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ORIGINAL ARTICLE

Field evaluation of the impact of *Sahlbergella singularis* Haglund infestations on the productivity of different *Theobroma cacao* L. genotypes in the Southern Cameroon

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Abstract

Mirids (*Sahbergella singularis* and/or *Distantiella theobroma*) are the major pests of cacao farms in Africa. Cocoa production losses due to these species have been widely documented in West Africa. However, their impact on cocoa production is unknown in Central Africa, especially in Cameroon. Moreover, no data are available on the threshold level of fruits tolerance to mirid attacks. For these reasons, we assessed the effect of *S. singularis* on the productivity of ten cacao genotypes as well as the threshold number of the lethal feeding punctures to fruits under a randomized experimental design. Observations were made on three categories of fruits (cherelle, immature and mature/ripe). A control trial was also set up per batch. The overall results showed that 68.0% and 0.4% of fruits aborted, respectively, in mirid and control trials. The percentages of aborted fruits were significantly ($p < 5\%$) different between cacao genotypes and ranged from 20 to 100%. Bonferroni test revealed six homogenous groups for cacao genotypes susceptibility to mirid attacks; SNK52 proved to be most tolerant/resistant, whereas two genotypes (UPA138 and SNK67) revealed more sensitive. In contrast, six genotypes (SNK07, IMC60 × SNK417, T60/887 × PA7, T79/501 × SNK479, UPA143 × ICS84, UPA143 × NA33) displayed similar sensitivity to mirid attacks. ANOVA showed that the threshold tolerance of tested fruits, expressed by the mean numbers of lethal feeding punctures, to *S. singularis* attacks was comparable between cacao genotypes. This new quantitative database improves our knowledge on the (i) threshold tolerance of fruits to *S. singularis* attacks and (ii) economic impact of this pest on cocoa production in Cameroon.

Keywords Cacao genotypes · Threshold tolerance · Productivity · Effect · Infestations · *S. singularis*

Introduction

Mirids, especially *Sahlbergella singularis* Haglund, 1895 and *Distantiella theobroma* (Distant, 1909), are known as the major pests of cacao trees in cocoa growing areas in West Africa (Entwistle 1972; Lavabre 1970, 1977; Babin 2018). These piercing-sucking species feed on all aerial parts of the cacao tree and cause cankers on the trunk and branches, and black spots on the fruits. During feeding, these insects inject their saliva into the wound and generally enhance histolytic effects (Williams 1953); this leads to necrosis of affected tissues where opportunistic fungi such as *Lasiodiplodia* spp., *Albonectria* spp. and *Fusarium* spp. are currently found (Adu-Acheampong et al. 2012, 2014; Anikwe and Otuonye 2015; Voula et al. 2018). Crowdy (1947), Adu-Acheampong et al. (2014), Anikwe and Otuonye (2015) and Voula et al. (2018) reported that the synergic actions of mirids and opportunistic fungi end up in some cases to the death of trees, commonly known as cacao dieback.

In nature, about 1500 different species of insect pests infect cacao trees, but less than 2% are of real economic importance (Entwistle 1972; Wood and Lass 1989). Species

