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RESEARCH ARTICLE

ASSESSMENT OF THE IMPACT OF DIFFERENT DEVELOPMENTAL STAGES OF *SAHLBERGELLA SINGULARIS* HAGL. (HEMIPTERA: MIRIDAE) ON CACAO TREES PRODUCTIVITY AND FRUITS GROWTH UNDER FIELD CONDITIONS IN THE CENTRE REGION OF CAMEROON (CENTRAL AFRICA)

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Abstract

Many studies have addressed the impact of mirids, particularly *Sahlbergella singularis* species on cocoa production in West Africa. However the effects of different developmental stages of this insect on host plant remain unknown. This work aimed to evaluate the impact of the different stages of *S. singularis* on the productivity and growth of the fruits of Amelonado and Trinitario cocoa varieties. We counted the number of feeding punctures on fruits as well as cherelles abortion, and measured the size of the surviving specimens then compared these with the control. The observations showed that all the development stages feed on cherelles. The number of feeding punctures upgraded from the larvae L1 stage to L5 irrespective to the cocoa variety, but Amelonado was more infested than Trinitario. Within each cocoa variety, significant differences in feeding punctures and aborted cherelles due to *S. singularis* developmental stages were observed. Trinitario was relatively more susceptible than Amelonado to all developmental stages of *S. singularis* and recorded a higher number of aborted cherelles, values ranged from 58% for L1 to 100% for L5. Irrespective of the cocoa variety, these results showed that older mirid larval stages (L3, L4 and L5) significantly induced more damage on cocoa productivity, regarding cherelles abortion and size reduction of the infected fruits. Overall, our findings showed that, compared to younger larvae (L1 and L2), older ones and adults are more damaging for cocoa production and the economy; this data should be incorporated in the IPM programs against *S. singularis*.

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Introduction:-

Native to tropical rainforests of Central and South America (Alverson et al., 1999, Whitlock et al., 2001), the cacao tree (*Theobroma cacao* Linnaeus, 1753) is cultivated mainly for its beans which represent one of the highest income-generating cash crops amongst others such as sugar cane, cotton, coffee, tea (Beg et al., 2017, Suh and Molua, 2022). In Central and West Africa, cocoa production providing about 90% of the income for rural

