of adult males by that of adult females. Final data were the cumulative numbers of RB and CBB (in all stages) per tree and per village, and/or sampling level.

Table 1: Agroecological, geographical coordinates and management characteristics of the selected plots

Characteristics	Study villages					
	Banki		**Mbile**		**Nteingue**	
	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6
Geographical coordinates and localization	5°25'N 10°03E	5°26'N 10°03'E	5°21'N 9°59'E	5°20'N 10°01'E	5°16'N 9°56'E	5°17'N 9°55'E
	Around Dschang City		Intermediary between Nteingue and Banki		Around Mboo plain	
Altitude (m)	1,439	1,440	831	835	732	737
Approximate age in year of plots (andfarmers)	40 (72)	30 (75)	45(65)	55(70)	50 (55)	35(58)
Approximate area (ha)	2	2	1.5	3	2	2
Origin of plants	Unknown	MINAGRI	Unknown	MINAGRI	Unknown	Unknown
Shade level	++	++	+	+	+	-
Weeding per year	None	Once	Twice	Once	Three time	Twice
Pruning per year	None	Once	Twice	Once	Three time	Twice
Post-harvest of RB per year	None	None	Once	Once	Twice	Twice
Chemical treatments	None*	None*	None*	None*	None*	None*
CBB within RB	**	**	**	**	**	**

Legend: -: no shade, +: low shade, ++: high shade. *: No chemical treatment for at least three years before the beginning of the study. **: very abundant. The shade within the robusta coffee farms was essentially ensured by *Manguifera indica*L., *Voacanga africana* Stapf and *Dacryodes edulis* (G. DON) H.J. Lam individuals. MINAGRI: Ministry of Agriculture, RB: Residual berry

2.3 Assessment of CBB fruit infestations

New coffee berries were harvested on coffee branches (sampling unit) from all compass directions at head height, from May 2015 to February 2016. Based on the internal characteristics observed after dissection, berries were classified into 3 categories: (i) Young berries (YB) - green, without nucleus, internal endocarp aqueous and highly milky; (ii) immature berries (IB) - green-yellow, with a nucleus not fully formed in the endocarp in many cases; and (iii) mature/ripe berries (MB) - red, with a nucleus fully formed in the endocarp.

Five fruits were randomly collected from each marked coffee tree at 2-week intervals for a total of 250 fruits per plot and 500 fruits per village (location) and per month.

Table 2: Comparison of the frequencies (% ± SE) of residual berries between the study localities in a given sampling level

Sampling levels	**Study localities**			**Total** (N)	**Statistics**
	Banki (n_1)	**Mbile** (n_2)	**Nteingue** (n_3)		
Branches (B)	45.07 ± 7.81 %^{a} (1,619)	27.53 ± 5.06 %^{b} (989)	27.40 ± 6.12 %^{b} (984)	100% (3,592)	$\chi^2 = 25.82$, *ddl = 2*, $P < 0.0001$
Soil (S)	37.76 ± 2.24 %^{b} (659)	18.34 ± 0.96 %^{c} (320)	43.90 ± 3.11 %^{a} (766)	100% (1745)	$\chi^2 = 48.60$, *ddl = 2*, $P < 0.0001$
B + S	42.68 ± 4.20 %^{a} (2,278)	24.52 ± 2.61 %^{c} (1,309)	32.80 ± 3.91 %^{b} (1,750)	100% (5,337)	$\chi^2 = 133.13$, *ddl = 2*, $P < 0.0001$

In each row, values followed with the same letter are not significantly different (Chi-2 test). SE: Standard Error, n_i = abundance of berries collected per sampling level and locality, N= total sample between the study localities

Collected fruits were preserved in polyethylene plastic bags (26 x 20 cm) according to their source. Once in the laboratory, berries were dissected with the same RB protocol as described above.The total number of infested berries per tree, plot and location was calculated and used for estimating monthly percentage of berries infestation.

