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BIOLOGICAL CHARACTERISTICS OF TWO SPECIES OF COCOA MIRIDS REARED ON *DESPLATSIA DEWEVREI* (De Wild & Th. Dur.) IN THE LABORATORY

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ABSTRACT
Studies were conducted on the development of two cocoa mirids. *Sahlbergella singularis* Hagl., and *Distantiella theobroma* Dist., on *Desplatsia dewevrei* and on cocoa pods, and the suitability of using the fruits to mass rear these cocoa mirids. Fertility, survival, longevity, number of instars and duration, sex ratio and feeding preferences were determined. A newly emerged adult female and two males of either species of mirids were isolated and fed different stages of maturity of the fruits of *D. dewevrei*. *S. singularis* laid eggs which hatched into nymphs, went through five nymphal stages and emerged as adults on only ripe fruits. *D. theobroma* could not reproduce on any of the stages of *D. dewevrei* fruits. Mortality in the nymphs of *S. singularis* was highest at the first instar but this reduced considerably as the nymphs got older. The mean longevities of 13.8 and 10.1 days were recorded for adult female and male respectively. The sex ratio varied from 1: 1.5 to 1: 2.5 (male: female). However, adults of *D. theobroma* that emerged from field collected specimen on the averaged lived 14.0 days in females and 12.0 days in males. Both species of mirids fed on all the stages of the fruits but fed more on the ripe fruits. Cocoa pods could not support the development of the two mirid species due to fungal rot. The fruits were found to contain protein and sugar. The biological characteristics observed in this study have shown that ripe fruits of *D. dewevrei* could be used to mass rear *S. singularis* in the laboratory but further work need to be done on that of *D. theobroma*.

KEYWORDS: *Desplatsia dewevrei*; mirids; Cocoa; *S. singularis*; *D. theobroma*; Ghana.

INTRODUCTION
Mirids are the most dangerous of all insects in the world that feed on and thus damage cocoa pods and shoots[1]. Africa is the top cocoa (*Theobroma cacao* L. Sterculiaceae) producing region, accounting for over 70% of global production. In West and Central Africa, this crop is attacked by two mirid pests, *i.e. Sahlbergella singularis* Hagl. (Heteroptera: Miridae) and *Distantiella theobroma* Dist. (Heteroptera: Miridae)[2]. These two bugs feed on cocoa pods and shoots, thus inducing tissue necrosis, and infested cocoa trees quickly become non-productive. In general, damage caused by feeding activities of nymphs and adults of these mirids reduce yield by outright killing of young shoots or delay establishment and fruit bearing by several years.These attacks are responsible for cocoa production losses of 25-40% in Côte d'Ivoire and Ghana, the largest producers in the region[1, 2]. Few studies have analysed the complex agro forestry systems in which cocoa trees are conventionally grown, and little is known about the ecology of *S. singularis* in these systems. In Ghana, more recent studies on crop losses indicate that about 25-30% of the national cocoa acreage is badly attacked by mirids leading to an annual crop loss of about 100,000 tonnes[3]. Currently, cocoa mirids in Ghana are controlled by means of chemicals. The use of chemicals is increasingly becoming unacceptable due to environmental and health hazards associated with chemicals, development of resistant strains of pest species, toxic residues in food, water or soil and destruction of beneficial organisms[4-6]. These considerations have stimulated interest to develop alternatives to chemical control of mirids especially at the Cocoa Research Institute of Ghana (CRIG). Such alternatives could be applied in an Integrated Pest management (IPM) programme, which may among others incorporate the use of insect growth regulators, behaviour modifying compounds and natural enemies including pathogens[7,8] and the use of botanical pesticides[9,10]. Development of these methods for an IPM programme involves intensive laboratory experimentation, which requires the use of large number of mirids. Normally, experimental mirids are obtained by hand from the field. However, this method of collection damages their mouthparts, legs and antennae creating variability problem in experimental insects. In addition, mirids generally have low population densities on cocoa, and even at peak period (July-December) only 6 mirids per 10 trees are obtained after the major rains. Thus, mirids collected may either be inadequate or unfit for experiments. Earlier rearing methods include the use of potted cocoa seedlings and green cocoa pods, potted cocoa seedlings, cut chupons of cocoa, and cut stems of *Ceiba pentandra*. The findings from these earlier methods helped to identify some problems associated with laboratory rearing of mirids. First, nymphs fed best in darkness however; they required