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populations (Abei and Van Rooyen, 2018) and approximately two million smallholder farming households depend on cocoa for sustenance (Schroth et al., 2016). In Cameroon, 600,000 individuals are estimated directly involved in cocoa cultivation, and this commodity, especially exported beans, annually generates about 250 billion CFA (US\$ 500,000,000); then contributes close to half of the country's export primary-sector exports (Ngong et al., 2019, Bomdzele and Molua, 2023). Despite its economic importance and the relatively increased annual cocoa production in the last decade, the Cameroonian cocoa sector, with an average 291,512 metric tons i.e. 6.5% of the total world production, is ranked fourth after Côte d'Ivoire with 1,472,313 (33.0%) metric tons, Ghana with 858,729 (19.2%) metric tons, and Indonesia with 656,817 (14.7%) metric tons (Beg et al., 2017, Suh and Molua, 2022). These statistics show that cocoa yield in Cameroon is low compared to that of the three countries mentioned above, even at the hectare level; e.g. cocoa annual yields were estimated between 500 to 600 kg per hectare in Côte d'Ivoire versus 300 to 400 in Cameroon (Wessel and Quist-Wessel, 2015, Suh and Molua, 2022, Ndah et al., 2023). Cocoa production is threatened by many biotic factors such as insect pests, which greatly reduces productivity worldwide (Entwistle 1972, Babin et al., 2010, Cilas et al., 2018, Awudzi et al., 2021, Suh and Molua, 2022). *Sahlbergella singularis* Haglund, 1895 (Hemiptera: Miridae) is recognized as one of the most economically harmful pest species in the plantations of the cocoa growing areas of West and Central Africa (Babinet et al., 2008, 2011, Anikwe 2009, Yede et al., 2012; Anikwe and Otuonye, 2015, Yede, 2016, Mahob et al., 2019, 2021, Awudzi et al., 2021; Suh and Molua, 2022). In Cameroon, Babin (2009) and Voula et al. (2018) have established that the development time of this insect from egg to imago is 20 to 49 days in the laboratory, and 30 to 58 days in the field. Its development includes five larval stages of variable duration depending on experimental conditions and/or rearing method (Babin et al., 2008, 2011, Voula et al., 2018). All mirid developmental stages, except eggs, feed on host plants via piercing-sucking tissues (Babin et al., 2008, Yede et al., 2012, Mahob et al., 2018, 2019, 2020). Their damage on cocoa trees are widely documented in West (Entwistle 1972, Idowu 1989, Ojelade et al., 2005, Anikwe, 2009) and Central Africa (Yede et al., 2012; Mahob et al., 2015, 2018, 2019, 2021). For instance, *S. singularis* and *Distantiella theobroma* (Distant, 1909) induce aggregated black spots on the surface around the peduncle i.e. the pod-stem interface on cacao trees; cause cankers on trunk and branches/twigs; dry leaves on canopy (Entwistle, 1972, Anikwe et al., 2009, Yede et al., 2012, Yede, 2016, Mahob et al., 2015, 2020, 2021), and in some cases, with the synergistic action of opportunistic fungi, the death of the host plant (Adu-Acheampong et al., 2012, Anikwe and Otuonye, 2015, Voula et al., 2018). These insect pests can cause up to 100% losses in cocoa yields in case of massive host plant infestations and inappropriate control measures (Mahob et al., 2015, 2019, 2021, Suh and Molua, 2022). Although several studies have been carried out in Central and West Africa on the ability of mirid attacks to reduce cocoa production and/or productivity in plantations (Entwistle 1972, Idowu 1989, Ojelade et al., 2005, Anikwe 2009, Yede et al., 2012, Yede, 2016; Babin, 2018, Cilas et al., 2018, Mahob et al., 2018, 2019, Cilas and Bastide, 2020), little is known on the influence of the different developmental stages of *S. singularis* infestations on cacao trees productivity and/or fruits growth. Therefore, the acquisition of such data would be useful to: (1) improve the knowledge on the effect of the feeding punctures of each mirid stage on the host plant productivity and/or the fruit's growth in order to determine the most economically harmful stage(s), and (2) ultimately address better integrated pest management (IPM) against the targeted insect pest in cocoa based-agrosystems in Africa in general, and particularly in Cameroon. We hypothesized that the impact of *S. singularis* on cacao trees productivity and fruits growth depend on the chosen varieties and specific development stage of the targeted insect pest. The objective of this study was to evaluate the impact of feeding punctures of different developmental stages of *S. singularis* on selected cocoa varieties productivity and fruits growth.

Material and Methods:-

Study locality and experimental plots description

This study was carried out from January to September 2021 in cocoa plantations in the village of Libelligoi (3°54.210'N; 10°55.610'E; 400 m a.s.l). Libelligoi is located in the subdivision of Ngog-Mapubi, Division of Nyong et Kéllé, Centre Region of Cameroon (Figure 1), and characterized by an equatorial climate with four seasons: two rainy and two dry seasons; these seasons alternate permanently with irregularities in intensity and period (Anonymous, 2020). Generally, the average annual temperature is 25.1°C and the annual rainfall more than 2100 mm (Kanmegneet al., 2006). Agricultural practices, flora and fauna composition, and pedological data of the study area are documented (Anonymous, 2020, Yede et al., 2023). In this locality, cocoa is grown under the canopy of natural trees, and planted without respect of the agronomic recommendations regarding the spacing of trees within and between rows, i.e. 2.5 x 2.5 m or 3 x 3 m for approximately 1,200 cocoa trees per hectare (Anonymous, 2002). The area of the farms selected for the experiments was measured using the Global positioning System (GPS), and covered approximately 3 hectares each; each plot was planted with both cocoa varieties, namely Trinitario or hybrids from the IRAD-Research Station of Yaoundé) and Forastero or Amelonado (German cocoa).