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belonging to Hemiptera order, commonly called true bugs, and particularly those of the Miridae family were reported to be more economically prejudicial to cacao farming compared to other species including those of other families of Hemiptera order, such as Pentatomidae, Coreoidae and Psyllidae commonly encountered in cacao farms (Entwistle 1972; Lavabre 1970, 1977; Anikwe et al. 2009; Yede 2016; Babin 2018). Damages due to mirids on cacao trees are clearly known (Entwistle 1972; Adu-Acheampong et al. 2014; Anikwe et al. 2009; Yede et al. 2012; Babin 2018). For example, *S. singularis* and/or *D. theobroma* cause damages on pods characterized by aggregated black spots on the surface around the peduncle basis, part in contact with the bark of the trunk or branches (Entwistle 1972; Anikwe et al. 2009; Yede et al. 2012; Mahob et al. 2015; Yede 2016). In contrast, *Helopeltis* (*Afropeltis*) spp. and other true bugs (e.g., Pentatomidae and/or Coreoidae) usually engendered black spots almost uniformly distributed over the entire surface of the pod (Yede et al. 2012) without any idea on the number of lethal feeding punctures for aborted/death fruits. In West Africa, the impact of mirids, especially *S. singularis* and *D. theobroma,* on cocoa production without any control measures has been ranged from 30 to 70% losses (Entwistle 1972; Idowu 1989; Ojelade et al. 2005; Anikwe 2009). To develop sound integrated pest management (IPM) programs against mirids, such as the recommendations on the timing of insecticide application (or other fight methods) on cacao farming, several studies on the biology and ecology of these two species have been conducted in vivo and in vitro in both West and Central Africa (Williams 1954; Lotodé 1969; Bruneau De Miré 1977; Lavabre et al. 1963; Lavabre 1970, 1977; Nwana and Youdeowei 1978; Babin et al. 2008, 2010, 2011; Anikwe et al. 2010; Bisseleua et al. 2011; Mahob et al. 2015). According to these authors investigations, the economic threshold level for control measures is based on mirid densities per cacao tree, 0.7 individual Cameroon/Central Africa (Decazy and Essono 1979) versus 0.6 individual Ghana/West Africa (Padi and Owusu 1998). However, no quantitative data are available on cacao genotypes threshold tolerance to mirid attacks, assessed in terms of the number of lethal feeding punctures on fruits. Yet, these data could complement those based on mirid densities per cacao and then optimize the IPM against mirids, especially *S. singularis*, as reported in the literature for other insect pests of plants such as the Tobacco budworm, *Heliothis virescens* (Fabricius, 1777), and the Corn earworm, *Helicoverpa zea* (Boddie, 1850), both insect pests of cotton (Greene, 2017). Therefore, a thorough evaluation of the effect of mirids on the productivity of different cacao genotypes remains necessary with the ultimate goal to improve the varietal breeding program against these pests, on the one hand, and to recommend the timing period for phytosanitary interventions, based on the phenology of fruits, on the second hand. We hypothesized that fruits threshold tolerance to mirid attacks varied between cacao genotypes. The objective of this work was to provide quantitative database on the threshold tolerance of cacao genotypes to *S. singularis* attacks as well as to determine the responsibility of this pest on cocoa production losses. AQ4

Material and methods

Study site and plot description

This work was carried out from April 2017 to January 2018, within four cacao blocks (54 m × 24 m each) located at the Research Station of Nkoemvone (2°40′N and 11°20′E; 630 m above sea level) (Fig. 1) in the semideciduous rain forest of Southern Cameroon. Cultural practices, vegetational composition, climatic and soil data of this site are already described by Mahob et al. (2011).

Plots for experiments were the Fisher's completely randomized blocks and contained fourteen different cacao varieties: (i) six clones (SNK7, SNK52, SNK67, SNK181, T79/501, UPA138) and eight hybrids (IMC60 × SNK417, T79/501 × SNK479, T79/501 × SNK41, UPA143 × SNK64, IMC60 × SNK417, T60/887 × PA7, UPA143 × ICS84, UPA143 × NA3), (ii) herbaceous species were *Chromolaena odorata* King & Robinson, 1970, *Crotalaria* sp. (Gramineae), *Pennisetum purpureum* Schumach, 1827 (Poaceae), *Mimosa invisa* Martius ex Colla, 1834 and *Mimosa pudica* Linné, 1753 (Mimosaceae). Shading was mainly provided by *Cassia spectabilis* DC. (Fabaceae), *Inga edulis* (Vellozo) Martius (Fabaceae) and *Maesopsis* sp. (Rhamnaceae). From one to another tree in the row and/or between adjacent rows, cacao was spaced by 3 m. Experimental plots were selected on the basis of their well-known cacao varieties, carrying all fruits stages and without chemical treatments for at least 4 years before the beginning of this work to avoid any bias in the data collection.