2.4 Statistical analysis

Frequencies of RB and the associated CBB numberswithin the collected fruitswere pooled per village and sampling levels, then compared with Chi-2 testbetween the studied villages or the sampling levelswithout any data transformation because the statistical conditions were satisfied. Pearson's correlation coefficients between RB frequency and associated CBB numbers were computed afterlog-transformation [log(x+1)] for Gaussian's distribution reasonto determine the linear relationship between both dependant (CBB abundance) and independent (FR) variables. For new fruits (NF), monthly numbers of infested berries were log-transformed [log(x+1)] before analysis to correct the unequal variances inherent in count data. Mean abundances of infested fruits were used in mixed model repeated measures ANOVA,due to our sampling method of NF (i.e. NF were collected only on marked productive trees), with village (i.e. locality) and date as the fixed factors and plots nested within village as the random factor. When significant differences were found in the analysis of variances (ANOVA) model, the Tukey post-hoc test was used for pairwise comparisons of means. ANOVA using the GLM procedure tested the main effects of categorical factors i.e. altitude and climatic data (temperature and rainfall), as independent variables, on the number of CBB infested berries (dependent variable). Pearson's correlation test was also used to estimate the linear relationship between fruit maturity (young, immature and mature/ripe fruits) and berries infestation levels under field conditions. All statistical analyses were performed with STATISTICA Software System, version 7, 2004. The differences were deemed at α = 5%.

3 Results

3.2 Residual berries

A total of 5,337 RB were harvested in the selected plots of the three study localities: 2,278 (42.7 ± 4.20 %) in Banki, 1,309 (24.5% ± 2.61 %) in Mbile and 1,750 (32.8 ± 3.91 %) in Nteingue (Table 2). The frequencies of RB were significantly higher in Banki compared to Mbile and Nteingue (χ^2 = *133.1, ddl = 2, P< 0.001*), except on the ground where valuesat Nteingue were significantly (χ^2 = *48.6, ddl = 2, P< 0.001*)higher than those obtained in Banki and Mbile (Table 2). Considering the sampling level, the frequencies of RB collected on branches were always significantly (*P<0.001*) higher compared to those found on the plantation floor (Table 3).

Table 3: Comparison of the frequencies (% ± SE) of residual berries between sampling levels per study locality

Sampling levels	**Study localities**		
	Banki	**Mbile**	**Nteingue**
Branches (n_1)	71.07 ± 7.81 %[a] (1,619)	75.55 ± 5.06%[a] (989)	56. 23 ± 6.12 %)[a] (984)
Soil (n_2)	28.93 ± 2.24 %[b] (659)	24.45 ± 0.96%[b] (320)	43.77 ± 3.11 %[b] (766)
Total (N)	100% (2,278)	100% (1,309)	100% (1,750)
Statistics	χ^2 = *211.68, ddl= 1, P<0.0001*	χ^2 = *182.91, ddl= 1, P<0.0001*	χ^2 = *13.63, ddl= 1, P<0.001*

In each column, values followed with the same letter are not significantly different (Chi-2 test). N: total sample in a given locality

A total of 9,525 CBB individuals were collected in the RB: 5,123 (53.7 ± 7.98 %) in Banki, 2,641 (27.7 ± 4.37 %) in Mbile and 1,171 (18.6 ± 3.89 %) in Nteingue (Table 4). The frequencies of CBB differed significantly (*P<0.001*) among villages and sampling levels. A decrease in the CBB frequency, regardless the sampling level, was observed from Banki to Nteingue; but on the plantation floor the CBB frequency in Nteignue was intermediate between Banki and Mbile (Table 4).

Table 4: Comparison of the average frequencies of *H. hampei* (% ± SE) collected in berries between the study localities in a given sampling level

Samplinglevels	Study localities			Total (N)	Statistics
	Banki (n_1)	Mbile (n_2)	Nteingue (n_3)		
Branches (B)	51.50 ± 14.02 %[a] (3,464)	33.16 ± 7.90 %[b] (2,230)	15.34 ± 6.07 %[c] (1,032)	100% (6,726)	$\chi^2 = 56.87$, *ddl = 2*, *P< 0.0001*
Soil (S)	59.06 ± 6.55 %[a] (1,659)	14.63 ± 2.58 %[c] (411)	26.31 ± 4.91 %[b] (739)	100%) (2,809)	$\chi^2 = 158.10$, *ddl = 2*, *P< 0.0001*
B + S	53.73 ± 7.98 %[a] (5,123)	27.70 ± 4.37 %[b] (2,641)	18.57 ± 3.89 %[c] (1,171)	100% (9,525)	$\chi^2 = 905.30$, *ddl = 2*, *P< 0.0001*

In each row, values followed with the same letter are not significantly different (Chi-2 test).