high humidity and very succulent food source. Secondly, severed pods and stems of potted seedlings were not suitable to support either an acceptable length of adult survival or fertility. Finally, eggs hatchability under laboratory conditions is generally lower than the field conditions. Fruits of *Desplatsia dewevrei* (De Wild and Th. Dur.) (Tiliales: Tiliaceae) have been reported as potential breeding host plant for cocoa mirids[8]. The present study is therefore aimed at investigating some biological characteristics (fertility, survival, longevity, number of instars and duration, sex ratio and feeding preferences) of two species of mirids and also to determine the stage of fruits of *D. dewevrei* suitable for rearing the mirids in the laboratory.This knowledge is useful in establishing laboratory mass rearing of two species of mirids to provide a reliable supply of adequate and healthy mirids of known ages to facilitate intensive laboratory experimentation for IPM programmes.

EXPERIMENTAL SECTION

Fruits of *D. dewevrei* at different stages of development were collected and categorized into unripe (freshly plucked from tree, dark green in colour), ripe (fruits, freshly dropped from tree, light green in colour) and overripe fruits (fruits that have stayed over 45 days on the ground, yellowish). They were washed with water to remove dirt and other insects and air-dried for 24 hours in the laboratory. Cherelles (immature pods), ripe (mature green) and overripe (mature yellow) cocoa pods were also collected so that their suitability as food source for rearing mirids could be compared with that of *D. dewevrei*. Healthy adult mirids used for the various studies were selected from hand-collected specimens from the field that had been fed on cocoa chupons and cherelles and kept overnight.

Fertility studies on D. dewevrei and Cocoa pods

An adult female and two adult males of either *S. singularis* or *D. theobroma* were put on unripe, ripe and overripe fruits of *D. dewevrei* and on cocoa pods in plastic bucket measuring 20cm in diameter by 17cm in height and covered with a lid with a hole (10.1cm x 10.1cm) sealed with very fine net to prevent escape and provide ventilation. The experiment was repeated 50 times for *S. singularis* and 20 times for *D. theobroma* between February and October 2011 with temperature range of 20.8 –33.7^{o}C and relative humidity of 56.9–84.4%. The unequal numbers were due to difficulty in obtaining adequate number of *D. theobroma*. To determine fertility, number of first instar nymphs produced were counted and recorded.

Nymph survival and sex ratio on D. dewevrei

First instar nymphs produced in the fertility studies were allowed to continue feeding on the same fruit, and sometimes on an added fresh fruit till they developed into adults. The number of nymphs that survived to adulthood and sexes of the emerged adults were recorded, and sex ratio determined.

Adult survival of longevity of D. theobroma on D. dewevrei

A batch of ten newly emerged adults females from nymphs obtained from the field, were isolated on unripe, ripe and overripe fruit in the laboratory. At the end of each week (7 days), the number of females that survived was counted and the percentage survival was determined. This was continued till all died. Longevity of each adult female was determined. The same procedure was adopted for the males. Experiments were replicated ten times.

Nymphal development, adult longevity and life-span of S. singularis on ripe fruits of D. dewevrei

A freshly emerged nymph from the fertility studies was placed on each ripe fruit. The nymph was examined daily for exuviae using stereoscopic microscope and hand lens. The presence of exuviae indicated ecdysis. The period for each ecdysis was recorded until the nymph emerged as an adult of either sex. Adult longevity was determined by observing the newly emerged adults till they died. Life span was determined by counting the days from hatching to death of adult. *D. theobroma* was not considered because no nymphs were obtained in the fertility studies.

Feeding preference of S. singularis and D. theobroma on D. dewevrei and nutrient of the fruits

First instar nymphs of either *D. singularis* or *D. theobroma* were released singly on unripe, ripe and overripe fruits of *D. dewevrei* placed in plastic containers in the laboratory. Records were taken of the number of lesions made on each fruit. At the end of each instar, the nymphs were transferred onto a fresh fruit and counting of lesions continued. The same procedure was repeated till the insect turned adult. At the adult stage, the number of lesions on the fruits was recorded for the first seven days. After the seventh day, there was the tendency for lesions to overlap hence counting was discontinued.

