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Laboratory Biology and Field Behaviour of *Epilachna punctipennis* Muls. (Coleoptera: Coccinellidae) on the bitterleaf, *Vernonia amygdalina* Del. in Southwest Nigeria

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ABSTRACT

The foraging behaviour and life cycle parameters of *Epilachna punctipennis* on the bitterleaf, *Vernonia amygdalina* were investigated in the field and in the laboratory at ambient temperature and humidity. *E. punctipennis* appeared to be a diurnal folivore and the adults were most active in cool and bright daylight condition. Adults did not fly often and mating took place on both adaxial and abaxial surfaces of leaves of *V. amygdalina*. Copulation occurred more than once in the adult. Eggs were deposited by the females in about 10 clusters of 25 eggs and glued firmly on to the adaxial surface of very young leaves. Actual fecundity per female averaged 218 eggs per lifetime. Unmated females laid very few eggs and these did not hatch. The first and second instar larvae fed on young leaves but migrated later to older leaves at the third instar. Adults and the immature stages were commonly distributed spatially on each branch at the uppermost 5-10 leaves from the apical bud. All the feeding stages were phytophagous. The life cycle consisted of an egg, four larval instars, a pupa and the adult. All larval stages lasted an average of five days except the fourth instar, which lasted only four days. The mean growth ratio was 1.46. The potential of the beetle to cross on to other feeding sites and cause damage to the host plant increased with age of the insect.

INTRODUCTION

The bitterleaf, *Vernonia amygdalina* Del. (Asteraceae) is a perennial shrub that is abundant in the humid and dry savanna ecosystem of Nigeria (Franklin and Ruberte, 1975). It is commonly grown as vegetable in homestead gardens in villages (Hills and Waller, 1988) and may also be harvested in the wild. This shrub is significantly important for its nutritional and medicinal values.

Relatively very little efforts have been expended to enhance production and protection of *V. amygdalina*. As a vegetable, the green leaves and the young stems are rich in essential vitamins that are made readily available to the consumer with medium cooking (Oyenuga and Fetuga, 1985). Other parts of the plant such as the roots and older stem are utilized as medicinal concoctions in treatment of various ailments. However, a complex of insect pests attacks this shrub like many other plants and based on field observations, the phytophagous ladybird beetle, *E. punctipennis* Mulsant; (Coccinelidae: Coleoptera) is a major vegetative pest attacking the much valued leaves and young stems of this important rainforest resource (Anikwe, 1998; Omoloye *et al*, 2000; Omoloye *et al*, 2002).

The biology and ecology of many other species of *Epilachna* have been studied particularly outside Nigeria but we are not aware of any bio-ecological study on this insect folivore of the bitter leaf. This knowledge is however basic for any program of control to be meaningful and effective. This study therefore investigated aspects of the field foraging and oviposition behaviour and laboratory biology of *E. punctipennis* on *V. amygdalina* in order to provide some background information necessary towards formulation of an appropriate control strategy for the pest.

MATERIALS AND METHODS

2.1 Study site

Field observations and laboratory experiments were conducted at the Crop Garden and Entomology Research Laboratory respectively of the Department of Crop Protection and Environmental Biology, Faculty of Agriculture and Forestry, University of Ibadan, Nigeria. Ibadan is located in the moist woodland savanna ecosystem with mean solar radiation of 18 mj/m^2/day. The annual rainfall averaged 2000mm with a bimodal pattern. It lies between the latitude 7° 30' N and longitude 3° 54'E at an altitude of 200m above sea level. The field study was conducted on a 75x105m plot containing 38 wild stands of *V. amygdalina* that had been left to fallow for four years.

2.2 Field studies

Field observations on the foraging activities and ovipositional behaviour of *E. punctipennis* on *V. amygdalina* were carried out over a 12-month period between May 1997 and April 1998. Observations were carried out twice (day 10 and day 20) each month between 7.00 and 18.00hrs local time. Data on the number of immature and adult stages of *E. punctipennis* per leaf, the spatial distribution of all developmental and adult stages on the plant as well as the number of eggs deposited per leaf were taken and described.