Moreover, and irrespective of locality, the sub-populations of CBB were always significantly (*P<0.001*) higher in RB still on coffee branches than in fallen RB (Table 5). CBB sex ratio was highly female-biased at both levels of sampling: (1) on branches - 1:30 at Banki, 1:20 at Mbile and 1:40 at Nteingue, and (2) on the ground - 1:20 at Banki, 1:19 at Mbile and 1:31 at Nteingue.

Table 5: Comparison of the frequencies of *H. hampei* (% ± SE) collected in berries between the sampling levels per study locality

Samplinglevels	Study localities		
	Banki	Mbile	Nteingue
Branches (n_1)	67.62 ± 14.02 %[a] (3,464)	84.44 ± 7.90%[a] (2,230)	58. 27 ± 6.07%[a] (1,032)
Soil (n_2)	32.38 ± 6.55 %[b] (1,659)	15.56 ± 2.58%[b] (n_2= 411)	41.73 ± 4.91 %[b] (n_2= 739)
Total (N)	100% (5,123)	100% (2,641)	100% (1,771)
Statistics	$\chi^2 = 211.68$, *ddl= 1, P<0.0001*	$\chi^2 = 182.91$, *ddl= 1, P<0.0001*	$\chi^2 = 13.63$, *ddl= 1, P<0.0001*

In each column, values followed with the same letter are not significantly different (Chi-2 test).

The results also showed,in the three study locations,a significant positive strong correlation between the number of RB and the number of their parasite: $r_\alpha = 0.728$ at Banki; $r_\alpha = 0.787$ at Mbile and$r_\alpha = 0.819$ at Nteingue.

3.3 New fruits

3.3.1 Temporal evolution of fruitsphenology

In the three localities, the temporal evolution profile of young, immature and mature/ripe berries was similar although month percentages varied in all the selected plots. The young berries (YB) percentagedecreased as of 78% to 0% from May to February in Banki, 59 to 0% from May to January in both Mbile and Nteingue(Fig. 2). The immature berries (IB) rate showed a monomodal profile and presented two evolution phases.In the first phase, their values increased as of 22 to 85% in Banki, 41 to 84% in Nteingue and 41 to 90%in Mbile from May to November within the three studylocalities. In the second phase, IB decreased progressively to1, 13and 23% in February 2016 inMbile, Nteingue and Bankirespectively. Mature berries (MB) appeared within the study plots in September at Mbile then in October at Banki and Nteingue;itsrate gradually increased up to January/February and ranged from 2 to 75%, 2 to 99% and 3 to 87% at Banki, Mbile and Nteingue respectively (Fig. 2).

