Academic Journal of Entomology 3 (1): 39-44, 2010
ISSN 1995-8994
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Feeding Preference and Morphometrics of *Sahlbergella singularis* (Hemiptera: Miridae) on Cocoa Pods at Different Stages of Physiological Development

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Abstract: The brown cocoa mirid, *Sahlbergella singularis* is the major insect pest of cocoa in Nigeria and this pest is capable of causing about 30-80% yield loss to cocoa in the field. Both nymphs and adult mirids feed on cocoa pods, twigs and chupons as well as trunks and flower chushions. Feeding activities by the nymphs and adults result in lesions on the pods with a resultant deformation, distortion or bean decay. With this breakthrough in the continuous rearing of *S. singularis*, homogenous populations of mirids were now available for intensive laboratory bioassays. Body morphometrics of mirids fed on pods of different physiological ages were recorded. The feeding preference of *S. singularis* to cocoa pods at different levels of physiological maturity was assessed in the laboratory using choice and no-choice assays. Cocoa pods of the amelonado variety (N38) were used for this experiment. The pods were classified into six age groups as follows: Very young cherelle (2 weeks old), Old cherelle (4 weeks), Young pod (6 weeks), Mature pod (8 weeks), Ripe pod (10 weeks) and Over-ripe pod (12 weeks). Also the proximate content of these cocoa pods were determined. In a choice experiment, *S. singularis* showed specific preference for ripe pod at the expense of cherelles, mature and over-ripe pods. Mean numbers of 29.8, 39.0 and 51.8 mirid lesions were counted on ripe pods 24, 48 and 72 hours after *S. singularis* introduction on pod. This is significantly different ($P<0.05$) from the number of feeding lesions found on the other pod types. In the no-choice experiment, *S. singularis* were observed to feed on all available pod ages presented as source of food. The protein content steadily increased from 2.66% in two weeks old cherelle to 6.05% in 3months overripe pod, almost three times the quantity of protein in the initial stage. This was even obvious from the pod colour that changed from green to yellow (ripe pod) and then orange when overripe. There were significant differences ($P<0.05$) in the crude fibre content from 7.98% (two weeks cherelle) to 6.49% (overripe pod). The crude fibre as well as the ash contents decreased with an increase in pod age. This study showed that though all pod ages were able to support day old nymph to adult, however, ripe pods were most preferred for feeding by the brown cocoa mirid.

Key words: *Sahlbergella singularis* · Feeding preference · Morphometrics · Proximate analysis

INTRODUCTION

The brown cocoa mirid, *Sahlbergella singularis* has been reported to be the major insect pest of cocoa in Nigeria. This pest is capable of causing about 30% yield loss to cocoa in the first year alone and up to about 80% loss in two consecutive seasons if farm is left unprotected [1-4].

Damage by the brown cocoa mirid is caused by the feeding activities of the nymphs and adults. Feeding causes characteristic dark markings on pods and shoots known as lesions. Lesions result from the collapse of plant tissues caused by the toxic saliva of mirid. Secondary damage symptomized by canker and dieback occurs when the feeding lesions are infected by a parasitic fungus, notably, *Calonectria rigidiuscula* [5]. The combination of mirid and fungal damage results in a quick decline of cocoa farms, especially when control measures are inadequate [6].

The use of synthetic insecticides has been a common method of mirid control in Nigeria. Several insecticides had been approved by the Cocoa Research Institute of Nigeria (CRIN) but recently the approvals for these insecticides have been withdrawn due to health and environmental hazards posed by them. Only two brands of insecticides (Thiamethoxam and Chlopyrifos active ingredients) were recently passed for use in protection of cocoa farms against mirids. This portends danger as the pest may develop resistance to such narrow groups of insecticides over time.

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S. singularis is the most widespread species of mirid attacking cocoa wherever cocoa is grown in Nigeria [7]. The population of *S. singularis* in mature fruiting farms is usually low with the abatement of rains when the atmospheric relative humidity is also usually low [8]. In Nigeria, mirid infestation is usually lowest between February and July and highest between August and November/December. This is dependent on the severity of the preceeding dry season or the onset of the 'August break' and the cessation of the early rains, respectively. However, infestation also depends on the availability of adequate number of suitable pods and young tissues as well as suitable canopy or shade required for resting, feeding and reproduction of cocoa mirids [1]. Opeke [7] reported that mirid feeds on pods, chupons and fan branches and causes extensive damage on the plant. The feeding points turn to brown or black with sunken, oval lesions on pods resulting in the malformation of pods, wilting of branches, die-back of shoots, scar (canker) on stems and eventual stag-headedness [1]. Mirid attack on pods may also cause pod deformation, distortion or bean decay depending on the severity of the attack [7]. If the more persistently attacked trees have the crown extensively reduced by die-back, the condition is described as "stag headed". Normal flowering and pod setting are severely affected as a result of the previous season's attack [2]. When the cocoa tree loses its dried twigs as a result of mirid attack, the damage progresses to a final stage that is referred to as "pole formation" with only the dead main trunk remaining [1].