Experimental design

The experimental design was completely randomized with five replications for each trial. Fruits or pods of ten cacao clones and/or hybrids (SNK7, SNK52, SNK67, T79/501, UPA138, IMC60 × SNK417, T60/887 × PA7, T79/501 × SNK479, UPA143 × ICS84, UPA143 × NA33) have been infested by mirids (one individual per pod) on the basis of their high productivity during the data collection period. The infested fruits previously showed good physiological growth and were free from pest attacks. Three different categories or growth stages of fruits (cherelle, immature and mature/ripe) were considered in this study according to Niemenak et al. (2010) and Mahob et al. (2018). According

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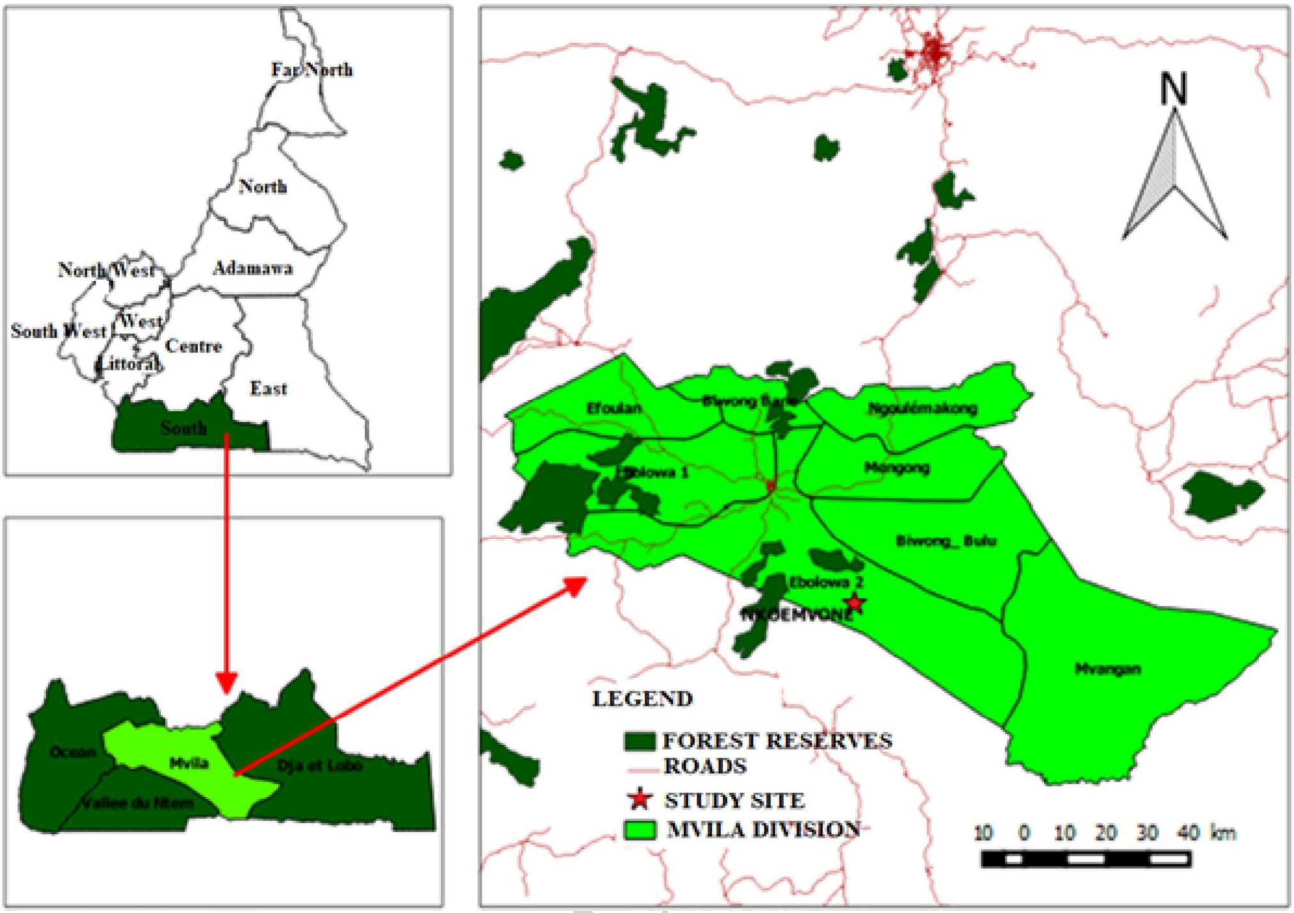