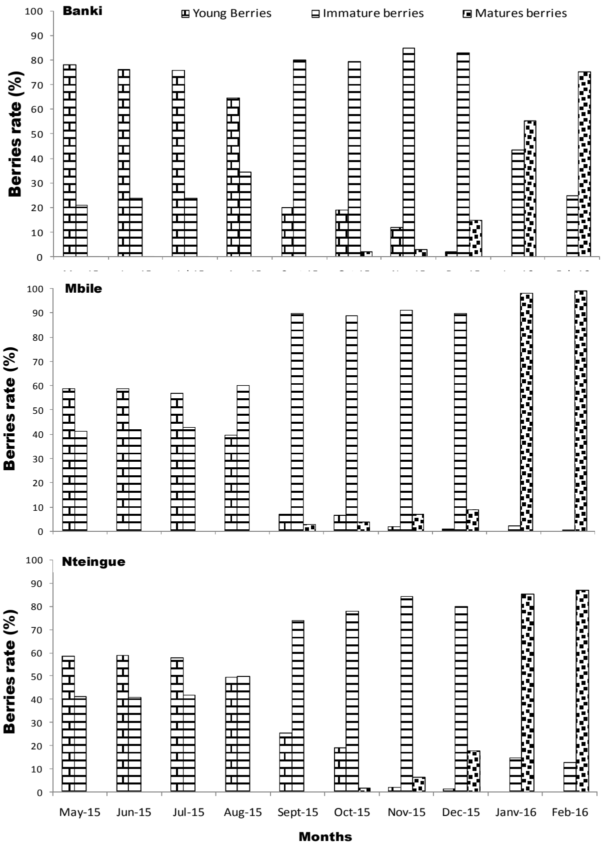

Fig. 2: Evolution of the fruits' maturation within plots of the three study locations

3.3.2Assessment of CBB berries infestation

Until December, the infection rate of NF by CBB (larvae only) was no more than 3% in all the selected plots. Then, the percentage of larvae inside the sampled NF increased and reached12, 13 and 14% in February at Nteingue, Mbile and Banki respectively (Fig. 3A). Berries infestation with CBB adults (females only) showed two peaks in all the selected plots: a small one in September with values ranging from 6%, 8% and 15% in Nteingue, Mbile and Banki respectively, and the main one in January: 30% in both Banki and Nteingue and 35% in Mbile. The overall prevalence of CBB adults in sampled berries ranged from 1 to 30% at Nteingue,2 to 30% at Banki and 2 to 35% at Mbile (Fig. 3B).The mean number of infected berries was significantly (*P< 0.001*) higher in Banki only from August to December (Fig. 4). In the selected plots, ANOVA revealed 5 different temporal NF infection levels in Banki, and 2 in both Mbile and Nteingue (Table 6). In addition, berries were significantly (*P< 0.001*) more infested by CBB at all locations in January and February i.e., when mature/ripe fruits were predominant in fields, suggesting a significant preference for this stage of berries compared to YB and IB ones (Table 6).

Table 6: Comparison of the monthly average (± SE) number of infested berries in the study localities during the sampling period

Months	**Number of infested berries (mean ± ES)**		
	Banki	**Mbile**	**Nteingue**
May	0.02 ± 0.14^{d}	0.02 ± 0.14^{a}	0.01 ± 0.10^{b}
June	0.03 ± 0.17^{d}	0.03 ± 0.17^{b}	0.02 ± 0.14^{b}
July	0.04 ± 0.19^{d}	0.04 ± 0.19^{b}	0.03 ± 0.17^{b}
August	0.07 ± 0.25^{cd}	0.05 ± 0.21^{b}	0.03 ± 0.17^{b}
September	0.15 ± 0.36^{b}	0.08 ± 0.22^{b}	0.06 ± 0.23^{b}
October	0.12 ± 0.33^{bc}	0.03 ± 0.17^{b}	0.02 ± 0.14^{b}
November	0.12 ± 0.32^{bc}	0.06 ± 0.24^{b}	0.03 ± 0.17^{b}
December	0.11 ± 0.31^{bc}	0.06 ± 0.24^{b}	0.02 ± 0.14^{b}
January	0.30 ± 0.46^{a}	0.35 ± 0.47^{a}	0.30 ± 46^{a}
February	0.30 ± 0.46^{a}	0.34 ± 0.47^{a}	0.29 ± 0.46^{a}
Statistics	$F_{(9,4990)}=50.80, P<0.0001$	$F_{(9,4990)}=105.76, P<0.0001$	$F_{(9,4990)}=102.44, P<0.0001$

Values expressed are mean ± standard error (SE). Values with different letters in the column are significantly different at P < 0.05, according to Tukey post-hoc test.

The number of CBB within berries was significantly (*P < 0.001*) and positively correlated to the number of MB (r_α= 0.93 for Banki,r_α = 0.92 for Mbile and r_α = 0.95 for Nteingue), but negatively correlated to that of YB (r_α = - 0.94 at Banki, r_α = - 0.87 at Mbile and r_α = - 0.89 at Nteingue)(Table 7).With IB this correlation was not significant (*P>0.05*) in the other study localities (Table 7).

