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Authors: Silva, Flávia A. C., and Panizzi, Antônio R.

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## OVIPOSITION OF THE NEOTROPICAL BROWN STINK BUG *EUSCHISTUS HEROS* (HETEROPTERA: PENTATOMIDAE) ON ARTIFICIAL AND ON NATURAL SUBSTRATES

FLÁVIA A. C. SILVA<sup>1</sup> AND ANTÔNIO R. PANIZZI<sup>2</sup>

<sup>1</sup>Universidade Federal do Paraná, Departamento de Zoologia, Caixa Postal 19020, Curitiba 81531-990, PR, Brazil

<sup>2</sup>Embrapa Soja, Laboratório de Bioecologia de Percevejos, Caixa Postal 231, Londrina 86001-970, PR, Brazil

Correspondent author: E-mail: panizzi@cnpso.embrapa.br; Phone: 55-43-3371-6123

The Neotropical brown stink bug, *Euschistus heros* (F.), a major component of the pest complex on soybean (*Glycine max* (L.) Merrill) (Panizzi et al. 2000a), can be reared under laboratory conditions with high rates of survivorship and fecundity (Peres & Corrêa-Ferreira 2001; Silva et al. 2008). However, it is critical to provide a suitable oviposition substrate in order to concentrate the egg masses and to reduce input to routine maintenance.

Pentatomids oviposit on a variety of artificial substrates (Shearer & Jones 1996; Bundy & McPherson 2000; Panizzi et al. 2000b; Panizzi et al. 2004; Silva & Panizzi 2008) and *E. heros* oviposits on cotton balls (Silva & Panizzi 2007). However, an earlier study did not test the preference of *E. heros* to oviposit on cotton balls versus other artificial substrates or its host plant, soybean. This study was conducted to compare oviposition of *E. heros* on artificial substrates of cotton, cheesecloth, polyester veil, and living soybean plants.

A laboratory colony was established from field collected *E. heros* by putting 30 pairs of *E. heros* on pods of green beans, *Phaseolus vulgaris* L., raw shelled peanuts, *Arachis hypogaea* L., and fruits (berries) of privet, *Ligustrum lucidum* Ait. (Oleaceae). The colony was maintained in an environmental chamber at 25 ± 1°C temperature, 60 ± 10% RH and with a photoperiod of 14:10 h (L:D). Food was replaced every other day.

Experiments were conducted to compare oviposition on several substrates, as follows: (1) Oviposition on a cotton ball (2 cm diameter, from Cremer S.A., Blumenau, SC, Brazil), a 3 × 3 cm-piece of extra fine cheesecloth (Têxtil São João Ltda, São João da Boa Vista, RS, Brazil), or a 3 × 3 cm-piece of polyester veil (mesh 1.0 mm, from Bankike—Com. e Ind. de Rendas Ltda, Nova Friburgo, RJ, Brazil). One male and 1 female were placed in a covered translucent plastic box (11 × 11 × 3.5 cm) lined with filter paper and containing pods of green beans and raw shelled peanuts ( $n = 12$  replicates). Egg masses, total eggs, and eggs/mass were recorded daily for 6 d on the 3 substrates. (2) Oviposition on a cotton ball or on a soybean leaflet, and (3) Oviposition on a cotton ball or a soybean pod. In these experiment 5 pairs of *E. heros* were placed (1 pair per box) in

boxes containing a cotton ball and a soybean leaflet (with the petiole placed in a vial containing water) or a cotton ball and a soybean pod (BRS 267 cultivar). Each box had a raw shelled peanut as food. Data on number of egg masses, eggs and eggs/mass were collected for 12 d. (4) Oviposition on a cotton ball, a soybean leaflet, or a soybean pod. One pair of *E. heros* in a box as described above were observed daily for 3 d, and eggs recorded ( $n = 8$  replicates). (5) Oviposition on cotton balls tied to a living, potted soybean plant (BRS 267 cultivar) at the full pod-filling stage, or on the potted plant itself. Two cotton balls were tied on each plant, 1 near the top and the other near the bottom. Ten pairs of *E. heros* were placed in each of 4 netted cages (50 × 50 × 30 cm) containing the soybean plant and cotton balls. Egg masses, eggs and eggs/mass deposited on the plant and on the cotton balls were recorded for 4 d.