Fig. 1 Geographic localization of the study site

to Niemenak et al. (2010) and our observations, the internal cavity of the cherelles (category 1) does not have beans; in addition, the thickness of its husk measures 7 to 10 mm whereas immature (category 2) and mature/ripe fruits (category 3) have, respectively, the beans being or fully physiologically and/or phenologically formed; the thickness of their husk measures 9–15 mm for category 2 and 12–23 mm for category 3. A total of 500 specimens for each category of fruits were used for experiments: 50 specimens per selected cacao genotype and 10 specimens per replication. A control containing fruits under sleeve without mirids was also set up on the same selected tree per batch. Larvae (stages 4, 5) and imagos of mirids obtained from field collection and/or rearing house of IRAD were used for fruits infestation, after having spent one fasting day in the laboratory (Anikwe and Otuonye 2015). Young larvae (stages 1, 2 and 3) were disqualified from this study due to their defenselessness for field experiments. Fruits selected for trials were previously confined in cloth sleeves (20 × 10 cm for cherelles, 30 × 20 cm for immature and 40 × 30 cm for mature/ripe fruits) to avoid any exogenous bias; then, each selected fruit in muslin cloth and sleeve was monitored during 15 days post-infestation (Fig. 2).

Evaluation of aborted fruits

The evaluation of 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 the phenologic cycle of fruits (Niemenak et al. 2010). The daily number of aborted fruits per category, genotype and trial was counted. A final score recorded was the cumulative number of aborted fruits for each category and genotype.

Evaluation of the threshold number of lethal feeding punctures of mirids to fruits

The threshold number of lethal feeding punctures of mirids toward infested fruits has been assessed by counting the number of black spots on the surface of the husk of each aborted fruits per genotype and category. Mirid damages

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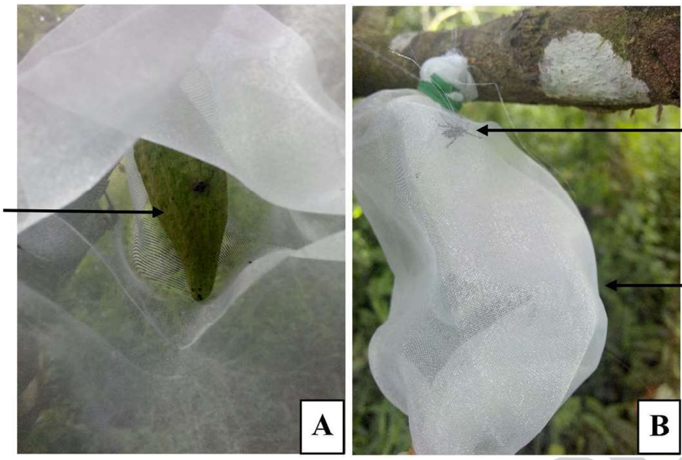

Fig. 2 Protocol of fruits infestation by mirid: **a** partial opening of the cloth sleeve protecting the infested fruit, **b** complete closure of the cloth sleeve with infested fruit

were characterized by the existence of the circular black spots on the fruits/pods (Yede et al. 2012).