It is obvious that mirids prefer cocoa pods to any other plant parts, however, it has not been established in any literature yet, the particular pod type that is most preferred by mirids for feeding. In the light of the foregoing, studies on the morphometrics and damage assessment of S. singularis were conducted on six cocoa pods of different physiological ages and the proximate analysis of these pod types were determined.

MATERIALS AND METHODS

Study Site: The experiment was conducted inside an improvised rearing chamber made up of ply-wood for raising cultures of the mirids, constructed within the Entomology laboratory of Cocoa Research Institute of Nigeria (CRIN) Headquarters, Ibadan, Nigeria. The dimension of the growth chamber is 300cm x 200cm x 240cm. A digital Panasonic air conditioner (Model CS-C120KF, 220-240V, 50Hz, R22) which was the temperature regulating system, an electronic ultrasonic humidifier (model V5100N 120VAC, 60Hz, 36W) that generates humidity and a portable digital enviro-meter 4 in 1 digital instrument that measures temperature, relative humidity, wind direction and light intensity were installed inside the growth chamber to obtain a well regulated environment for the mirid culture.

Insect Culture: The rearing procedure was done in two phases. In the first phase, day old nymphs were collected from the field and transferred into the growth chamber equipped with regulated conditions of 90±5% Relative Humidity and Temperatures of 22°C at night and 24°C during the day. The temperatures and relative humidity chosen for the study of mirid lifecycle were derived from the mean records taken under the cocoa canopies during the day and at night (n=10). The day old nymphs were fed on cut chupons in plastic cages and placed inside the growth chamber. The cut chupons were replaced every other day until adult mirids emerged. The adults were placed separately in small plastic cages (13x8x6cm) and fed with fragmented pieces of chupons (5cm long) for 6 days, which corresponds to the period of sexual maturity of females. Thereafter, the mirids were sexed and pairs maintained in the plastic cages for another 24 hours. The second phase was carried out on cocoa trees under natural conditions. Mated females from the first phase were carefully transferred onto pods on cocoa trees in the field and covered carefully with a mesh-sleeve cage. Field observation for F_2 emergents was carried out at 14 days after introduction of the gravid female and thereafter daily till the last eclosion. The day old nymphs were collected and fed on cut chupons in plastic cages in the growth chamber. The pod habouring the emergents was also plucked and transferred back to the growth chamber. This procedure was repeated to maintain a continuous culture.

Assessment of damage potential of *S. singularis* to pods of different ages:

The feeding response of *S. singularis* to cocoa pods at different levels of physiological maturity was assessed in the laboratory using choice and no-choice assays. Cocoa pods of the amelonado variety (N38) were used for this experiment. The pods were classified into six age groups as follows:

- Very young cherelle (2 weeks old)
- Old cherelle (4 weeks)
- Young pod (6 weeks)
- Mature pod (8 weeks)
- Ripe pod (10 weeks)
- Over-ripe pod (12 weeks)

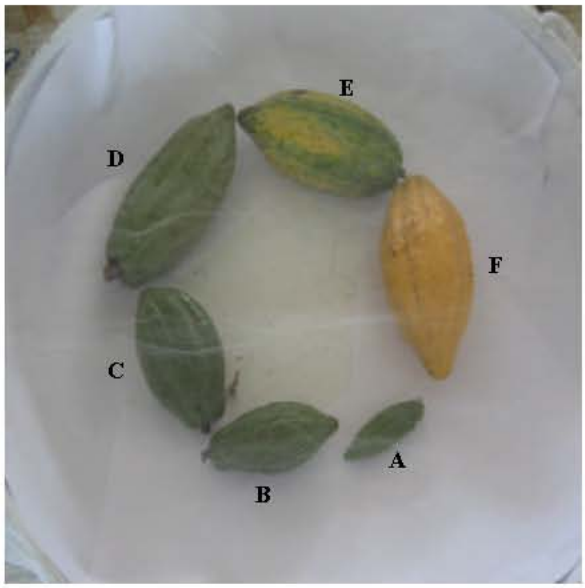

Fig. 1: Feeding preference of *S. singularis* on six different pod ages in the laboratory
A = 2 weeks old cherelle, B = 4 weeks old cherelle, C = 6 weeks old young pod, D = 8 weeks old mature pod, E = 10 weeks old ripe pod, F = 12 weeks old over-ripe pod

Choice Assay: The choice experiment was done by placing the pods of different ages in an improvised choice chamber (Figure 1). Ten day-old adults were introduced to the center equidistant to all the pods and replicated five times. The container was thereafter covered with muslin cloth and held in place by means of twines. Data of the number of feeding lesions on pods were collected and recorded 24, 48 and 72 hours after.