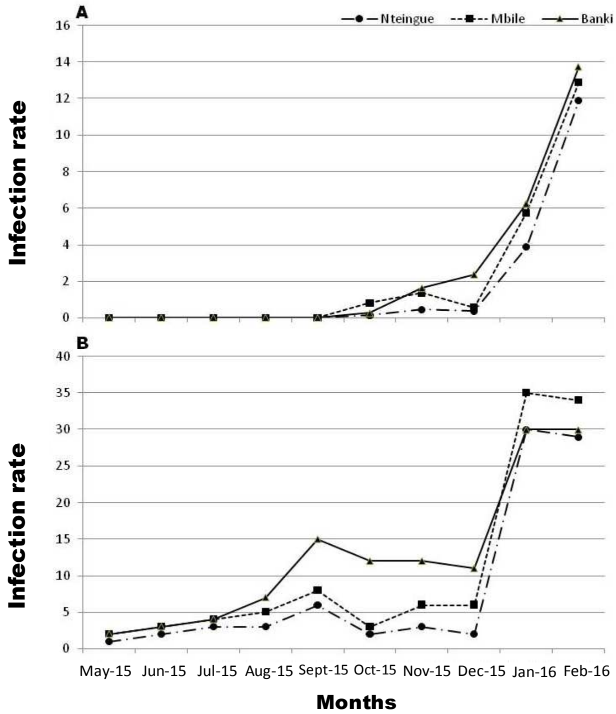

Fig. 3: Spatiotemporal infestation dynamics of berries by CBB: A) immature stages (larvae only) and B) Adult females only.

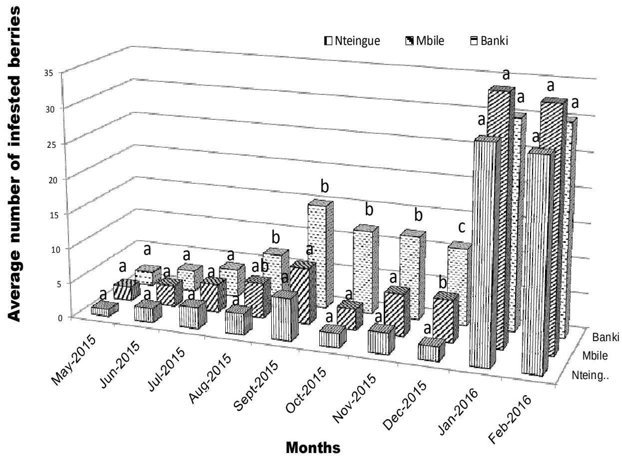

Fig. 4: Monthly comparisons of the average numbers of infested berries between the study localities.

The bars with the same letter per month mean that average numbers of infested berries are not significantly different (Tukey post-hoc test)

Table 7: Correlation between the abundance of CBB and number of berries at different developmental stages in the study localities

Study localities	Development stages of berries		
	Young	Immature	Mature/ripe
Banki	r_α= - 0.94, *P< 0.0001*	r_α= 0.35, *P = 0.31*	r_α= 0.93, *P< 0.0001*
Mbile	r_α= - 0.87, *P< 0.001*	r_α= - 0.11, *P = 0.09*	r_α= 0.92, *P< 0.0001*
Nteingue	r_α= - 0.89, *P< 0.001*	r_α= 0.26, *P = 0.46*	r_α= 0.95, *P< 0.0001*

Among the factors considered in this study e.g. temperature and rainfall, only the date significantly (*P < 0.0001*) showed effect on the parasitism of NF by CBB under field conditions in the three study localities (Table 8).