Fruits selection for experiments

During the experimentation, only one-month-old fruits (cherelle stage) of both varieties were used, based on their physiological and phenological health i.e. absence of stings, good vigour and normal volume (Niemenak et al., 2010; Mahob et al., 2018, 2019), also because they are known as the only fruit stage sensitive to mirid attacks under field conditions (Yede et al., 2012, Mahob et al., 2018, 2019).

Sampling procedure and conditioning of mirids

In the plantations surrounding the experimental plots, different developmental stages of mirids (larva stages 1 to 5, namely L1, L2, L3, L4, L5, and adults) were collected on the aerial part of cocoa trees using fine tweezers from 6:00 to 9:00 a.m, due to the high activities of this targeted species at this time (Bisseleua et al., 2011, Mahob et al., 2021). They were transported to the laboratory in aerated transparent plastic boxes (24x19x26 cm) containing cocoa pods or chupons as food source (Babin et al., 2008). In the laboratory, mirids were grouped by developmental stage (Babin et al., 2008; Babin 2009), and starved for 24 hours before the trials.

Assessment of the number of feeding punctures and aborted fruits

At total of 30 cherelles per cocoa variety and per developmental stage (one individual per trial) were infested for 24 hours on cocoa trees, under a cloth sleeve to avoid any exogenous bias; a negative control was also used i.e. cherelles in the cloth sleeve without mirid (Yede et al., 2012, Mahob et al., 2018, 2019). At total of 3 replications (10 cherelles each) were realized per variety and per developmental stage of *S. singularis*. The number of feeding punctures i.e. number of black spots on infested fruits was evaluated daily (Yede et al., 2012, Yede, 2016), whereas the aborted fruits were recorded weekly for a period of 1 month (Mahob et al., 2018, 2019).

Evaluation of the impact of mirid developmental stages on the growth of surviving cherelles

The impact of each *S. singularis* development stage on the growth of the surviving cherelles was evaluated 1 month post infestations in both trials (with mirids and control). The length and the diameter of the fruits were measured using a Vernier caliper and data were pooled per cocoa variety and per developmental stage of mirid for analysis.

Data analysis

Cumulative numbers of aborted cherelles due to each mirid developmental stage per cocoa variety, as well as the number of feeding punctures on cherelles, the length and diameter of surviving cherelles post mirid attacks were recorded and for the control. The average numbers of feeding punctures inflicted by each mirid developmental stage per cocoa variety were compared by the Kruskal-Wallis (H) test followed by the Wilcoxon (W) test for pairwise comparisons of the medians/mean ranks, if necessary. The average numbers of aborted cherelles per cocoa variety, as well as the length and diameter of the surviving cherelles, due to each mirid developmental stage infestation were compared using One-way ANOVA through the Generalized Linear Model (GLM), after log-transforming the data for normality reasons. When differences were found between means, the Tukey's Post Hoc test was used for pairwise comparisons of multiple means of the studied parameters. The average numbers of aborted cherelles due to each developmental mirids stage between the cocoa varieties were compared using the Student t test because the conditions were satisfied. All the statistical analyzes were performed with the STATISTICA (version 10) software. The differences were deemed to be significant for $p < 0.05$.

