



**Original Paper**

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**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)**

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**ACKNOWLEDGEMENTS**

We thank the Institute of Agricultural Research for Development (IRAD) for providing financial and logistic supports.

**ABSTRACT**

The impact of mirid true bugs on cocoa production is widely assessed for *Sahlbergella singularis* and *Distantiella theobroma* species in the cocoa growing area in Africa. No study has been focused on the impact of another common mirid species in cocoa farming, such as *Helopeltis* sp., on the cocoa productivity. Thus, the main objective of this work was to assess the effect of *Helopeltis* sp. attacks on cocoa productivity of ten genotypes. Observations were made on infested fruits (cherelles, immature and mature fruits) under a randomized experimental field design. A control assay was also used in our investigations. The overall results revealed that only fruits infected by mirids aborted: 80.0% for cherelles and 0.4% for immature fruits. The numbers of aborted fruits were statistically comparable between cocoa genotypes and their rates varied from 60 to 96%. In contrast, ANOVA showed that the feeding lethal punctures of *Helopeltis* sp. were significantly ( $p < 5\%$ ) different between fruits of the tested cocoa genotypes; the mean values ranged from  $41.5 \pm 5.5$  to  $76.0 \pm 4.6$  and were classified in three homogenous groups, with a significant sensitivity of clone/hybrids T79/501, UPA143 x SNK64 and T79/501 x SNK413 compared with clone SNK16. The proposal of including *Helopeltis* sp. as one of the most important pest in cacaoculture is discussed.

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**Keywords:** Cocoa genotypes, production, impact, attacks, mirids, *Helopeltis* sp.

**INTRODUCTION**

Cocoa is one of Cameroon's main export crops (Jagoret, 2011). According to the International Cocoa Organization, this commodity represents 30% of the Gross Domestic Product in the Agricultural Products Subsector for Export and Processing. Cocoa

production increased from 180, 000 to 232, 000 metric tons from the year 2005 to 2015 (Anonymous, 2014, 2015). Despite this temporal increasing yield, cocoa production per hectare remains low, 250 to 350 kilograms (Jagoret, 2011) compared to other great producing countries such as Côte d'Ivoire

where the yields per hectare ranged from 260 to 600 kilograms (Assiri Assiri et al., 2012).

Cocoa production is constrained by several factors including aged trees, declining soil fertility levels, poor maintenance practices, predominance of old farmers (Jagoret, 2011) and attack by pests and diseases (Dormon, 2006; Sonwa et al., 2005, 2008; Mahob et al., 2014). Mirids have been reported as the main pest for cacao culture (Dibog et al., 2008; N'Guessan et al., 2010; Anikwe et al., 2009, 2015; Adu-Acheampong et al., 2014; Mahob et al., 2011, 2014, 2015). Apart from species belonging to the *Helopeltis* genus which are confined only on cocoa pods (Entwistle, 1972), mirids feed on fruits, twigs/branches, chupons, shoots and trunks of their host plant. The feeding activities of mirids are characterized by (1) the direct effects such as dark markings or lesions (commonly called cankers), the black spots, the dry leaves and in some cases the stagheaded cocoa (i.e. cocoa trees with numerous small crown branches but forming a poor canopy because of the absence of the leaves) and these last symptoms were generally observed or more prevalent where cocoa is growing without shade (Entwistle, 1972); (2) the indirect effects due to the invasion of the resulting lesions by the wound parasitic fungi such as *Lasiodiplodia* spp., *Albonectria* spp. and *Fusarium* spp. leading lastly to the death of trees, commonly known as cocoa dieback (Adu-Acheampong et al., 2012, 2014; Anikwe et al., 2015). In West Africa, many taxa of pests such as *Distantiella theobroma* (Distant, 1909), *Sahlbergella singularis* Haglund, 1895, *Bryocoropsis laticollis* Schumacher, 1917 and *Helopeltis* spp. were found in cocoa farms; but two most important and/or dominant species, *D. theobroma* and *S. singularis* were widely reported as most economically prejudicial to cacao culture (N'Guessan et al., 2010; Anikwe et al., 2009, 2015; Adu-Acheampong et al., 2014). In Central Africa and particularly in Cameroon, the same species were usually found in cocoa farms except *B. laticollis* (Yede, 2016). However, while researchers have widely studied the biology and ecology of *S. singularis* and *D. theobroma* in both West and Central Africa (N'Guessan and Coulibaly, 2000; N'Guessan et al., 2008,

