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## BEHAVIOR OF FIRST INSTAR NYMPHS OF *EDESSA MEDITABUNDA* (F.) (HEMIPTERA: PENTATOMIDAE) ON THE EGG MASS

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The brown-winged stink bug, Edessa meditabunda (F.) (Heteroptera: Pentatomidae) is a Neotropical pentatomid associated mostly with several species of cultivated and non-cultivated Fabaceae, Solanaceae, and Asteraceae (Silva et al. 1968; Rizzo 1971; Lopes et al. 1974; Krinski et al. 2012). It has been reported as a pest of several crops such as soybean, sunflower, and potato (Rizzo 1971; Galileo & Heinrichs 1979; Panizzi & Machado-Neto 1992). Description of the egg mass, nymphs, and adult (Rizzo 1971), effect of temperature on development and reproduction (Gonçalves et al. 2008), and feeding behavior and damage caused to soybean (Galileo & Heinrichs 1979, Panizzi & Machado-Neto 1992, Silva et al. 2012) have been investigated.

As nymphs of *E. meditabunda* emerge, they stay on the top of the egg shells (chorions), as most pentatomid nymphs do, and later move off the eggs, but remain nearby, facing the empty eggs (Sánchez et al. 1999). The early association of the first instar nymphs with the empty eggs is believed to help in possible symbiont acquisition (e.g., Abe et al. 1995). Female stink bugs pass bacteria to their progeny by smearing cells on the egg mass during oviposition (Buchner 1965).

To our knowledge, the behavior of sitting on the top of the eggs and positioning themselves adjacent to the eggs has not been investigated in detail. Therefore, the objective of this study was to observe and describe the sequence of behavior presented by *E. meditabunda* nymphs from the time of their emergence from the eggs, including the time spent and the number of eggs/chorions visited, and the total time required to finally aggregate in the typical position adjacent to the egg shells.

From Feb to Apr 2010 a colony of *E. meditabunda* was established in the laboratory at the National Soybean Research Center of Embrapa in Londrina, Paraná. Adults were collected from soybean and sunflower fields cultivated at the Embrapa field experiment station (S  $23^{\circ}$  11' W  $51^{\circ}$  10'). Specimens were taken to the laboratory and placed in plastic rearing boxes ( $25 \times 20 \times 20$ 

cm) in an environmental room kept at  $26 \pm 1$  °C temperature,  $70 \pm 10\%$  RH and 14:10 h L:D. They were fed green bean pods of *Phaseolus vulgaris* L.; peanuts, *Arachis hypogaea* L.; soybean, *Glycine max* (L.) Merrill; and sunflower, *Helianthus annus* L., seeds and mature fruits (berries) of privet, *Ligustrum lucidum* Aiton (Lamiales: Oleaceae). Paper towel was used to receive the egg masses. Every other day, egg masses were collected, and placed singly in Petri dishes ( $6.5 \times 2.0$  cm) lined with filter paper. A small plastic lid with wet cotton was placed in each dish to provide humidity to prevent egg desiccation.

The emergence of nymphs and their movement on the top of the eggs/chorions, and their final position adjacent to the chorions were examined using MiView USB microscopy digital (200X, 8pcs led) and recorded using a 1.3 mega pixel camera (35x). The video editing was done using Microsoft® Windows® Movie Maker Version 5.1 program. Graphical material is shown online in color in a supplementary document at http://purl.fcla. edu/fcla/entomologist/browse.

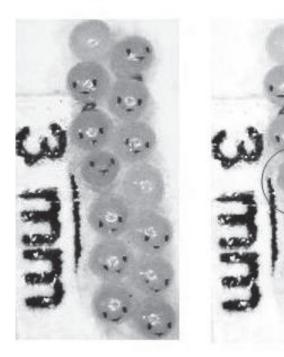
Five egg masses were used, each containing 14 eggs (total of 70 eggs), and 39 nymphs were individually recorded. Egg masses were observed in the laboratory at room temperature ( $26 \pm 2$  °C). Nymphs that emerged while others were still in the eggs moved onto the top of the egg shells and/ or onto the top of the unhatched eggs. A chronometer was used to measure the time each nymph spent on the top of each egg/chorion visited, and the time needed from emergence to final position adjacent to the chorions, facing them (Fig. 1a-d). The number of eggs/chorions visited by each nymph was recorded.