Results:-

Assessment of the feeding punctures per developmental stage of mirid with regards to the two cocoa varieties studied

Over all, the number of feeding punctures on the cherelles due to mirids infestations varied, depending on the variety of cacao trees; values of Amelonado were higher than those of Trinitario for each mirid developmental stage. Significant differences of this parameter were observed in cases of infestations of cherelles by the larvae L2, L3 and L5 (the Amelonado variety being more affected); while comparable values were obtained for infestations by L1, L4 and Adults (Figure 2). The peak abundances of feeding punctures were 17 (L3, L5 and adults) for Amelonado and 14 (L5) for Trinitario. Within a given cocoa variety, the average numbers of the feeding punctures varied depending on the mirid developmental stages (Table 1). In Amelonado, L4, L3 and L5 larvae inflicted comparable numbers of lesions on the cherelles (10.05 ± 0.62 , 11.13 ± 0.78 and 11.29 ± 0.70 respectively), in the same way the L2 larvae and adults (7.28 ± 0.70 and 7.75 ± 0.89), and finally the L1 larvae (4.95 ± 0.84), in decreasing order. In Trinitario, three levels of cherelles attacks were also noticed; the highest were by L4 and L5 larvae (9.44 ± 0.70 and 9.97 ± 0.42

respectively), followed in decreasing order by adults and L3 larvae (6.25 ± 0.42 and 7.32 ± 0.72), then L2 and L1 larvae (4.32 ± 0.43 and 4.37 ± 0.64) (Table 1).

Evaluation of aborted cherelles post mirid infestations

Irrespective of the variety of cocoa tree, the number of cherelles aborted varied according to the mirid developmental stages. In Amelonado, four homogeneous subsets of values were observed (Table 2); L5 larvae caused more abortions (81.67 ± 4.41), followed in decreasing order by L4 (70.00 ± 5.29), L3 and adults (63.33 ± 4.91 and 60.33 ± 1.76 respectively), then L2 and L1 larvae (53.33 ± 2.60 and 50.00 ± 6.02 respectively). In Trinitario five homogeneous subsets of values were obtained; L5 and L4 larvae caused more abortions (100 ± 00 and 86.66 ± 2.40 respectively), followed in decreasing order by adults (83.33 ± 4.40), L3 (78.33 ± 4.40), L2 (66.33 ± 6.96), then L1 (58.00 ± 4.72) larvae (Table 2). The susceptibility of the cherelles of the two cocoa varieties to each mirid developmental stage was comparable, except for adult infestations which were significantly higher ($P=0.02$) for Trinitario (Table 2).

Assessment of the diameter and length of surviving cherelles post mirid infestations

Irrespective of the cocoa variety, the influence of mirids on the length and diameter of cherelles varied according to the developmental stages of these insects. In Amelonado, the reduction in length decreased significantly ($P < 0.0001$) under the effect of L3 and L4 larvae (14.15 ± 0.48 and 14.21 ± 0.35 respectively); L5 (14.63 ± 0.36); adults (15.68 ± 0.48); L2 and L1 (16.91 ± 0.44 and 17.22 ± 0.39); and the control. Regarding the diameter, the effect of mirids gradually decreased ($P < 0.0001$) under the action of L5 and L4 (5.47 ± 0.18 and 5.91 ± 0.25); L3 (6.42 ± 0.16); adults (6.87 ± 0.26); L2 (7.71 ± 0.21); and the control (7.79 ± 0.13) (Table 3). In Trinitario, the length of cherelles also gradually decreased ($P < 0.0001$) from the control (18.88 ± 0.32), L2 (18.78 ± 0.48) and L1 (18.59 ± 0.46) to 16.30 ± 0.85 , 15.62 ± 0.27 and 15.52 ± 0.57 under the action of adults, L3 and L4, respectfully. Regarding the diameter, the measurements decreased significantly ($P < 0.0001$) as follows under the action of L1 (8.50 ± 0.24); L2 (7.98 ± 0.21); control (7.66 ± 0.14); L3, L4 and adults (6.45 ± 0.24 ; 6.05 ± 0.13 and 6.34 ± 0.25 respectively) (Table 3).