Atago refractometer (type 1T, 4T) was used to measure the sugar content present in the juices of unripe, ripe and overripe fruits of *D. dewevrei*. The nitrogen content in 0.5m of fresh weight in the three stages of the fruits was measured by two methods, namely digestion and distillation. The products of digestion were distilled and the products of distillation were titrated with standardized 0.02M H_2SO_4 and titre values used to determine percentage of nitrogen in each sample using the formula;

$$\frac{14 \times T \times 100 \times 10}{1000 \times 0.5g}$$

Where T= titre values of 0.02M H_2SO_4

RESULTS

Fertility of S. singularis and D. theobroma

Isolated adults of both sexes of *S. singularis* survived over a period from 11-15 days and reproduced on only ripe fruits of *D. dewevrei*. Eggs were laid horizontally into the epidermis of the fruits. Not all eggs laid were located on the fruits, thus giving lower figures that the numbers of instar nymphs produced (Table 1). The highest number of eggs recorded was 36 (March-April) whilst the lowest was 15 per female (July-August).

TABLE 1. Number of eggs, first instar nymphs produced, nymphal survival and sex ratio of *S. singularis* on fruit *D. dewevrei*

Age of fruit	Period test was Conducted	Number of eggs located on fruit	Number of first instar nymph	Number of nymphs survived to adulthood	Percentage of nymphs survived to adulthood	Sex ratio of adults (Male: Female
Unripe	February-March (T_1)	-	-	-	-	-
	March-April (T_2)	-	-	-	-	-
	May-June (T_3)	-	-	-	-	-
	July-August	-	-	-	-	-
	September-October (T_5)	-	-	-	-	-
Ripe	February-March (T_1)	30 [6]	133	48	36.1	1:22 {15/33}
	March-April (T_2)	36 [6]	103	*28*	27.2	1:2.5 {18/20}
	May-June (T_3)	35 [5]	66	47	71.2	1.1.6 {18/29}
	July-August	15 [5]	68	61	89.7	1.1.4 {25/36}
	September-October (T_3)	16 [4]	40	34	82.5	1.1.2 {15/19}
Overripe	February-March (T_1)	-	-	-	-	-
	March-April (T_2)	-	-	-	-	-
	May-June (T_3)	-	-	-	-	-
	July-August (T_4)	-	-	-	-	-
	September-October (T_5)	-	-	-	-	-

{ } =Females/Males ( ) = Period of test [ ] = Number of females observe

Incubation period (estimated from the day of locating exposed eggs to the day of appearance of nymphs) was between 14 and 17 days. Paired adults of both sexes of *D. theobroma* did not survive beyond 12 days on any of the stages of *D. dewevrei* fruits. No eggs or first instar nymphs were recorded on the fruits. Again, adults of both sexes did not survive on severed cocoa pods. The number of female *S. singularis* that produced offspring was 26 out of the total of 50 females. The number of first instar nymphs produced by these females ranged from 6 to 25. The highest number of instar nymphs recorded in a period was 133 (February-March) whereas the least was 40 (September-October). Nymphal survival to adulthood ranged from 27.2% (March-April) – 89.7% (July-August). The sex ratio (male: female) on ripe fruits varied from 1:1.5 to 1:2.5 (Table 1).

Mortality in nymphs of *S. singularis* was highest at the first nymph instar and reduced considerably as the nymphs got older. Between February and March, mortality at the first instar was 52.6% but this decreased to 11.1% at the third instar. Again between March and April percentage mortality at the first instar was 53.4 falling to as low as 0.4 at the fifth instar (Table 2). High mortality occurred at the early part of the year in the first instars but decreased at the latter part of the year. In February-March, mortality was 52.6% but this decreased to 7.4% in July-August, suggesting that the rearing period affected the mortality of the insects.

Adult survival and longevity of D. theobroma on fruits of D. dewevrei

Survival of adult of both sexes was generally high in the first week of emergence but decreased considerably by the third week (Table 2). Furthermore the adults survived better on the ripe fruits than on the unripe and overripe fruits (Table 3). Adult longevity of female of *D. theobroma* averaged 5.4 days on unripe fruits 14.1 days on ripe fruits and 7.3 days on overripe fruits (Table 4). The means were significantly different (LSD = 0.05). For the male adult longevity were 9.1 days on unripe fruits, 12.0 days on ripe fruits, 7.2 days on overripe fruits. Again, the means were significantly different (LSD = 0.05) (Table 4).