Table 8: Evaluation of the temperature, rainfall and altitude effects on berries infestation by CBB

Categorial factors	*F-value*	*Df*	*P-value*
Temperature	*2.87*	*2*	*0.102*
Rainfall	*3.48*	*2*	*0.073*
Altitude	*3.49*	*2*	*0.073*
Date	*5.84*	*9*	*0.0001*
Locality	*1.45*	*2*	*0.251*
Date*locality	*0.14*	*18*	*1.000*

4Discussion

4.1 Residual berries

In this study, the frequency of RB varied significantly among the 3 locations and the sampling levels. RB frequencies were overall significantly higher at Banki's plots followed by Nteingue, and then Mbile's plots. These differences in RB frequencies could be explained by the diversity in the coffee farms management. Indeed, since the decrease of the annual income of this commodity, around 1990s, due to the drastic decline or volatility of coffee beans prize in the world market [35], farmers pay less attention to the RB harvest or they do not simply harvest these berries as in Banki's case (Table 1). This farmers' behaviour clearly justifies the highest number of RB on branches (1,619 specimens; 45.05 ± 7.81 RB)at Banki for example (Table 2). It is known that for a suitable harvest, less than five berries can remain per coffee tree per season [14, 36, 37]. Taking into account this threshold of RB on coffee tree for effective berries harvest in farms, the number of berries recorded in all the surveyed locations during the current study (average value always higher than 16 RB per coffee tree) indicated that the harvest was incomplete [36, 37]. This result agrees with previous published data found in the literature [14, 17, 37]. Besides the economic reason which, among others, deprives farmers to pay young individuals to manage farms in order to have good outcomes, this result could be also linked to the farmers' old age; these no longer have enough energy to rigorously remove RB on coffee trees, especially for plantations with high elevation such as those of Banki (Table 1).The frequency distribution of CBB varied between the study localities and sampling levels; the values obtained ranged with the following profile: Banki > Mbile > Nteingue (Table 4). This situation seems to establish a positive relationship between the availability of food resources (high number of berries remaining due to ineffective or none harvest during the inter-campaign) and the abundance of CBB (Table 2). Indeed, irrespective of the study village, RB, especially those from branches constitute the main parasite reservoirs for new fructifications as previously reported by Zelaya and Vargas [38]in Guatemala, Villanueva [39] in Mexico, Bustillo *et al.* [36], Aristizábal *et al.* [37], Saldarriaga [40] and Benavides *et al*. [41] in Colombia. Considering the sampling level, CBB sub-population sizes were significantly higher on the branches than on the ground (Table 5). This result confirms the fact that CBB populations and/or their infestation pattern in berries are closely related to the different mesological factors of coffee fruits (i.e. dry matter content, humidity rate or berry moisture, presence/absence of natural enemies, etc…) between those still set to branches and those on ground [1,3,4,8,42]. Several authors reported that within the coffee plantations throughout the World, CBB populations in RB are naturally limited by many antagonistic organisms such as: hyper/parasitoids, predators, nematodes, and fungal entomopathogens[3,4,7,8,9,42,43]. Based on the statements of the latter authors, these CBB's natural enemies are broadly numerous on ground than on the branches. For example, Jaramillo*et al.* [9] found that 97% of the parasitoid*Prorops nasuta*Waterston (Hymenoptera: Bethylidae), an important natural biocontrol agent vs CBB, originates from RB collected on the soil versus ≈ 3% from those recorded on the trees. The results obtained in the current study are hence justified, and confirm those of Vega *et al.* [4], Jaramillo *et al.* [43], Ticheler [44], Borbón-Martinez [45] and Bustillo [46].

The sex ratio of CBB was female biased. This situation seems to diverge from the expectation that living organisms, including insects, which are exposed to the same environmental conditions, should have a balanced sex ratio 1:1 [47]. It is likely that CBB female-biased reproduction and survival are linked to enhanced fitness [48, 49]. Another explanation is the longevity of adult CBB; males have shorter life span than females [1,3,14]. However, our results support numerous findings with numerical difference values [4,14,50]. The discrepancy in sex ratio values between studies is probably caused by the heterogeneity of (a) reproduction strategies of different CBB populations, (b) host-plant nutritional values, and (c) experimental and/or environmental conditions. It is well known that parasite populations' structure varies spatio-temporarily with the heterogeneity of the host populations and/or macrohabitat [24].