Discussion:-

The objective of this work was to assess the impact of each developmental stage of *S. singularis* on cocoa trees productivity and fruits growth, through the enumeration of the aborted fruits on the cocoa varieties, and the characterization of surviving fruits damage by measuring the surviving cherelles length and diameter, after the insect pest infestations, including a control. The tested cocoa varieties (Amelonado and Trinitario) showed differences in their susceptibility to *S. singularis* attacks as well as in: (i) the number of feeding punctures (Figure 2), (ii) cocoa production losses i.e. number of aborted cherelles (Table 2), and (iii) the length and diameter of surviving cherelles post mirid developmental stage infestations (Table 3).

From our findings, irrespective of mirid developmental stage, the highest number of feeding punctures, expressed by the number of circular black spots on the pericarp (Yede et al., 2012; Mahob et al., 2019), were observed on cherelles of Amelonado compared to Trinitario. This situation might be linked to the differential susceptibility of these cocoa varieties to mirid infestations. It is suggested from our results that cherelles from Amelonado are preferred by/more attractive to the targeted insect pest compared to those from Trinitario due to their intrinsic properties, such as nutrients quality, primary/secondary metabolites, etc...; nonetheless complementary studies are needed on the food nutritional components of these cocoa varieties to elucidate their trophic interactions with mirids to better understand the feeding ecology of this insect species. In addition, mirid developmental stages trophically exploit differently the host plants. The feeding of Larvae L3, L4 and L5 cause more black spots than larvae L1 and L2 and Adults on the studied cocoa varieties (Amelonado and Trinitario). Notwithstanding, the mechanism involved in the feeding polymorphism remains unknown, the different numbers of feeding punctures due to the mirid developmental stages could reflect specific differences in nutrient resource requirements for the optimal growth of the different developmental stages of *S. singularis*. Apart from female and male adults that move from one host plant to another after oviposition and do not feed constantly (Entwistle, 1972), it is known that mirid larvae are sedentary and remain attached to the host organ where they hatched; and their main interaction with the host plant is trophic (Babin et al., 2008; Mahob et al., 2015). Taking into account the duration of each developmental stage, 2 to 9 days for older Larvae (L3, L4 and L5) versus 2 to 4 days for young Larvae L1 and L2 (Babin et al., 2008), it is clear that physiologically/genetically, older larvae are more adapted to feeding on the host plant than young larvae. Consequently, it is obvious that severity of damage caused by *S. singularis* depends on the variety of the cacao tree

and the developmental stage of this insect. This result supports previous studies by Sounigo et al. (2003), Anikwe et al. (2009), Badegana et al. (2005), N'Geussanet al. (2008, 2010) and Mahobet al. (2019, 2020, 2021); who reported that the susceptibility and/or the number feeding punctures, expressed as attractiveness/repellency, of mirids varied between cocoa genotypes.

In the current work, average cherelle losses due to *S. singularis* developmental stage infestations ranged from 50% to 100% (Table 2). The number of aborted cherelles was relatively higher in the Trinitario variety compared to Amelonado; it also varied significantly between the mirid developmental stages. Although Babin (2018) has globally established that all cocoa varieties are sensitive to mirid attacks, to our knowledge, the current work provides the first data on the impact of each mirid development stage. It appears that L5 larvae of *S. singularis* caused more production cocoa losses followed, on one hand, by L4, L3 larvae and adults for Amelonado, and on the other hand by L4, adults and L3 larvae in Trinitario; while in both cocoa varieties, L1 larvae caused fewer abortions. It is therefore suggested that older larvae, especially L5, could inject more hemolytic saliva in the host tissues resulting in more aborted cherelles on the cacao trees. The abortion rates of cherelles obtained (50% to 100%) confirm that mirids are a potential cause of significant production and economic losses in West and Central Africa (Entwistle, 1972, Idowu, 1989, Ojelade et al., 2005, Anikwe et al., 2009, Yede et al., 2012, Yede, 2016, Mahob et al., 2019).