Data analysis

Cumulative percentages of aborted fruits per category and cocoa variety as well as numbers for lethal feeding punctures of fruits due to mirids attacks were computed for each trial; ten box plots were made for the both studied parameters using STATISTICA software (Statistica 2011, Version 10). After ranking relative frequencies (percentages), median values of aborted specimens were separated by the Kruskal–Wallis test, whereas means of lethal feeding punctures, after Poisson's correction of counting data, by ANOVA through the general linear model (GLM) procedure using once again STATISTICA software. When the statistical significant differences were found between the multiple comparisons of percentages and/or means, Bonferroni's and Tukey's post hoc tests were used for pairwise comparisons to determine which were significantly different. Data of immature and mature/ripe fruits for both treatments (mirid infestations and control) as well as those of cherelles for the control trial were excluded from the statistical analysis due to the fact that no or few aborted fruits were collected. The differences were appreciated at the 5% confidence level.

Results

Overall

From our investigations, 342 aborted cherelles were recorded: 340 (68.0% out of the 500 infested by mirids) and 2 (0.4% out of the 500 used as control). No aborted pod has been found in both categories 2 and 3 of infested fruits and control. Mirid damages in categories 2 and 3 of fruits were superficial and characterized by the presence of the circular black spots on the surface of the fruits' husk.

Assessment of the percentages of aborted cherelles per cacao genotype

Significant difference ($H = 38.16$; $p < 0.0001$) was obtained between the tested cacao genotypes with regard to the percentages of aborted cherelles (Table 1), and values ranged from 10% for SNK52 to 100% for SNK67 and UPA138, respectively (Fig. 3). The sensitivity/tolerance/resistance of the cacao genotypes tested can be classified into four groups, with a significant higher sensitivity of clones SNK67 and UPA138 and resistance/tolerance of clone SNK52 (Table 1). The other cacao genotypes showed an intermediary sensitivity/tolerance/resistance to mirid attacks (Table 1).

Assessment of the threshold number of lethal feeding punctures of cherelles due to mirid infestations

The threshold number (mean ± SE) of the lethal feeding punctures of cherelles to mirid attacks did not significantly ($F_{(9324)} = 1.11$; $p = 0.36$) vary between the cacao genotypes tested (Table 2); box plots values ranged from 30 punctures for clone T79/501 to 72 for hybrid UPA143 × SNK64 (Fig. 4).

Discussion

From our investigations, the sensitivity/tolerance of cherelles of different cacao genotypes to mirid infestations with regard to the threshold number of feeding lethal punctures, ranging from 30 to 72 according to cacao varieties, did not show any significant difference between the tested specimens. This result rises, on the one hand, the fact that all cacao varieties tested in this study are susceptible to mirid attacks; it also supports assertion

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Table 1 Comparison of the percentages of aborting cherelles in function of cacao genotype, after mirid infestations

Cocoa genotype	Number of infested cherelles (n_i)	Rates (%) of aborting cherelles			Average values grouping according to Bonferroni test
		Min	Average ± SE	Max	
SNK52	50	10	20 ± 3.2	30	A
T 79/501	50	30	40 ± 3.2	50	B
T79/501 × SNK 479	50	50	60 ± 3.2	70	C
T 60/887 × PA 7	50	50	60 ± 3.2	70	C
UPA 143/NA 33	50	60	70 ± 4.5	80	CD
IMC 60/SNK 64	50	60	70 ± 5.5	80	CD
UPA 143 × SNK 64	50	70	80 ± 3.5	90	D
SNK07	50	70	80 ± 3.5	90	D
UPA138	50	100	100 ± 0.0	100	E
SNK 67	50	100	100 ± 0.0	100	E

Average values with the same letters in the most right column are not significantly different at $p < 5\%$, according to Bonferroni test. SE: Standard error. Min: Minimum, Max: Maximum