4.2 New fruits

Coffee fruit maturation varied among the three study localities. The rate of MB was lower in Banki (during the harvest period i.e. from December to February) compared to the other localities (Fig. 2). Besides other potential factors, environmental conditions such as altitude may naturally favour or unfavour coffee plant development [21,51]. Indeed, it is known that Robusta coffee grows best at lower (less than 1,000 m a.s.l) elevations [21,51,52]. Robusta Coffee plantations located in Mbile and Nteingue are therefore within favorable altitude compared to those in Banki; this could explain differences in berry ripening among the locations. In the total absence of temperature, rainfall and/or altitude effect on the dynamics of berries infestation (Table 8), our result in Banki is mainly linked to agronomic practices of farmers in terms of the RB post-harvest (Table 1). RB represent a real danger forNF because a strong positive correlation wasfoundbetween their abundance(food resources) and the intensity of CBB within berries. Another argument, but least important compared to the first one due to its controversial character, is related to shade versus sun grown coffee on CBB berries infestation[53,54,55]. In the three study localities, the higher infection rates of berries and the average numbers of infested berries in January and February in all the selected plots (Fig. 3 and Table 6) are justified by the higher rates of MB during these months. This finding is in accordance with the statement that the abundance of the infesting stage of a given parasite species correlates positively with the intensity and/or prevalence of that parasite in a biocenosis containing the targeted host population [24].Our finding support the recommendation that after the harvest period (in inter-campaign), all berries needed to be completely removed from the trees and ground because they constitute the parasite reservoirs (PR) i.e. the vital reinfestation sources of berries in the next campaign [3,5,16,36,56]. However, some authors reported on the contrary the effect of abiotic factors (temperature and rainfall) as well as altitude on berries infestations [1,3,5,12,22,23,26]. This divergence could be explained by the heterogeneity of the experimental/environmental conditions and/or the heterogeneity in the exposure/susceptibility of the host populations to different CBB populations [24,25].

In this work, CBB highly preferred the MB (fruits with red color) to YB (fruits with green color) and IB (fruits with green-yellow color) (Table 7). Adult CBB females represent the only stage of this insect pest which colonizes coffee berries [3,5,11,36]. Thus, CBB preference forMB is governed by their dry matter content (>20%) and the presence of fully formed nucleus and consistent endosperm which represent a good quality of food and/or habitat to themselves and/or to their offsprings [3,4,5,8,36]. It is also known that CBB prefer the red color both in natural and artificial conditions [3,4,20]. Therefore MB, which are the harvested stage for farmers, are at high risk of infestation due to the fact that CBB can annually cause up to 50% losses in coffee yields [6]. Taking into account the high preference of MB (red fruits) by CBB, coffee plantations needed IPM [1,2,7,8,13,15,16, 17,18,19,20,21] when berries reach at least the immature stage to optimize annual coffee yields.

5 Conclusion

This study focused on the identification of key factors involved in berries infestation by CBB under contrasting field conditions. Our findings showed that climatic data (temperature and rainfall) and altitude do not affect the CBB infestation dynamics and/or their abundance in coffee plantations. But the key factor of the bio-ecology of CBB is the coffee fruits' phenology (biotic factor). From our findings, we suggest: (a) varietal screening studies against CBB in the plants breeding programs to reduce production losses due to CBB without the chemicals intervention in order to promote organic coffee; (b) other IPM methods such as Brocap®and/or pheromone traps for the mass trapping of adult females due to their high abundance compared to males, and especially the fact that they are the only development stage which colonizes MB in plantations, and consequently causes a lot of damage related to coffee trees productivity. Residual berries shelter the CBB populations in coffee plantations, and therefore constitute the primary parasite reservoir or the main source of berries reinfestation in the next campaign. Systematic post harvest of RB by farmers during inter-campaign are recommended (on the branches and ground) to significantly reduce the prevalence and/or abundance of CBB inNF. Our investigations raise that rigorous harvest of remaining berries in coffee plantations associatedwith Brocap® traps or chemical treatments in some cases, when berries reach immature stage, could substantially improve the coffee yields. This new database of the berries infestation pattern by CBB under contrasting field conditions should be incorporated in the IPM program against this targeted insect pest in the coffee growing area, especially Central Africa.

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