2010; Anikwe et al., 2010; Babin et al., 2008, 2010, 2011; Bisseleua et al., 2011), data related to mirids (*S. singularis* and *D. theobroma* only) damage are available only in West Africa (Entwistle, 1972; Ojelade et al., 2005; Anikwe, 2009). According to these authors, annual damage assessed in terms of cocoa production losses due to *S. singularis* and *D. theobroma* ranged from 30 to 50% without any control measures (insecticides treatments). However, to the best of our knowledge, no quantitative data are available in literature with regard to *Helopeltis* spp. damage, especially those concerning the cocoa production losses. Yet, collecting these data will undoubtedly improve our knowledge on the impact and/or the responsibility of this pest on the cocoa productivity. Therefore, the objective of the present work was to provide quantitative data on the effect of *Helopeltis* sp. attacks on the productivity of different cocoa genotypes as well as the lethal feeding punctures for infested fruits.

## MATERIALS AND METHODS

### Study site and plot description

This study was conducted from May 2017 to January 2018, in the semideciduous rain forest of Southern Cameroon, in four cocoa blocks (60 m x 30 m each). Cocoa farms were located at the Institute of Agricultural Research for Development, Nkoemvone Research Station (2°40'N and 11°20'E; 630 m above sea level) (Figure 1). The agronomic structures of the experimental plots as well as their vegetational composition, climatic and soil data have been previously documented by Mahob et al. (2011).

Experimental plots were the Fisher's completely randomized blocks. Plots contained: (1) different cocoa genotypes, eight clones (IMC60, SNK7, SNK10, SNK16, SNK52, SNK67, SNK181, T79/501) and five hybrids (T79/501×IMC60, T79/501×SNK413, T79/501×SNK479, UPA143×NA33, UPA143×SNK64), (2) herbaceous species: *Chromolaena odorata* King & Robinson, 1970 and *Crotalaria* sp. (Gramineae), *Pennisetum purpureum* Schumacher, 1827 (Poaceae) and *Mimosa invisa* Martius ex Colla, 1834 and *Mimosa pudica* Linné, 1753 (Mimosaceae), and (3) shade trees such as

*Cassia spectabilis* DC. (Fabaceae), *Inga edulis* (Vellozo) Martius (Fabaceae) and *Maesopsis* sp. (Rhamnaceae). The study plots were chosen on the basis of the presence of well-known cocoa genotypes, which present all fruit stages and which have not been treated for at least four years.

### Experimental design

The design was completely randomized with five replications for each cocoa clone and/or hybrid per trial. Fruits of ten genotypes, five clones (IMC60, SNK7, SNK10, SNK16, T79/501PA138) and five hybrids (T79/501×IMC60, T79/501×SNK413, T79/501×SNK479, UPA143×NA33, UPA143×SNK479) were infested by mirids, one individual per fruit, on the basis of their high productivity compared with three others (SNK52, SNK67, SNK181) during the trial period. Fruits chosen for assay previously showed good physiological growth and were free from mirid bites (i.e. fruits without any black spots). Three different growth stages of fruits (Young/cherelle, immature and mature) were considered in this work according to Niemenak et al. (2010) and our observations of the internal cavity, after dissection. These observations were based on the physiological/phenological state of the seeds. According to Niemenak et al. (2010) and our observations, the internal cavity of the cherelle (stage 1) does not have cocoa beans or seeds and the thickness of its husk measures 6 to 9 mm while immature (stage 2) and mature fruits (stage 3) have the seeds being or completely physiologically formed respectively; the thickness of their husk measures 9 to 16 mm for stage 2 and 13 to 25 mm for stage 3. For each fruit stage and selected cocoa genotype, 10 specimens were infested per replication. A control (clothing sleeve without mirid) was also set up per batch. Larvae of stages 4, 5 and imago of mirids obtained from field collection and the rearing house (insectarium) of IRAD were used for fruits infestation, after a fasting period of 24 hours in the entomology laboratory of IRAD at Nkoemvone station. Young larvae (stages 1, 2 and 3) were excluded from this study due to their vulnerability for field work. Fruits selected for

assays were previously confined in cloth sleeves (20 x 10 cm for cherelles, 30 x 20 cm for immature and 40 x 30 cm for mature fruits) to avoid any exogenous bias; then each sleeve was partially opened to allow infestation of fruits (Figure 2). For each infested fruit, mirids were removed after 15 days post-infestation.

