ECOLOGY, BEHAVIOR AND BIONOMICS
Survivorship and Egg Production of Phytophagous Pentatomids in Laboratory Rearing

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Keywords
Pentatomidae, insect colony, natural food, reproductive performance

Abstract
Survivorship and reproductive performance of the pentatomids *Euschistus heros* (F.) (EH), *Nezara viridula* (L.) (NV), and *Dichelops melacanthus* (Dallas) (DM) were tested in the laboratory. A mixture of natural foods (pods of green beans, *Phaseolus vulgaris*, raw shelled peanuts, *Arachis hypogaea*, and fruits of privet, *Ligustrum lucidum*, and 50 pairs/box (25 x 20 x 20 cm) were used, observed for 30 days, and replicated three times. Thirty days after emergence, mean female survivorship was 91% (EH), 60% (NV), and 30% (DM). More egg masses were deposited during 11-20 days after emergence, with mean number of 45.1 (EH), 5.3 (NV), and 11.8 (DM). These values were smaller during the first 10 days (25.5, 2.1, and 4.7) and last 10 days (21-30 days) (39.4, 3.9, and 4.9), respectively. Mean maximum number of eggs/day was 489 (EH) on day 29, 474 (NV) on day 11, and 153 (DM) on day 14. Mean monthly fecundity (egg masses/box) was 985 (EH), 92 (NV), and 193 (DM), and mean number of eggs/box was 8,490; 5,147, and 2,042.7, respectively.

Introduction
Phytophagous pentatomids are important pests causing economic losses on an array of crops and fruit trees all over the world (Panizzi et al 2000). In the Neotropical region, several species are key pests, including the Neotropical brown stink bug, *Euschistus heros* (F.), and the southern green stink bug, *Nezara viridula* (L.), on soybean (Panizzi & Slansky 1985), and *Dichelops melacanthus* (Dallas) on corn (Ávila & Panizzi 1995, Gomez 1998) and wheat (Chocorosqui & Panizzi 2004, Manfredi-Coimbra et al 2005).

Due to the above mentioned importance of these pentatomids, there has been a dramatic increase in the need of these bugs to perform field and laboratory trials to select potential insecticides and new varieties. Furthermore, biological control of these bugs using egg parasitoids is an important issue that demands laboratory colonies (Peres & Corrêa-Ferreira 2004, Silva et al 2008).

*Nezara viridula* is known to be reared in the laboratory using raw shelled peanuts and green beans in several laboratories worldwide (references in Panizzi et al 2000). However, Brazilian populations of this insect cannot be continuously maintained on this diet in laboratory conditions (A.R. Panizzi, personal observation). The relative success in rearing *N. viridula* is achieved using a combination of whole soybean plants, dry soybean seeds and raw shelled peanuts (Corrêa-Ferreira 1985). *Euschistus heros* is more easily reared in the laboratory using green bean pods, dry soybean, peanuts and sunflower seeds (Silva et al 2008), while *D. melacanthus* can be maintained on dry soybean seeds and corn seedlings (R. Bianco, personal communication).

This study was conducted to provide information on the survivorship and reproductive performance (i.e., fecundity) of the three before mentioned pentatomids using a mixture of natural foods, with a selected number of couples/container for a fixed period of time, in order to establish a

Neotrop Entomol 40(1): 35-38 © 2011 Sociedade Entomológica do Brasil
successful routine for rearing these insects under laboratory conditions to support egg parasitoid production for the implementation of biological control programs.

Material and Methods

Insect colony

Adults of *E. heros*, *N. viridula*, and *D. melacanthus* were collected at the Embrapa (Empresa Brasileira de Pesquisa Agropecuária) Farm in Londrina Co., northern Paraná State, Brazil (latitude 23º 18' S), from soybean fields. They were taken to the laboratory and individualized couples (n = 50) were placed in clear plastic boxes (25 x 20 x 20 cm), and provided with pods of green beans, raw shelled peanuts, and fruits (berries) of privet, *Ligustrum lucidum* (Oleaceae). Food was replaced every other day. Every two days the egg masses were collected and placed in plastic boxes (11 x 11 x 3 cm), containing moistened filter paper to prevent desiccation. The nymphs were provided with the same food as those used for adults. When nymphs started molting to the fifth instar, they were transferred to plastic boxes (25 x 20 x 20 cm) to complete their development. Boxes were kept in an environmental chamber at 26 ± 1°C temperature, 70 ± 10% RH and with a photoperiod of 14:10 h (L:D).