No-Choice Assay: In a no-choice experiment, day-old adult mirids (n=10) were introduced into plastic rearing cages shown in Plate 1B. Each cage contained one pod belonging to a specific age and the numbers of feeding lesions were counted 24, 48 and 72 hours after. There were five replicates for each of the pod type used and were arranged in a completely randomized design. In another experiment, day old nymphs (n=10) were fed on the different ages of cocoa pods till adult stage. Data collected were as follows:

- number of feeding lesions on pods
- morphometrics of life stages

Determination of proximate content of cocoa pods at different stages of development:

Cocoa pods at six different stages of physiological development were plucked from zone 1 of CRIN headquarters, Ibadan. The age classes of the pods were as follows:

- Very young cherelle (2 weeks old)
- Old cherelle (4 weeks)
- Young pod (6 weeks)
- Mature pod (8 weeks)
- Ripe pod (10 weeks)
- Over-ripe pod (12 weeks)

The pericarp of each pod type was separately peeled and dried under-shade for a period of one week. After a week, the cocoa peels were separately blended in the laboratory using an electric blender. 50g each of ground material was poured into separate labelled test tubes, sealed and taken to the International Institute of Tropical Agriculture, Ibadan, for proximate analysis. Proximate content of cocoa pods were determined as follows:

Determination of Protein Content: The protein content of the samples was determined by the method of Hach. The procedure involves digesting the material with concentrated tetraoxosulphate VI acid (H_2SO_4) to dehydrate and char the sample (carbonisation) and hydrogen peroxide (H_2O_2) to complete sample decomposition by providing a reducing environment, which helps in converting the nitrogen to ammonium salts. On treatment with a dispersing agent (polyvinyl alcohol (PVA)), the ammonium salt decomposes to liberate ammonia, which in the presence of Nessler's reagent gives an orange colour, which is read at 460nm according to the specification of the Hach procedure manual 0.25 g sample was weighed into Hach digestion flask and 4ml of conc. tetraoxosulphate VI acid was added. The sample was transferred to the fume hood and heated for 5minutes at 440°C. To the charred sample was added 16ml of H_2O_2 to clear off the brown fumes and make the digest colorless. The flask was taken off the heater, allowed to cool and the contents made up to 100ml mark with deionized water and mixed. To 1ml of the digest, 3 drops of mineral stabilizer and 3 drops of polyvinyl alcohol dispersing agent was added. It was mixed, made up to 25ml and 1ml of Nessler's reagent was added. The colour was read within 5minutes at 460nm on the Hach spectrophotometer against deionized water blank. The absorbance gives mg/l apparent Nitrogen. The true Kjeldahl nitrogen is calculated as follows:

$$\% N = \frac{0.005625 \times A}{B \times C}$$

Where:

A = mg/l (reading displayed), B = ml or g sample digested and C = ml digest analysed.

The protein content is determined by multiplying percentage Nitrogen by a constant factor of 6.25 [9].

Determination of Fat Content: This was determined using Automated Method (Soxtec system HT2). The sample was ground and dried properly before loading 2g of the sample in a thimble and plugged with cotton wool. Thereafter, the thimbles were dried and inserted into Soxtec. HT. The extraction cups (with boiling chips) were dried and weighed before 25ml of solvent was added. The cup was inserted into the Soxtec.HT and extracted for 15mins in boiling position and for 30mins in 'Rinsing' position. Thereafter solvent was evaporated and the cups are released and dried at 100°C for 30mins. The sample was cooled in a dessiccator and the following calculation was done.