### Evaluation of aborted fruits

The evaluation of the aborted fruits was carried out during 15 days for cherelles and immature fruits, and 30 days for mature/ripe ones because of their differential susceptibility to mirid attacks (Yede et al., 2012) and phenologic cycle of fruits (Niemenak et al., 2010). The daily number of aborted fruits per stage, genotype and assay were counted. A final score was the cumulative number of aborted fruits recorded for each stage per genotype.

### Evaluation of the number of lethal feeding punctures of mirid's to fruits

The number of lethal feeding punctures of mirids has been expressed by counting the black spots on the surface of the husk of each aborted fruits per genotype and category. Mirid damages on fruits were characterized by the presence of black spots on their surface (Yede et al., 2012).

### Data analysis

The cumulative numbers of aborted fruits per stage and genotype, as well as the lethal feeding punctures were computed for each treatment (mirid infestation and control) using STATISTICA software (STATISTICA, 2011, Version 10). Mean numbers of aborted fruits and lethal feeding punctures were separated by ANOVA using STATISTICA via the General Linear Model (GLM) procedure. The mean values for pairwise comparisons were compared and ranked using Newman-Keuls post-hoc test. Data of immature and mature/ripe fruits for both treatments as well as those of cherelles for the control assay were excluded from the statistical analysis because no or very few aborted fruits were recorded. The differences were deemed to be significant when  $p < 5\%$ .

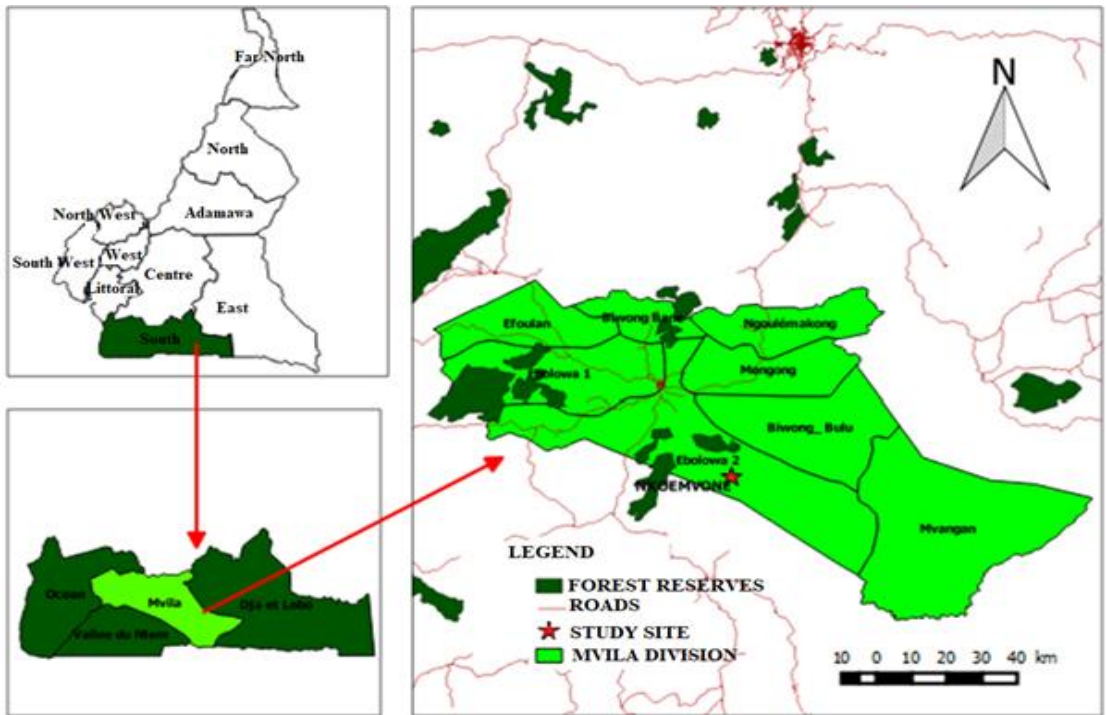


Figure 1: Geographic localization of the study site.



Figure 2: Protocol of fruits infestation with mirids: A) beginning of fruits infestation with partial opening of the cloth sleeve protecting the infested fruit B) post infestation of fruit with complete closure of the cloth sleeve.