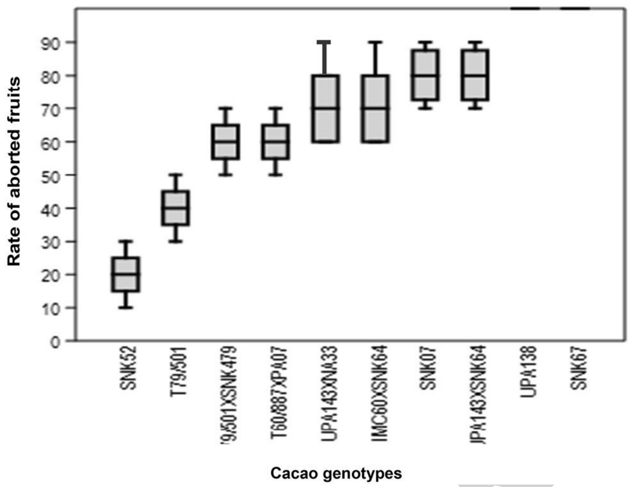

Fig. 3 Range of the percentages of aborted cherelles in function of cocoa genotypes, after mirid attacks

Table 2 Comparison of the numbers (mean ± SE) of the lethal feeding punctures of fruits due to mirid attacks, in function of cacao genotypes; the minimum and maximum values of studied parameter are also given

Cacao genotypes	Numbers of lethal feeding punctures		
	Min	Max	Mean ± SE
UPA 143 × SNK 64	31	72	51.5 ± 2.5[a]
PA 143 × NA 33	39	58	48.5 ± 1.4[a]
UPA	45	62	53.5 ± 1.7[a]
T 79/501 × SNK479	39	65	52.0 ± 2.2[a]
T 79/501	30	57	43.5 ± 3.2[a]
T60/887 × PA7	34	65	49.5 ± 2.2[a]
SNK 67	38	63	50.5 ± 2.7[a]
SNK 52	39	57	48.0 ± 3.2[a]
SNK7	37	63	50.0 ± 2.0[a]
IMC 60 × SNK 417	32	63	47.5 ± 2.4[a]

Means within the column followed by the same letters are not significantly different at $p < 5\%$, according to Tukey test. Min: Minimum, Max: Maximum

that no cacao genotype up to date substantially resists to insect pests in the nature contrary to some cacao diseases such as the witches' broom disease caused by *Moniliophthora perniciosa* in Brazil, for which varietal solutions have been found (Babin 2018). On the other hand, it recommends the integrated pest management (IPM) programs through the researches focused on the timing period for cacao protection against mirids, to establish the phytosanitory treatments (by chemicals, for example) of cacao trees carrying cherelles with less than 30 black spots to avoid serious cacao production losses, therefore optimizing yields in cacao farms and/or incomes of farmers.

Our results obtained in Southern Cameroon, averagely 68% of losses of cherelles, first clearly show that mirids, especially *S. singularis*, are economically prejudicial to cacaoculture and secondly agree with the finding in West Africa by Entwistle (1972), Idowu (1989), Ojelade et al. (2005) and Anikwe et al. (2009), who showed that in case of the massive fruit infestations and the absence of chemical treatments, mirids cause production losses from 30 to 70% in cacao farming. However, in terms of the percentages of fruits lost, our results numerically diverge from those obtained by the above authors. Indeed, it is known that cacao genotypes through antixenosis mechanisms (N'Guessan et al. 2010) and others intrinsic parameters or characteristics poorly understood such as those which involve primary/secondary metabolites and/or genetics, differently tolerate/resist to toxic saliva (with their histolytic effects) of mirids injected through their rostrum (stylet) during their feeding activity on the host plant. This also justifies the different percentages of production losses observed and highlights the divergence in the susceptibility/tolerance/resistance of cacao genotypes to brown cocoa mirid infestations (Sounigo et al. 2003; Anikwe et al. 2009; N'Guessan et al. 2008, 2010).