Weight of the cup with the extracted oil = W_3.
Weight of the empty cup = W_2
Weight of sample = W_1

$$\% \text{ fat/oil} = \frac{(W3 - W2) \times 100}{W1}$$

Determination of Ash Content: This was determined by weighing 2.0g of the sample into a porcelain crucible. This was transferred into the muffle furnace set at 55°C and left for about 4 hours. About this time it had turned to white ash. The crucible and its content were cooled to about 100°C in air, then room temperature in a desiccator and weighed. This was done in duplicate. The percentage ash was calculated from the formula below

$$\text{Ash content} = \frac{\text{Wt. of ash}}{\text{Original Wt.}} \times \frac{100}{1} \quad \text{of sample}$$

Determination of Fibre Content: 2.0g of the sample was accurately weighed into the fibre flask and 100ml of 0.255 N_2SO_4 added. The mixture was heated under reflux for 1 hour with the heating mantle. The hot mixture was filtered through a fibre sieve cloth. The filtrate obtained was thrown off and their residue was returned to the fibre flask to which 100ml of (0.312N Na0H) was added and heated under reflux for another 1 hour. The mixture was filtered through a fibre sieve cloth and 100ml of acetone added to dissolve any organic constituent. The residue was washed with about 50ml hot water on the sieve cloth before it was finally transferred into the crucible. The crucible and the residue were oven-dried at 105°C overnight to drive off moisture. The oven-dried crucible containing the residue was cooled in a desiccator and later weighed to obtain the weight W_1. The crucible with weighed sample W_1 was transferred to the muffle furnace for ashing at 55°C for 4 hours. The crucible containing white or grey ash (free of carbonaceous material) was cooled in the desiccator and weight obtained is W_2. The difference W_1 -W_2 gives the weight of fibre. The percentage fibre was obtained by the formula.

$$\%\text{Fibre} = \frac{W_1 - W_2}{\text{wt of sample}} \times 100$$

Dry Matter (DM) and Moisture Determination: 2g of the sample was weighed into a previously weighed crucible. The crucible plus sample taken was then transferred into the oven set at 100 °C to dry to a constant weight for 24 hours overnight. At the end of the 24 hours, the crucible plus sample was removed from the oven and transferred to dessiccator, cooled for ten minutes and weighed.

If the weight of empty crucible is W_o
Weight of crucible plus samples is W_1
Weight of crucible plus oven-dried sample is W_3

$$(\%DM)\%\text{Dry Matter} = \frac{W_3 - W_o}{W_1 - W_o} \times \frac{100}{1}$$

$$\%\text{Moisture} = \frac{W_1 - W_3}{W_1 - W_o} \times \frac{100}{1}$$

Or%Moisture = 100 -%DM.

Determination of Carbohydrate Content: This was done by subtracting (%Protein +%fat +%Moisture +%Ash) from hundred i.e. 100-(%M+%P+%fat+%ash).

RESULTS

Results showing feeding punctures of *S. singularis* on different ages of pods is presented in Table 1. In a choice experiment, *S. singularis* showed specific preference for ripe pod at the expense of cherelles, mature and over-ripe pods. Mean numbers of 29.8, 39.0 and 51.8 mirid lesions were counted on ripe pods 24, 48 and

Table 1: Number of feeding lesions on cocoa pods of different ages exposed to *S. singularis* in a choice and no-choice experiments

	Number of feeding lesions					
	Choice			No- Choice		
Pod Age	24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
1st Cherelle	2.20d	6.00e	7.20d	27.24d	52.05a	82.61b
2nd Cherelle	1.60d	4.60e	5.80e	31.76c	54.36a	85.54b
Young Pod	7.60c	10.00d	15.80c	29.96c	51.55a	92.18ab
Mature Pod	14.40b	17.60b	26.40b	34.34b	53.78a	97.66a
Ripe Pod	29.80a	39.00a	51.80a	37.62a	54.79a	98.42a
Overripe Pod	7.00c	15.00c	19.60c	33.75b	52.73a	98.25a

Means followed by the same letter in the same column are not significantly different (P>0.05)

Tukey's Studentized Range (HSD) Test

Table 2: Morphometrics of the life stages of *S. singularis* on different ages of cocoa pod

Parameters						
Overripe Pod	Life Stage	1st Cherelle	2nd Cherelle	Young Pod	Mature Pod	Ripe Pod
Body length	1st Instar	2.30f 2.30f	2.00f	2.33f	2.28f	2.21f
2nd Instar	3.20e	3.15e	2.95e	3.32e	2.97e	2.35e
3rd Instar	4.20d	4.15d	4.40d	4.12d	3.98d	4.41d
4th Instar	5.25c	5.30c	5.80c	5.27c	4.85c	5.72c
5th Instar	7.20b	7.00b	7.35b	7.32b	7.12b	7.26b
Adult	7.85a	7.92a	7.70a	7.93a	7.67a	7.69a
Abdominal1st Instar	1.00d	1.10d	1.12d	1.24d	1.17d	1.20d
Width 2nd Instar	2.05c	1.95c	1.90c	2.19c	1.98c	2.05c
3rd Instar	2.90b	2.85b	2.94b	2.61b	2.72b	2.90b
4th Instar	3.10b	2.90b	3.12b	3.02b	2.96b	3.12b
5th Instar	4.35a	4.30a	4.45a	4.48a	4.35a	4.55a
Adult	4.40a	4.39a	4.50a	4.53a	4.44a	4.58a
Antennal 1st Instar	2.00d	1.95d	1.80d	2.02d	1.83d	2.01d
Length 2nd Instar	2.95c	2.85c	2.65c	2.99c	2.72c	2.94c
3rd Instar	3.05c	3.02c	2.98c	3.15c	2.88c	3.19c
4th Instar	4.05b	4.12b	4.25b	4.27b	4.30b	4.42b
5th Instar	5.20a	5.15a	5.24a	5.33a	5.25a	5.26a
Adult	5.25a	5.16a	5.31a	5.45a	5.37a	5.34a