**RESULTS**

**Overall**

The general results of the descriptive analysis revealed that 402 fruits aborted out of the 500 infested by mirids per growth stage, either 400 for cherelles (80.0% of the total) and 2 (0.4% of the total) for immature fruits. No aborted fruit has been recorded in the infested fruits of stage 3 and the control. Damages due to mirids in most cases for stage 2 and all cases for stage 3 of the development fruits were superficial and characterized by the presence of black spots on pods.

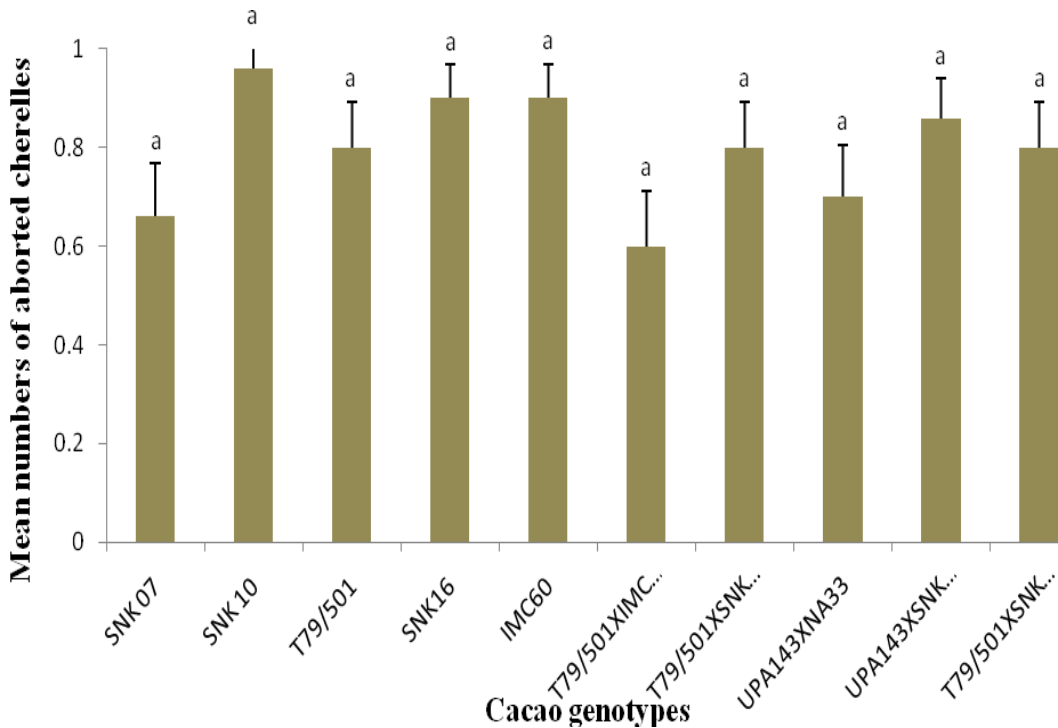
**Assessment of the mean numbers of aborted cherelles per cocoa genotype**

The mean numbers of aborted cherelles numerically varied from  $0.60 \pm 0.11$  for T79/501xIMC60 to  $0.96 \pm 0.05$  for SNK10 (Figure 3); but no significant difference ( $F_{(9,40)} = 1.64; p = 0.11$ ) was found between the tested cocoa genotypes in terms of aborted

cherelles due to *Helopeltis* sp. attacks (Figure 3).

**Evaluation of the number of mirids lethal feeding punctures for cherelles**

The numbers of the mirids lethal feeding punctures for cherelles varied from 8 to 115 punctures for cocoa genotypes UPA143xSNK64 and T59/501xIMC60 respectively (Table 1). Significant differences ( $F_{(9,389)} = 962.14; p < 0.0001$ ) were found between mean numbers of the *Helopeltis* sp. lethal feeding punctures on cherelles among the different cocoa genotypes tested (Table 1). Cocoa genotypes sensitivity/tolerance to mirid attacks can be classified in three different groups, with significant sensitivity of clone/hybrids T79/501, UPA143xSNK64 and T79/501xSNK413 compared with clone SNK16. The others clones/hybrids revealed themselves comparable sensitivity/tolerance as well as with the previous ones in some cases (Table 1).



**Figure 3:** Comparison of the aborting cherelles (means ± SE) in function of cocoa genotype tested, after mirid infestations.

**Table 1:** Comparison of the lethal feeding points numbers (means  $\pm$ SE) in function of cocoa genotypes, including the minimum and maximum values of studied parameter.