TABLE 2. Mortality at the nymphal stages of *S. singularis* reared on the ripe fruit of *D. dewevrei*

Test period	Percentage mortality recorded at each instar				
	Instar 1	Instar 2	Instar 3	Instar 4	Instar 5
Feb. -Mar.	52.6 (70) *	14.3 (9)	11.1 (6)	-	-
Mar. -April	53. 4 (55)	11.4 (13)	11.4 (4)	0.06 (2)	0.04 (1)
May - June	12.1 (8)	19.0 (11)	-	-	-
July – Aug.	7.4 (7)	3.2 (2)	-	-	-
Sept. – Oct.	5.0 (6)	3.0 (1)	-	-	-

* Number of nymphs in parentheses

TABLE 3. Percent weekly survival of *D. theobroma* on *D. dewevrei* fruits

	Female			Male		
State of fruit	Week 1	Week 2	Week 3	Week 1	Week 2	Week 3
Unripe	40	0	0	80	12.5	0
Ripe	100	50	20	100	30	0
Overripe	60	0	0	70	0	0

TABLE 4. Mean longevity (days ± s.e) of adult *D. theobroma* on *D. dewevrei fruit* (*n* = 10)

State of fruit	Female	Male
Unripe	5.4 ± 1.0b (2-10)	9.1 ± 0.9b (5-14)
Ripe	14.1 ± 1.2a (8-12)	12.0 ± 0.5a (10-14)
Overripe	7.3 ± 0.8b (4-12)	7.2 ± 0.8b (3-10)

LSD ≤ (0.05). Mean followed by the same letters in a column are not significantly different.
( ) =Range of longevities.

Development of nymphs and adult longevity in S. singularis on ripe fruits of D. dewevrei

Both sexes went through 5 nymphal instars. For the females, the total nymphal period, adult longevity and total life span were 23-26 days (mean: 24.4 days), 5-22 days (mean: 13.8 days) and 30-46 (mean: 38.2) respectively (Table 5). For males, the total nymphal period, adult longevity and total life span were 20-35 days (mean: 22.5 days), 6-15 days (mean: 10 days) and 26-35 days (mean: 32.6 days) respectively (Table 5). The means for both males and females were not significantly different.

TABLE 5. Mean duration (± S.E) of development instars and longevity (days) of male and female *S. singularis* reared on ripe fruits of *D. dewevrei* (*n* = 15)

	Species/Treatment	
Duration of life stage	Female	Male
Nymphal instars		
I	5.0 ± 0.2	4.4 ± 0.1
II	4.3 ± 0.2	4.4 ± 0.2
III	5.3 ± 0.2	5.1 ± 0.2
IV	6.1 ± 0.2	5.1 ± 0.2
V	3.7 ± 0.1	3.6 ± 0.1
Total nymphal period	24.4 ± 0.2	22.5 ± 0.2
Total longevity	13.8 ± 1.1	10.1 ± 0.7
Total life span	38.2 ± 1.1	32.6 ± 0.8

Feeding preference in nymphs of S. singularis and D. theobroma

Nymphs of both *D. theobroma* and *S. singularis* fed on all the stages of *D. dewevrei* fruits. However, the results on both insects indicated preference for the ripe fruit. For instance, *S. singularis*, significantly higher number of feeding lesions on ripe fruit than on the overripe and unripe fruits were recorded for 2nd, 3rd, 4th and 5th instar nymphs ($F_{instar\ 2}$ = 9.8, df = 29, P≤ 0.05, $F_{instar\ 3}$ = 31.4, df = 29, P≤ 0.05; $F_{instar\ 4}$ = 35.3, df = 29, P≤ 0.05; $F_{instar\ 5}$ = 5.8, df = 29, P≤ 0.05) (Table 6). No significant difference was recorded for the first nymph. For *D. theobroma*, feeding lesions made the 4th and 5th instar nymphs were significantly higher on ripe fruits than on unripe and overripe fruits ($F_{instar\ 4}$ = 7.9, df = 29, P≤ 0.05; $F_{instar\ 5}$ = 28.8, df = 29, P≤ 0.05; (Table 6). However, 1st, 2nd and 3rd instars did not discriminate among the three stages of the fruits.