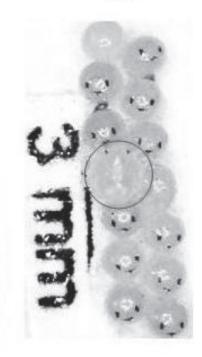
Results indicated that as the emergence of the nymphs is completed, they remain on the top of the egg/chorion for about 3 min (179.3  $\pm$  43.1 s; n = 39 nymphs recorded). This time is thought to be used for possible acquisition of symbionts from the upper surfaces of the eggs, and ingestion of liquids that remain inside the egg shells. Symbionts are known to be present on the egg surface of many species of pentatomids (references in Hirose



(b)

(c)





(d)



Fig. 1. Egg mass of *Edessa meditabunda* showing the nymphs inside the chorions (a); nymph emerging from the corion [circled in red (b)]; nymph on the top of the corion after complete emergence [circled in red(c)]; and nymphs positioned around the chorions and facing them (d). This figure is shown in color in the supplementary document in Florida Entomologist 97(1) (2014) online at http://purl.fcla.edu/fcla/entomologist/browse.

et al. 2012), and their effects on the bugs' biology is variable, and depends on several factors such as temperature (Prado et al. 2009, 2010). Another species of Heteroptera, *Megacopta cribaria* (F.) (Plataspidae), which lays its egg masses in a similar pattern (i.e., in 2 parallel rows) is known to de-

posit symbiont capsules under the eggs (Jenkins et al. 2010; see also Fukatsu & Hosokawa 2002).

The mean number of eggs/chorions visited by each nymph was about 8 (7.8  $\pm$  1.46 eggs/chorions; n = 5 nymphs that emerged first from each of the 5 egg masses observed) before moving out of the shells and positioning adjacent to them. The movement of nymphs on the top of the eggs/ chorions and stopping on several for a period of time reinforces the hypothesis of symbiont acquisition. Hirose et al. (2012) discovered a dramatic difference in the egg surfaces of several species of pentatomids by comparing chemically sterilized eggs with non-sterilized ones. The main difference was that non-sterilized eggs showed an array of microorganisms on their surface, strongly suggesting the presence of symbiotic microorganisms which may play a role in the bugs' biology.

The total time from nymphal emergence to the final position adjacent to the egg mass (chorions) was ca. 25 min (1,510.8 ± 133.9 seconds: n = 39 nymphs). We based our observations on early emerged nymphs that can move more freely, nymphs that emerged later may not be able to move about as easily due to crowding, which might increase this total time. All nymphs eventually positioned themselves adjacent to the chorions, facing them. This behavior is also shown by other species of pentatomids, e.g., the brown marmorated stink bug, Halyomorpha halys (Stål) (Northeastern IPM Center 2013), and is speculated to be related to defense, i.e., the nymphal display may be mimetic with bird droppings or with poisonous caterpillars. Aggregations of first instars might provide protection against desiccation and against predators (Lockwood & Story 1986; Hirose et al. 2006). However, to this date this behavior has not been fully explained.

In conclusion, first instar nymphs of *E. meditabunda*, immediately following emergence from the eggs, visit several eggs/chorions and spend a relatively large amount of time on top of each one before moving off to the side and settling into their final position adjacent the chorions.

### SUMMARY

Laboratory studies were conducted on the behavior and time budget of first instar nymphs of the brown-winged stink bug [*Edessa meditabun*da (F.) (Heteroptera: Pentatomidae)] from egg emergence to their final position adjacent to the egg shells (chorions). Results indicated that right after emergence the nymph stays on the top of each chorion for about 3 min; the mean number of chorions or eggs visited by each nymph was 7.8 before moving off the shells or eggs and positioning itself adjacent to them. The total time required for from emergence of each nymph to its final position adjacent to the chorions was ca. 25 min. All nymphs relocated from on top of the egg mass to a position in which they faced it. This behavior of moving on to the chorions or eggs and positioning adjacent to them is speculated to be related to possible acquisition of symbionts from the surfaces of the chorions and to the defense of the egg mass.

Key Words: Insecta, Pentatomidae, brownwinged stink bug, egg mass, nymph behavior symbiont acquisition, defense

### Resumo

Estudos laboratoriais foram conduzidos sobre o comportamento e a alocação do tempo de ninfas de primeiro instar do percevejo asa marrom, Edessa meditabunda (F.) (Heteroptera: Pentatomidae) da emergência até o posicionamento final ao redor das cascas dos ovos (córions). Os resultados indicaram que logo após a emergência as ninfas ficaram sobre cada córion ou ovo por cerca de 3 minutos; o número médio de córions/ovos visitados por cada ninfa foi de 7,8 unidades, antes de se deslocarem e se posicionarem ao redor dos mesmos. O tempo total da emergência até o posicionamento final ao redor dos córions foi cerca de 25 minutos. Todas as ninfas se posicionaram ao redor e voltadas para os córions. Esses comportamentos de se movimentarem sobre os córions ou ovos e estacionarem ao redor dos mesmos podem estar relacionados com a aquisição de simbiontes e com defesa.

Palavras Chave: Insecta, Pentatomidae, percevejo, massa de ovos, comportamento ninfal, adquisición simbiontes, defesa

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