Genotype	Number of lethal feeding points			Means grouping according to Newman test
	Min	Max	Means $\pm$ SE	
UPA143XSNK64	8	78	41.5 $\pm$ 5.5	B
T79/501	20	80	46.2 $\pm$ 4.7	AB
T79/501XSNK413	13	90	49.1 $\pm$ 7.5	AB
UPA143XNA33	13	86	56.6 $\pm$ 6.4	ABC
IMC60	29	91	58.4 $\pm$ 4.6	ABC
SNK 10	21	112	58.8 $\pm$ 6.0	ABC
T79/501XSNK479	15	98	61.4 $\pm$ 5.4	ABC
SNK 07	18	115	67.6 $\pm$ 8.3	AC
T79/501XIMC	30	115	70.3 $\pm$ 6.6	AC
SNK16	45	109	76.0 $\pm$ 4.6	C

Means followed by different letters in the most right column were significantly different at  $P < 5\%$ , according to Newman-Keuls test. SE: Standard error, Min: Minimum, Max: Maximum.

## DISCUSSION

Significant production losses were recorded in the attacks of cherelles by *Helopeltis* sp.: 80% of the total number of infested fruits. This result shows that this species is also an important pest to cocoa trees in terms of the cocoa productivity losses, and this support the assertion that mirids are very prejudicial economically to cocoa farming without any control measure (Entwistle, 1972; Dibog et al., 2008; N'Guessan et al., 2010; Anikwe et al., 2009, 2015; Adu-Acheampong et al., 2014; Mahob et al., 2011, 2014). Taking into consideration the cocoa genotypes, our results numerically varied from 0.60 to 0.96 i.e. 60% to 96% (Figure 3). These values differ from those (30 to 50%) of *S. singularis* and *D. theobroma* obtained in cocoa farms in West Africa (Entwistle, 1972; Ojelade et al., 2005; Anikwe, 2009). This numerical discrepancy should be due to genetic determinism and this substantially highlights the divergence in the susceptibility/tolerance/resistance of different cocoa plant varieties to mirid attacks (Sounigo et al., 2003; Dibog et al., 2008; N'Guessan et al., 2008, 2010), although the mechanisms

involved in the study of this parameter (susceptibility/tolerance to mirid attacks) in the host plant are poorly understood.

*Helopeltis* sp. attacks have presented no effect on immature and mature fruits, except the presence of black spots on the cocoa husk. This result departs from the hypothesis of Padi (1997) that fruits of three months at most (i.e. cherelles and immature fruits according to Niemenak et al. (2010)) have reduced chance of surviving to mirid attacks. The keystone to explain this situation would undoubtedly be linked to the phenology of cocoa fruits, although the mechanisms involved remain to be clarified. Indeed, the internal cavity of cherelles contains a zygote attached to the husk with the preliminary development of the embryo (Niemenak et al., 2010). Conversely, immature and mature fruits are respectively the intermediate and final growth stages; their internal cavities contain ripening and/or fully mature cocoa beans with nucleus located in the pericarp (Loor Solorzano, 2007). Moreover, according to Loor Solorzano (2007) and our observations in the present work, the husk of cherelle is less thick (7.5 mm in average) and

tender due to the lack of lignin; therefore it is permeable to liquid diffusion, such as the toxic saliva of mirids. In contrast, the husk of immature and mature fruits is thicker, 12.5 mm and 19 mm respectively; it is also hard due to the presence of lignin and therefore impermeable to liquid diffusion. When feeding on cacao trees, mirids inject their toxic saliva (Willians, 1954) in the host tissues at a depth that is less than 2 mm through their short (1.7 mm long on average) rostrum (Delvare and Alberlenc, 1989). Saliva injected by mirids has the hemolytic effects, diffuses right to the zygote through the permeable husk of cherelles, causing the destruction of the latter and consequently the death/abortion of the infested cherelles. In contrast, immature and mature/ripe fruits prevent the diffusion of the mirid's saliva injected into the host tissues. Due to their imperviousness, mirids damage are thus restricted to the surface of the husk of the infested pod, and appear as black spots. Our findings undoubtedly highlight the differential sensitivity of the fruits of stage 1 (i.e. cherelles) compared with both stages 2 (i.e. immature) and 3 (i.e. mature) of fruits to mirid, especially in *Helopeltis* sp. attacks, and support previous works of Yede et al. (2012) carried out in cocoa plantations in the Centre Region of Cameroon, where hexapod infestations induced 4 to 60% abortion of cherelles against none for ripe fruits. However, numerically our results discrepancy from those obtained by Yede et al. (2012) and Bos et al. (2007) in the Indonesian cocoa plantations, where physiological abortions of cherelles, commonly called wilt, caused 50% of the total cocoa productivity losses. This numerical divergence is explained on the one hand by the fact that Bos et al. (2007) and Yede et al. (2012) conducted their works under different environmental conditions; on the other hand, cocoa genotypes observed by these authors were different from ours. This situation confirms the fact that cocoa varieties have different susceptibility/tolerance/resistance to mirid infestations (Sounigo et al.,

2003; Anikwe et al., 2009; N'Guessan et al., 2008, 2010).