This work also demonstrated the influence of mirids on the length and diameter of cherelles. Once more, older larvae (L3, L4 and L5) revealed more harmful than the younger ones (L1 and L2) (Table 3). The reduction in the size of cherelles ultimately leads to a reduction in the number of beans in the mature/ripe fruits and consequently the cocoa yields in farms (Yede et al., 2012). However, the determinism of the high harmful of old developmental stages of mirids, especially the L4 and L5 larvae, towards to the host plant (especially fruits growth) remains to be elucidated in our future investigations.

Fig. 1:- Geographical location of the study site.

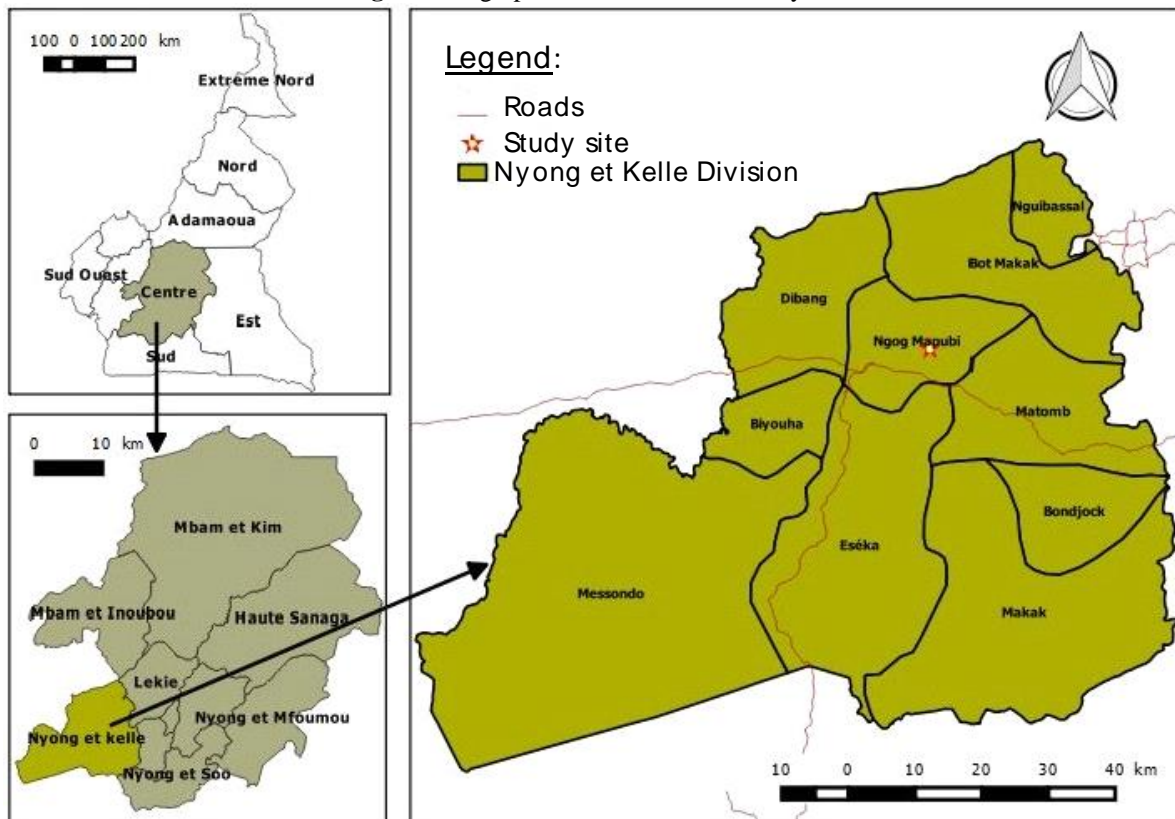
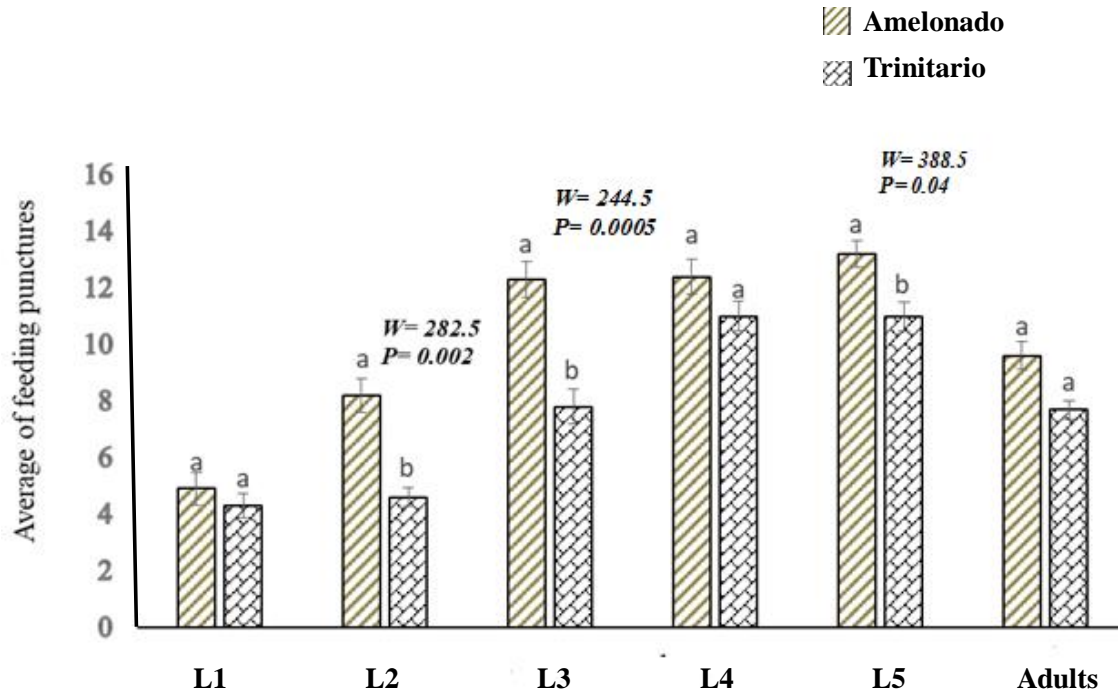