Survivorship and reproduction

Fifty newly emerged couples of *E. heros*, *N. viridula*, and *D. melacanthus* were selected from the established laboratory colony and placed in plastic boxes (25 x 20 x 20 cm) (three boxes for each species), provided with the same food and kept at the same environmental condition as the insect colony. A ball (2 cm diameter) of commercial absorbent cotton was used as ovipositional substrate for *E. heros* and *D. melacanthus* (Silva & Panizzi 2007), and a piece of paper towel was provided for *N. viridula* (Shearer & Jones 1996). Boxes were daily observed for 30 days and dead females and males were recorded, as well as the number of egg masses and eggs. The mean survivorship (%) of adults and the mean number of egg masses and eggs were calculated on a daily basis up to 30 days of adulthood.

Statistics

Data on the total mean number of egg masses and eggs were calculated and submitted to the analysis of variance (ANOVA) and compared using the Tukey test (P ≤ 0.05). These analyses were performed using the statistic program SAS 8.2 (SAS Institute 1981, Zar 1984).

Results and Discussion

Survivorship

Comparing the survivorship of females, a significant difference was observed between the three pentatomid species. The percentage of survivorship of *E. heros* females was 91% 30 days after emergence, followed by *N. viridula* (60%) and *D. melacanthus* (30%) (Fig 1). Chocorosqui & Panizzi (2008) reported similar survivorship (about 29%) of *D. melacanthus* fed on mature soybean seed.

The mean monthly percentage of female survivorship per box was significantly higher for *E. heros* (95.7%), than for *N. viridula* (78.3%) or *D. melacanthus* (68.3%) (Table 1).

![Graph](image-url)

**Fig 1** Female survivorship (%) and mean number of eggs per day up to 30 days after emergence of three pentatomid species under laboratory conditions (26 ± 1°C, 70 ± 10% RH, photophase of 14h).
Silva et al (2008) observed lower survivorship of *E. heros* females (about 60%), in similar conditions, and suggested that mortality occurred independently of age.

**Reproductive performance**

*Euschistus heros* produced more egg masses and eggs than *N. viridula* and *D. melacanthus* in the laboratory conditions used in a 30-day period. During the first 10 days, *E. heros*, *N. viridula*, and *D. melacanthus* laid a mean number of egg masses of 25.5, 2.1, and 4.7, respectively. The three pentatomid species deposited more egg masses during the second 10-day period after emergence (11-20 days), with 45.1 egg masses for *E. heros*, 5.3 for *N. viridula*, and 11.8 for *D. melacanthus*. These values decreased in the third period (21-30 days) to 39.4, 3.9, and 4.9 egg masses, respectively (Table 2). A similar trend of egg production was observed by Silva et al (2008) when *E. heros* was rearne with different densities of pairs per box.

The highest number of eggs laid/day was 489 eggs for *E. heros* on the 29th day, 474 eggs for *N. viridula* on the 11th day, and 153 eggs for *D. melacanthus* on the 14th day (Fig 1). These results demonstrate that *E. heros* can keep their egg production high over a period of 30 days, as also observed by Peres & Corrêa-Ferreira (2001) and Silva et al (2008). On the other hand, *N. viridula* and *D. melacanthus* decreased their fecundity during the last 10-day period of observation and couples should be replaced by younger insects in order to maintain a desirable egg production.

Comparing monthly fecundity, *E. heros* deposited 984.7 egg masses per box, followed by *D. melacanthus* (193.3) and *N. viridula* (92). The mean number of eggs monthly produced per box was 8,480; 5,147, and 2,043 for *E. heros*, *N. viridula*, and *D. melacanthus*, respectively (Table 1). In similar conditions, Silva et al (2008) reported a lower egg production by *E. heros*, but females were not fed fruits of privet, which is an important host of several pentatomids species (Panizzi & Grazia 2001). Nymphs develop well and adults show higher fecundity on privet than on other host plants commonly used to rear pentatomids (Panizzi & Mourão 1998, Panizzi & Mourão 1999, Coombs 2004, Fortes et al 2006).