TABLE 6 . Mean (± s.e) of feeding lesions made by nymphal instars of *S. singularis* and *D. theobroma* on fruits of *D. dewevrei* (*n* = 10)

	Species/Treatment					
	S. singularis			*D. theobroma*		
Life stage	unripe	ripe	overripe	unripe	ripe	overripe
Nymphal instars						
I	3.9 ± 0.3	4.6 ± 0.3	3.9 ± 0.3	4.2 ± 0.4	4.8 ± 0.6	4.0 ± 0.4
II	13.2 ± 0.6b	17.1 ± 0.7a	13.4 ± 0.8b	9.9 ± 0.9	13.4 ± 1.3	11.3 ± 1.0
III	11.7 ± 0.9b	20.6 ± 0.8a	18.4 ± 0.8a	15.1 ± 1.0	18.3 ± 1.3a	15.8 ± 1.2
IV	14.5 ± 0.5b	22.2 ± 0.8a	20.4 ± 0.7a	14.5 ± 0.9b	24.2 ± 1.7a	17.3 ± 1.2ba
V	19.4 ± 0.8b	24.1 ± 1.3a	20.4 ± 0.9a	18.3 ± 1.2b	26.8 ± 2.2a	20.1 ± 2.2b

Means followed by the same letters (a or b) in a row for each of the species are not significantly different

Feeding preference in adult S. singularis and D. theobroma

Adult mirids also fed on all the stages of fruit of *D. dewevrei*. The male *S. singularis*, made significantly more feeding lesions on ripe and overripe fruits than on unripe fruits (F = 117.5, df = 29, P≤ 0.05) (Table 7). Similarly, the female also made significantly more feeding lesions on ripe fruits than on unripe and overripe fruits (F = 138.3, df = 29, P≤ 0.05). Similar results were obtained for *D. theobroma*. In general, feeding lesions recorded on unripe, ripe and overripe fruits of *D. dewevrei* were higher in the female than in the male for both *S. singularis* and *D. theobroma*.

TABLE 7. Mean feeding lesions ± s.e made by adult *D. theobroma* and *S. singularis* on fruits of *D. dewevrei* (*n* = 10)

	S. singularis		*D. thebroma*	
Stage of fruit	Male	Female	Male	Female
Unripe	10.3 ± 0.9	16.5 ± 2.0	56.6 ± 4.5b	75.3 ± 5.5c
Ripe	57.8 ± 2.9a	139.5 ± 6.0a	89.4 ± 5.5a	109.2 ± 4.8a
Overripe	53.0 ± 2.9a	120.7 ±7.5b	58.3 ± 5.2b	99.6 ± 5.1b
LSD ≤ (0.05)	7.0	16.4	14.8	15.0

Means followed by the same letters (a or b or c) in a column are not significantly different

Concentration of sugar and nitrogen in fruit juices of D. dewevrei

All the stages of the fruits contained some amount of sugar and nitrogen. Ripe fruits had highest level of sugar content (18%) followed by overripe fruits (10%) and lowest level was in unripe fruits (4%) (Table 9). Furthermore, ripe fruits had the highest level of nitrogen content (0.13%) while the lowest level occurred in unripe fruits (0.01). the presence of nitrogen in the fruits could be used as a measure of the amount of protein (amino acids) in the fruits of *D. dewevrei*

TABLE 9. Concentration of sugar and nitrogen in fruits juices of D. dewevrei

Stage of fruit	Nitrogen	(%) Level Sugar
Unripe	0.01 (0.13gm) *	4.0
Ripe	0.13 (0.12gm)	18.0
Overripe	0.11 (0.11gm)	10.0