Ndoubè-Nkeng and Sache (2003), Ndoubè-Nkeng et al. (2004) and Yede et al. (2012), working in the cocoa farms of the Centre Region in Cameroon, reported that black pod disease cause the important production losses per year, ranged from 12 to 100% in case of no chemical (fungicides) treatments. As far as stramenopile genus (*Phytophthora*) is concerned, the percentages of fruits mortality due to this disease were similar to those obtained in our study. These results show that cocoa farming in Cameroon is confronted to two major constraints: Black pod rot and mirids, which constitute up to date, an economically serious pest to cocoaculture. This is in conformity with previous studies conducted by Varlet and Berry (1997) and Mahob et al. (2014) in Cameroon. However, it should be noted that *Helopeltis* sp. causes cherelles mortality only, while black pod rot causes mortality at all stages of the fruit development; for that reason, it is though that black pod disease could be an economically most important than mirids, although data to confirm or refute this hypothesis remain to be collected in different growing cocoa area.

From our investigations, the numbers of lethal feeding punctures due to mirids were different amongst the cocoa genotypes tested. This result suggests that albeit the susceptibility/tolerance of cherelles of our cocoa genotypes (cherelles) to mirid attacks was comparable in general, the mean numbers of feeding punctures were significantly different; the minimal and maximal values were observed for UPA143×SNK64 and SNK07 then for SNK16. This situation could be linked to the intrinsic characteristics of the different cocoa genotypes tested (e.g. physical structure of fruits, composition of primary and secondary metabolites of fruits, etc.); it also confirms the recognized intra and interspecific susceptibility of biological populations to exogenous aggressions, such as mirid attacks to cocoa trees (Sounigo et al., 2003; Anikwe et al., 2009; N'Guessan et al., 2008, 2010).

## Conclusion

At the end of our investigations, *Helopeltis* sp. is economically a major pest of cocoa farm, as its homologous *D. theobroma* and *S. singularis*, with regards of the significant cocoa production losses (60 to 96% according to cocoa genotypes), especially fruits of stage 1 currently called cherelles. The infestation by this pest has no economic impact on immature and mature fruits, but leads to superficial damage that is characterized by the presence of black spots on the husk of the infested fruits. The sensitivity/tolerance of the cocoa genotypes tested to mirid attacks are comparable with regards of the rate of mortality/abortion of cherelles. Our finding suggests that all the cocoa genotypes tested are equally sensitive/tolerant to mirid attacks. This result could be taking into consideration in breeding programs of plant varieties against mirids, especially *Helopeltis* sp. Cocoa genotypes tested are also differently susceptible/tolerant to the feeding mirid bites. Two genotypes out of ten tested, UPA143XSNK64 and SNK16, are the most and less sensitive/tolerant respectively for feeding lethal punctures of mirids. This new data improves our understanding of the field ecology of mirids and highlights responsibility of *Helopeltis* sp. on cocoa production losses. Therefore, this study presents evidence of the negative impact of mirids on cocoa productivity and justifies cocoa mirid control recommendations such as chemicals used in the integrated pest management (IPM) against this pest under field conditions to protect fruits on cacao trees, especially cherelles, and finally to optimize the annual cocoa yields.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## AUTHORS' CONTRIBUTIONS

MRJ, DL, BR, BD and BBCF conceived and designed research. MRJ, NEPB, AVV, FTYG, BL and ONPA conducted experiments. MRJ were involved in

the data analysis. MRJ wrote the manuscript and the co-authors reviewed it. BD, DL, BL and ONPA also provided finance and logistics during the study. All authors read and approved the manuscript.

## ACKNOWLEDGEMENTS

Support from Hermine MAHOT, Irène NKOTTO and Damien Eyenet was especially valuable in the realization of this study. Special thanks to Professor Gideon Ajeegah for his contribution to the scientific writing of this paper.

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