*Euschistus heros* females present higher values of survivorship and egg production than *N. viridula* and *D. melacanthus*. In soybean fields all over Brazil, *E. heros* is, in

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**Table 1** Monthly mean (± SE) number of female surviving, egg mass size, total number of eggs, and number of eggs laid/female/box by three pentatomid species under laboratory conditions (26 ± 1°C, RH 70 ± 10%, photophase of 14h).

<table>
<thead>
<tr>
<th>Species</th>
<th>Female survivorship (%)</th>
<th>Monthly (mean ± SE)/box</th>
<th>Eggs</th>
<th>Eggs/female&lt;sup&gt;ts&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. heros</em></td>
<td>95.7 ± 0.58 a</td>
<td>984.7 ± 8.05 a</td>
<td>8,480.0 ± 67.20 a</td>
<td>187.1 ± 0.49</td>
</tr>
<tr>
<td><em>N. viridula</em></td>
<td>78.3 ± 2.33 b</td>
<td>92.0 ± 1.01 b</td>
<td>5,147.0 ± 67.44 b</td>
<td>166.0 ± 0.60</td>
</tr>
<tr>
<td><em>D. melacanthus</em></td>
<td>68.3 ± 4.15 c</td>
<td>193.3 ± 2.19 b</td>
<td>2,042.7 ± 25.00 c</td>
<td>136.2 ± 0.21</td>
</tr>
</tbody>
</table>

Means (n = 3) followed by the same letter in each column do not differ significantly using the Tukey test (P > 0.05); “ns” non significant.

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**Table 2** Mean (± SE) number of egg masses and eggs laid by pentatomid species in laboratory rearing, up to 30 days after emergence (26 ± 1°C, 70 ± 10% RH, photophase of 14h).

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Egg masses (mean ± SE)</th>
<th>Eggs (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>E. heros</em></td>
<td><em>N. viridula</em></td>
</tr>
<tr>
<td>0-10</td>
<td>25.5 ± 7.02 b</td>
<td>2.1 ± 0.80 b</td>
</tr>
<tr>
<td></td>
<td>[50]</td>
<td>[46]</td>
</tr>
<tr>
<td>11-20</td>
<td>45.1 ± 2.43 a</td>
<td>5.3 ± 0.49 a</td>
</tr>
<tr>
<td></td>
<td>[48]</td>
<td>[38]</td>
</tr>
<tr>
<td>21-30</td>
<td>39.4 ± 2.98 ab</td>
<td>3.9 ± 0.33 ab</td>
</tr>
<tr>
<td></td>
<td>[46]</td>
<td>[32]</td>
</tr>
</tbody>
</table>

Means (n = 3) followed by the same letter in each column do not differ significantly using the Tukey test (P > 0.05); “ns” non significant. Number of females in brackets.
In general, the most abundant stink bug, which suggests that it is better adapted to different environmental conditions. Apparently, it is more resistant to abiotic (temperature, humidity, sun radiation, rain impact, etc.) and biotic factors (lack of preferred food plants, natural enemies, starvation and handling, etc.) than the two other species studied. The fact that *N. viridula* performed relatively poorly (and this has been observed in several laboratories in Brazil) needs to be further investigated, since this species is reared with great success using similar foods in other parts of the world.

In conclusion, our study demonstrated that the Neotropical brown stink bug, *E. heros* is better fitted to be raised in the laboratory than the former two species as egg provider for parasitoid rearing.

**Acknowledgments**

We thank D R Sosa-Gómez for critically reading the manuscript. This research was supported by a scholarship to GSC from the National Council for Scientific and Technological Development (Conselho Nacional de Desenvolvimento Científico e Tecnológico). This paper was approved for publication by the Editorial Board of Embrapa Soja as manuscript number 16/2009.

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