Fig. 2:- Average number of feeding punctures per development stage *S. singularis* on cocoa varieties. L1 to L5: Larvae of stage 1 to 5 and Adult stage.



S. singularis development stages

Table 1:- Comparison of the medians values (± ES) of the feeding punctures of the different development stages of *S. singularis* and the both tested cocoa varieties.

Development stages	Number of cherelles	Medians values (± SE) of feeding punctures of mirids	
		Amelonado	Trinitario
L1	30	4.95 ± 0.84 ^c	4.37 ± 0.64 ^c
L2	30	7.28 ± 0.70 ^b	4.32 ± 0.43 ^c
L3	30	11.13 ± 0.78 ^a	7.32 ± 0.72 ^b
L4	30	10.05 ± 0.62 ^a	9.44 ± 0.70 ^a
L5	30	11.29 ± 0.70 ^a	9.97 ± 0.42 ^a
Adults	30	7.75 ± 0.89 ^b	6.25 ± 0.42 ^b
Statistics		H= 32.31, df=5, P<0.0001	H= 17.65, df = 5, P=0.003

In columns 3 and 4, medians values with the same letter were not significantly different at (P<0.05), SE: Standard error

Table 2:- Comparison of the average number of the aborted cherelles between the different development stages of mirides and the two cocoa varieties.