2.3 Life cycle studies

Laboratory culture of *E. punctipennis* was established from field collected eggs laid on young leaves of *V. amygdalina* and reared in plastic cages (6.7x8.8x6.4 cm) on Whatman No. 2 filter paper pretreated with fungistatic M-tegosept solution as described by Omoloye *et al.* (2000). Thereafter 40 pairs of newly emerged adults were confined separately for mating and oviposition on leaves of *V. amygdalina* in plastic cages and observed for pre-mating, mating and post-mating behaviour. The leaves were held in water in a glass-vial fixed firmly to a side of each cage at ambient temperature (27 $\pm 2^0$) and 70-88% relative humidity. The leaves were replaced daily until oviposition commenced. Ten pairs of ovipositing adults were set aside for a daily count of the number of eggs deposited (actual fecundity) per female. Afterwards, 100 day-old eggs were taken at random from 10 pairs of ovipositing adults and reared till adult. A cohort of 20 pairs of emergent was taken from this and confined in plastic cages in five replicates till death to assess longevity of mated adults while 20 newly emerged adults of each sex were separately confined in like manner to assess longevity of unmated adults. All the eggs laid by the remaining 20 pairs of emergent were collected and incubated for 5 days in 15cm diameter Petri dishes to hatch and reared till adult. From this set, 20 eggs and thereafter 20 larvae were taken daily, killed in ethyl acetate fumes and measured daily through the developmental stages using an Olympus SH-ZT (x60) Stereo binocular microscope for life cycle parameters such as the body length, abdominal width and the head capsule. These data were subjected to appropriate one-way analysis of variance and mean separation using the Tukey test (Gomez and Gomez, 1984). The growth ratio was determined for each larval instar by dividing the mean width of one instar with the one preceding it (Ewete, 1990; Emehute and Odiete, 1991).

RESULTS

3.1 Field behaviour

E. punctipennis appeared to be a diurnal folivore and was easily observed between 8.00 and 12.00h and later between 16.30 and 18.00 h feeding on leaves and young stems of *V. amygdalina*. The adults were very active in cool and bright daylight condition. However, they hid under the abaxial surfaces of older leaves as the day advanced between 12.00 and 16.30h. Adults did not fly often and mating took place on both adaxial and abaxial surfaces of leaves. Each ovipositing female selected very young leaves for oviposition. The first and second instar larvae fed on young leaves but migrated later to older leaves at the third instars. The quiescent pupa was commonly seen glued firmly either to the base of the twig on which it has fed or on the abaxial surface of mature leaf. Adults and other mobile developmental stages were found on virtually all leaves of infested plant. However they were more spatially distributed on the upper crown of each branch particularly at the uppermost 5-10

leaves from the apical bud. All the feeding stages were phytophagous. They bit and chewed off the chlorophilous portion of the leaves in somewhat straight or oblique lines leaving behind brown patches of transparently scraped network of veins on the leaf surface.

3.2 Pre-oviposition and oviposition behaviour of *E. punctipennis* **on** *V. amygdalina*

The summary of the ovipositional and life history parameters of *E. punctipennis* on *V. amygdalina* is presented in table 1. Mating took place 2-3days after adult emergence. Copulation occurred more than once. The male followed the female for three to eight minutes till copulation was achieved. Each successful mating lasted about three minutes. Before oviposition in the laboratory, each gravid female migrated to the apex of each branch within the plastic cage and selected a very young leaf. Actual fecundity per female averaged 218 eggs and these were deposited in about 10 clusters of 24.7 ± 0.7 eggs and glued firmly on to the adaxial surface of the leaf. Unmated females laid very few eggs (42 eggs) and these did not hatch (Table 1).

Table 1: Oviposition, longevity and fecundity parameters of *Epilachna punctipennis* reared on *Vernonia amygdalina*.

Adult Character	Mean ± S.E.		
	Sample size (n)	Duration (days)	Number/female
Pre-oviposition period	40	2.4 ± 0.74	-
Oviposition period	40	5.7 ± 1.07	-
Actual Fecundity	10	-	217.5 ± 5.20
Duration of mating	10	2.8 ± 0.62	-
Longevity of mated female	10	8.2 ± 1.78	-
Longevity of unmated female	10	14.3 ± 2.16	-
Number egg cluster deposited/mated female	10	-	9.5 ± 0.60
Number egg cluster deposited/unmated female	10	-	0.1± 0.62
Number of eggs/ cluster	10	-	24.7 ± 0.71

3.3 Developmental life history

Development of *E. punctipennis* on *V. amygdalina* was completed in 30 ± 2.7 days in the laboratory. The life cycle (Fig.1) consisted of an egg, four larval instars, a pupa and an adult. Freshly laid egg had an oval, smooth and milk-white resilient surface glued at the broader end to the leaf surface. This turned pale gradually before eclosion on the fifth day. A newly emerged first instar was white and elliptical in shape. The colour turned creamy with age. It had three pairs of walking legs with 10 abdominal segments. Each thoracic and abdominal segment had a set of spines that were arranged in six rows on both lateral and dorsal part of the body. The first instar was 1.7-1.9 mm long with a mean vertex of 0.3 mm. Whereas the second instar larva is 3.03 mm long with a mean vertex of 0.45 mm, the third instar measured 4.7 mm in length with a mean vertex of 0.7 mm while the fourth instar is 5.7 mm long also with a mean vertex of 5.7 mm (Table 2). All larval stages lasted an average of five days except the fourth instar, which lasted only four days. Before pupation, the last instar larva carefully selected a convenient spot on the branch on which it has been feeding or withdrew to the side of the plastic cage as recorded in the laboratory. The pupa was ovoid in shape and quiescent with a mean body length of 4.6 mm. The pupa gradually turned from light yellow at pupation to dark brown with age. It migrated to a side within the plastic cage in which it was cultured. The pupal duration lasted six days. The male appeared similar to the female in size, body markings and color. Each elytron has a characteristic 9 dotted marks on a dull milky yellow background. Both male and female were 4.6mm long. The mean growth ratio was 1.40 (Table 2).