* Dry weight of fruit juice in parentheses

DISCUSSION

Host plants used in insect breeding experiments are usually chosen on the basis of their capacity to sustain breeding of the female and its offspring while withstanding the often high humidity which they are subjected to in closed containers. The present study seems to suggest the *Desplatsia dewevrei* has the capacity to sustain breeding of *Sahlbergella singularis* under the laboratory conditions. *S. singularis* successfully survived and reproduced on the ripe fruits. *Distaniella theobroma* on the other hand survived on the fruits but could not reproduce. Feeding, development and survival of mirids are largely influenced by water status of the source of food [11]. *D. theobroma* has been reported to have a rapid development and high percentage survival on potted cocoa seedlings having higher water status than on those with intermediate and low water status[11]. Cut sections of *D. dewevrei* fruits revealed that ripe and unripe fruits were fresh and juicy whereas overripe ones were fibrous and dry. Furthermore, chemical analysis showed that *D. dewevrei* contains sugar and nitrogen in variable quantities in all the stages of the fruits. Ripe fruits had the highest sugar content (18%) while unripe fruits had the least (4%). The nitrogen content varied from 0.13% in ripe fruits to 0.01% in unripe fruits. The nitrogen content suggests the presence of amino acids (protein). Thus the ripe fruits contain sufficient nutrients to better support the feeding, development, survival and reproduction of *S. singularis* than unripe and overripe fruits in the laboratory. The nutrient content of the unripe fruits is probably unsuitable for mirid growth. This assertion probably explains why both *D. theobroma* and *S. singularis* failed to establish on unripe fruit *D. dewevrei* [8]. Both mirid species did poorly on cocoa pods because the pods wilt more rapidly. Nutrient availability and composition is an important factor in the biology of insects for instance, sugar has been found to greatly increase fertility and longevity in *Pieris rapae* (L)[12], *Pieris brassicae* (L)[13] and in *Euploea core corina* [14]. Again cane sugar solution increases oogenesis, fat body size and reproductive performance in *Colias philodice Boisduval* (Lepidoptera: Pieridae)[15], while the presence of sugar in the diet of adult *Euphydryas editha* (Doubleday) increased longevity, body weight and egg production[16].

There are conflicting reports on importance of amino acids in insects. Johnson[17] concluded that adult Lepidoptera had lost the need for nitrogenous food and that their nitrogen requirements are met by stored food in their fat body. Again, no marked effect was observed on fecundity and longevity of *Euphoea core corina* (Lepidoptera: Danaidae) when fed on adult diet containing amino acids [14] and on egg weight of *Euphydryas editha* (Lepidoptera: Nymphaidae) [18], when fed on adult diet containing amino acids. In contrast [19] reported that females of *Heliconius charitonius* (L) laid eggs for several weeks on pollen diet containing natural amino acid in adult diet of *Euphydryas editha* increased egg weight[16]. Thus is appears, importance of amino acids vary with different insects. The observed five nymphal stages and adult stage recorded in the present study for *S. singularis* agrees with Cotterel's observation on young cocoa trees. The total nymphal period of 23-26 days for females and 20-25 days for males recorded compare favourably with the 23-24 days reported by Cotterell. This implies that fruits of *D. dewevrei* are as good as young cocoa trees for laboratory rearing of *S. singularis*.

Data on the adult longevity in cocoa mirids are generally unreliable because of the difficulty in maintaining mirids in captivity. Adults of both *D. theobroma* and *S. singularis* live for kless than one month and longevity of 16.6 days on cut shoots of *C. pentandra* has been reported[20]. The present study recorded mean longevities of 13.8 and 10.1 days for female and male *S. singularis*, respectively. However[8] reported relatively longer longevities averaging 26.5 days for female and 15.0 days for adult male of *S. singularis* on ripe fruits of *D. dewevrei* under ambient temperature of 20.3-34.0^{o}C and humidity of 87.3% in the insectary. The relatively longer longevities recorded on *D. dewevrei* may have been enhanced by the better nutrient content of the fruits.

CONCLUSION

The results demonstrate that the availability of nutrients such as sugar and nitrogen in fruits of *D. dewevrei* probably played an important role in the nutrition and growth of both species of mirids and more importantly in reproduction of *S. singularis*. The biological characteristics observed in this study on two mirid species of *D. dewevrei* have shown that the ripe fruits are suitable food source for the breeding of cocoa mirid *S. singularis* in the laboratory. Also, the unripe, ripe and overripe fruits could be used to feed the nymphs and adults of both species.

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