Development Stages	Number of Samples	Rate Number (\pm ES) of aborted cherelles		Statistics
		Amelonado	Trinitario	
L1	30	50.00 \pm 6.02 c ^a	58.00 \pm 4.72 c ^a	$t=-1.109$, $df=1$, $P=0.38$
L2	30	53.33 \pm 2.60 c ^a	66.33 \pm 6.96 BC ^a	$t=-1.508$, $df=1$, $P=0.27$
L3	30	63.33 \pm 4.91 B ^a	78.33 \pm 4.40 B ^a	$t=-1.614$, $df=1$, $P=0.25$
L4	30	70.00 \pm 5.29 AB ^a	86.66 \pm 2.40 A ^a	$t=-3.467$, $ddl=1$, $P=0.07$
L5	30	81.67 \pm 4.41 A ^a	100 \pm 0.00 A ^a	$t=1.960$, $df=1$, $P=0.19$
Adults	30	60.33 \pm 1.76 B ^b	83.33 \pm 4.40 AB ^a	$t=-7.155$, $df=1$, $P=0.02$
Statistics		$F_{(5,174)} = 1217.45$, $P < 0.0001$	$F_{(4,145)} = 1881.04$, $P < 0.0001$	

In the rows (letter on top the bar) and columns (capital letters in the bottom of the bar), values with the same letter are not significantly different at ($P < 0.05$), according to Student test in the rows and ANOVA in the column following by the Tukey post hoc test for pairwise multiple comparison tests.

Table 3:- Comparison of the length and diameter mean values (\pm SE) of surviving cherelles of two cocoa varieties post mirid infestations, by the different mirid larva stages.

Cocoa varieties	Development stages	Mean values (\pm SE)	
		Length	Diameter
Amelonado	L1	17.22 \pm 0.39 ^{ab}	7.83 \pm 0.15 ^a
	L2	16.91 \pm 0.44 ^{ab}	7.71 \pm 0.21 ^{ab}
	L3	14.15 \pm 0.48 ^c	6.42 \pm 0.16 ^{bc}
	L4	14.21 \pm 0.35 ^c	5.91 \pm 0.25 ^c
	L5	14.63 \pm 0.36 ^{bc}	5.47 \pm 0.18 ^c
	ADULTS	15.68 \pm 0.48 ^b	6.87 \pm 0.26 ^b
	CONTROL	18.01 \pm 0.37 ^a	7.79 \pm 0.13 ^a
		$F_{(6,91)} = 7118.52$; $P < 0.0001$	$F_{(6,91)} = 7487.17$; $P < 0.0001$
Trinitario	L1	18.59 \pm 0.46 ^a	8.50 \pm 0.24 ^a
	L2	18.78 \pm 0.48 ^a	7.98 \pm 0.21 ^{ab}
	L3	15.62 \pm 0.27 ^c	6.45 \pm 0.24 ^c
	L4	15.52 \pm 0.57 ^c	6.05 \pm 0.13 ^c
	ADULTS	16.30 \pm 0.85 ^b	6.34 \pm 0.25 ^c
	CONTROL	18.88 \pm 0.32 ^a	7.66 \pm 0.14 ^b
			$F_{(5,61)} = 4980.54$; $P < 0.0001$

In the columns 3 and 4, mean values with the same letter are not significantly different at ($P < 0.05$) according to Tukey pairwise comparison

Conclusion:-

The current study shows once again that the susceptibility of young fruits to *S. singularis* varied according to the cocoa variety. The Amelonado variety was more preferred than Trinitario, because it was relatively more attacked by this insect pest. *Sahlbergella singularis* causes important losses on cherelles production; older developmental

stages (L3, L4, and L5) among the six tested are particularly dangerous for cocoa production with regard to the number of feeding punctures and/or aborted fruits. This result can therefore be taken into consideration in varietal breeding programs against mirids in general, and especially *S. singularis*. More field studies in contrasting locations are needed to know the growth dynamics of the targeted insect pest, in order to determine the timing of each mirid stage to reduce their economical threshold, especially older larvae L3, L4, L5 and adult stages that are more damaging for cocoa production and economically most important.

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Compliance with ethical standards

Conflict of interest: All authors declare that they have no conflicts of interest that could have appeared to influence the work reported in this paper.

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