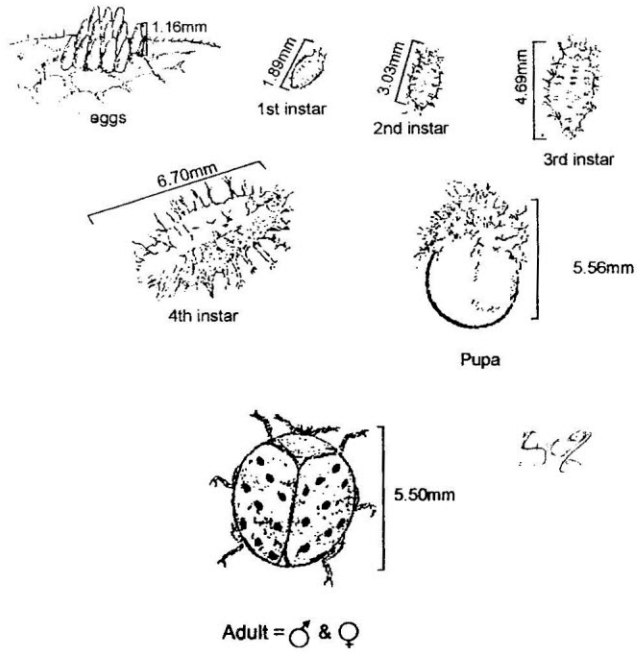

Fig. 1: Life cycle of *Epilachna punctipennis*

Table 2: Body morphometrics, width across head capsule (mm ±SE), growth ratio and duration of developmental stages of *Epilachna punctipennis* on *Vernonia amygdalina*

Growth stage	Sample size (n)	Length (mm)	Width (mm)	Width of vertex (mm)	Growth Ratio	Duration of Stages (days)
Egg	20	1.16 ± 0.14	0.39 ± 0.01			5
1st instar larva	20	1.89 ± 0.18	0.57 ± 0.07	0.31± 0.02d		5
2nd instar larva	20	3.03 ± 0.17	0.91 ± 0.05	0.45± 0.01c	1.43	5
3rd instar larva	20	4.69 ± 0.14	1.23 ± 0.07	0.65± 0.02b	1.44	4
4th instar larva	20	6.70 ± 0.14	2.09 ± 0.10	0.87± 0.01a	1.34	5
Pupa	20	5.56 ± 0.04	4.66 ± 0.14			8
Adult	20	5.50 ± 0.02	4.21 ± 0.10			
Mean Growth Ratio					1.40	

Means followed by the same letter in the same column are not significantly different (p< 0.05) by Turkey's Honestly Significant Difference (HSD)

DISCUSSION

E. punctipennis was most active within the early hours of the day but generally hid under the leaves as the day became brighter. This could be a survival mechanism to avoid both the unfavorable climatic conditions caused by increase in ambient temperature as the day advanced or an escape mechanism from the preying activities of natural enemies that had been identified to be associated with insect pests of *V. amygdalina* in Southwest Nigeria (Akinlosotu, 1977; Hills, 1983).

The foraging activities of the larval stages unleashed a great damage on the plant particularly when the different instars crossed from one leaf to other feeding sites. The potential of the beetle to cross on to other feeding sites and cause damage to the host plant increased with age, the adult being the most actively mobile and destructive (Aide, 1991; Omoloye *et al.*, 2000).

Multiple mating by the female as found in this study was necessary in order to sustain oviposition. This result agreed with Emehute and Odiete (1991) who found that *Conchyloctenia nigrisparsa* Boh. required multiple mating to sustain oviposition. Unmated female *E. punctipennis* lived longer than mated female. The early death of

mated females could be due to expended energy during reproductive activities. Omoloye *et al.* (1998) reported similar observation in the African rice gall midge, *Orseolia oryzivora* H.&G. in which the unmated female had longer life span than the mated female.

The head capsule measurements increased by a factor of 1.4. This agreed with Dyar's law (Wigglesworth, 1965; Ewete, 1990; Emehute and Odiete, 1991). It was therefore relatively easy to separate the larval instars accurately by the head capsule measurements. Also the conspicuous exuviae left after each moult further confirmed the number of instars. The fact that the larval stages and the adult especially the ovipositing female carried out their foraging activities usually on the adaxial surface of leaves increased the prospect of a successful management of this pest with most of the available control options. Yet the presence of natural enemies indicated strict caution in the use of insecticides against this pest.

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