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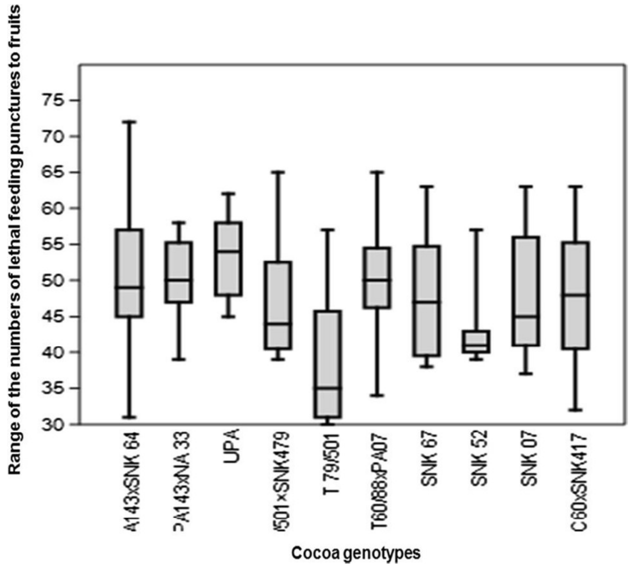

Fig. 4 Range of the numbers of lethal feeding punctures of fruits (cherelles) in function of the cocoa genotypes, after mirid infestations

Data for fruits mortality due to black pods disease (12 to 100% in case of the absence of chemical treatments) obtained by Ndoumbe-Nkeng et al. (2004) and Yede et al. (2012) in cacao farming in Centre Regions of Cameroon are similar to ours (20 to 100% of cherelles' mortality); this result confirms that cacaoculture in Cameroon is confronted at two major pests: mirids and black pod disease (Varlet and Berry 1997; Mahob et al. 2014). However, pending to elucidate that black pods disease mostly induces the mortality of immature and/or mature/ripe pods, it is suggested that this disease does not have the same economic inconveniences as mirid infestations in cacao farming. Thus, the comparative economic effects of these two major constraints in cacaoculture remain to be clarified.

From this work, it appears that mirids only cause losses of young fruits (cherelles) as asserted by Padi's (1997) who argued that fruits less than 3 months old (i.e., cherelles mainly according to Toxopeus (1985) and Niemenak et al. (2010)) have a very little chance to survive of mirid attacks. The pinpoint for the explanation of this result undoubtedly focuses on the phenology of cocoa fruits as documented by Mahob et al. (2018), although the mechanisms involved are still poorly elucidated.

Conclusion

In our experimental conditions, *S. singularis* is a major insect pest for cocoa farming in Cameroon; it causes important production losses, especially on young fruits up to 100% depending on the cacao genotype infested. Mirid attacks induce no economic effect on immature and mature/ripe fruits, but just cause superficial damage characterized by the presence of black spots on the surface of fruits/pods. The cacao genotypes tested differ in sensitivity/tolerance to mirid attacks because of the significant discrepancy of mortality/abortion rate of cherelles. One genotype (SNK52) among the ten tested was most resistant/tolerant, whereas two others (UPA and SNK67) were most sensitive to *S. singularis* infestations with regard to the numbers of aborted fruits. This result can therefore be taken into consideration in varietal breeding programs against mirids in general, especially *S. singularis*. Whatever the cacao genotype, the threshold level of tolerance of tested fruits, expressed by the number of lethal feeding punctures, to *S. singularis* infestations was comparable. These new data could undoubtedly improve our knowledge on the acceptable economic threshold level for mirid damages on fruits, especially cherelles; it also suggests that cacao tree carrying cherelles must be protected with the conventional prevention (chemical treatments for example) against mirids when an averagely less than thirty mirids punctures (black spots) are observed on cherelles; and this should be incorporated into IPM programs against *S. singularis* under field conditions.

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Compliance with ethical standards

Conflict of interest All authors agree that paper be published for the benefit of the scientific community and farmers and other stakeholders in the cocoa sector.

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