Means followed by the same letter in the same column and along the same row are not significantly different (P>0.05)

Tukey's Studentized Range (HSD) Test

Table 3: Proximate composition of cocoa pods at different stages of physiological development

Pod age	Protein%	Fat%	Ash%	Crude fibre%	Moisture%	Carbohydrate%
1st Cherelle	2.66d	2.00ab	6.82a	7.98a	8.90a	71.65d
2nd Cherelle	2.90cd	2.09a	6.81a	7.55ab	8.62bc	72.05c
Young Pod	3.10bc	2.19a	6.62ab	7.27bc	8.72ab	72.04c
Mature Pod	3.26b	2.14a	6.44b	7.10bcd	8.45c	72.63b
Ripe Pod	3.35b	1.85b	5.57c	6.67cd	7.87d	74.71a
Overripe Pod	6.05a	2.07ab	4.95d	6.49d	8.82ab	71.64d

Means followed by the same letter in the same column are not significantly different (P>0.05)

Tukey's Studentized Range (HSD) Test

72 hours after *S. singularis* introduction on pod. This is significantly different ($P<0.05$) from the number of feeding lesions found on the other pod types. However, the result in the choice tests contradicts the results in a no-choice test experiment. In the no-choice experiment, *S. singularis* were observed to feed on all available pod ages presented as source of food to the insect. Table 1 also shows that there was no significant difference in the number of feeding punctures on the different pod ages 48 hours after infestation in the no-choice experiment. The morphometrics of the life stages of *S. singularis* seeded on pods of different ages (Table 2) show that the different pod ages were able to support growth and development of *S. singularis* from first instar nymph to adult mirid.

Table 3 shows the proximate analysis of cocoa pods at different stages of growth. The protein content steadily increased from 2.66% in two weeks old cherelle to 6.05% in 3months overripe pod, almost three times the quantity of protein in the initial stage. This was even obvious from the pod colour that changed from green to yellow (ripe pod) and then orange when overripe. There were significant differences ($P<0.05$) in the crude fibre content from 7.98% (two weeks cherelle) to 6.49% (overripe pod). The crude fibre content decreased with age of the pod (Table 3). A decrease in the ash content was also observed with increase in pod age. In a choice experiment, the number of feeding lesions recorded on each pod type showed that ripe pod was most preferred (51.8), followed by mature pod (26.4), over-ripe pod (19.6), young pod (15.8), 1st week old Cherrelle (7.2) and 2nd week old Cherrelle (5.8) as shown in Table 1.

DISCUSSION

The different pod ages were able to support mired life cycle from one day old 1st instar nymphs to adult mirid. Though records of the duration of cocoa mirid life cycles are sparse, however, Entwistle [8] reported the life cycles of six genera, three from West Africa and one each from Central America, Malaya and Papua New Guinea. These were *D. theobroma* (Ghana), *S. singularis* (Ghana), *Bryocoropsis laticollis* (Ghana), *Helopeltis ceylonensis* (Ceylon), *Pseudodoniella typicus* (New Britain), *Monalonion annulipes* (Costa Rica) and *Monalonion shaefferi* (Brazil). The life cycle of *S. singularis* from egg to adult was 41.1 days and this result is in consonance with the report of Entwistle [8] who reported the life cycle range of 37-41 days for *S. singularis*. This study showed that ripe pods were most preferred for feeding by mirid as portrayed in the choice experiment however, when mirids are faced with a no-choice situation, they will feed on any available pod type. This could be the reason why large number of cherelles wilt in cocoa plantations as a result of mirid attack in the absence of ripe pods. The result of the proximate analysis of the different pod types is also informative as ripe pods have the most carbohydrate content as well as a steady increase in protein.

ACKNOWLEDGEMENT

The author acknowledges the permission by the Executive Director of Cocoa Research Institute of Nigeria to publish this paper.

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