Data were analyzed by (ANOVA), and means were separated by Tukey's test for multiple comparisons, or by Student's *t* test when comparing only 2 means. SAS 8.2 (SAS Institute 1981, Zar 1984) was used for the analyses.

Tables 1 and 2 show that more eggs were laid on a cotton ball than any other substrate including leaves or pods from soybean plants. The mean number of eggs/mass did not differ significantly ( $P < 0.05$ ) on the different substrates. Only 1 egg mass was laid on the soybean pod, and consequently data were excluded from analyses. When cotton balls were hung at the top and bottom of a soybean plant, *E. heros* laid 58% of the egg masses on the balls, despite the much greater area of foliage available for egg masses. Less than 45% of the egg masses were laid on the leaflets and pods, with pods receiving significantly fewer egg masses (6%). *Euschistus heros* is known to prefer to deposit its eggs on soybean leaves rather than on pods (Villas Bôas & Panizzi 1980). These results conclusively demonstrate that cotton balls are a suitable oviposition substrate for the Neotropical brown stink bug, and show that cotton balls are as acceptable as whole soybean plants. With all egg masses concentrated on cotton balls, routine maintenance of laboratory colonies is greatly simplified.

TABLE 1. MEAN ( $\pm$ SE) NUMBER OF EGG MASSES AND EGGS LAID BY *EUSCHISTUS HEROS* ON ARTIFICIAL SUBSTRATES IN A MULTIPLE CHOICE TEST IN THE LABORATORY ( $N = 15$  PAIRS; 6 D).

Substrate	Mean number ( $\pm$ SEM) <sup>1</sup>		
	Egg mass	Eggs	Eggs/mass
Cotton ball	10.9 $\pm$ 0.8 a	73.3 $\pm$ 6.6 a	6.9 $\pm$ 0.6 a
vs.			[163] <sup>2</sup>
Cheesecloth	0.13 $\pm$ 0.1 c	0.4 $\pm$ 0.4 c	3.0 $\pm$ 0.0 a
vs.			[2]
Polyester veil	1.7 $\pm$ 0.3 b	9.5 $\pm$ 2.5 b	5.4 $\pm$ 0.5 a
			[25]

<sup>1</sup>Means in each column followed by the same letter do not differ significantly using the Tukey test ( $P < 0.05$ ).

<sup>2</sup>Number of egg masses laid on each substrate in brackets.

TABLE 2. MEAN ( $\pm$ SE) NUMBER OF EGG MASSES AND EGGS LAID BY *EUSCHISTUS HEROS* ON ARTIFICIAL AND NATURAL SUBSTRATES IN A DUAL CHOICE TEST ( $N = 5$  PAIRS; 12 D), AND IN A MULTIPLE CHOICE TEST IN THE LABORATORY ( $N = 8$  PAIRS; 3 D).

Substrate	Mean number ( $\pm$ SEM)		
	Egg mass	Eggs	Eggs/mass
	Dual choice test <sup>1</sup>		
Cotton ball	8.2 $\pm$ 1.9 a	39.2 $\pm$ 10.8 a	4.2 $\pm$ 0.6
vs.			[41] <sup>3</sup>
Soybean leaflet	0.2 $\pm$ 0.2 b	0.4 $\pm$ 0.4 b	2.0 $\pm$ 0.0
			[1] <sup>4</sup>
Cotton ball	2.2 $\pm$ 2.0 a	10.2 $\pm$ 6.4 a	5.0 $\pm$ 0.6 a
vs.			[11]
Soybean pod	0.4 $\pm$ 0.4 b	2.2 $\pm$ 1.9 b	5.5 $\pm$ 2.8 a
			[2]
	Multiple choice test <sup>2</sup>		
Cotton ball	3.2 $\pm$ 0.6 a	30.9 $\pm$ 3.9 a	11.0 $\pm$ 1.1 a
vs.			[26] <sup>3</sup>
Soybean leaflets or	1.0 $\pm$ 0.3 b	6.4 $\pm$ 3.3 b	6.5 $\pm$ 0.9 b
Soybean pods	0.1 $\pm$ 0.1 b	1.2 $\pm$ 1.2 c	10.0 $\pm$ 0.0
			[1] <sup>4</sup>

<sup>1</sup>Means in each column followed by the same letter do not differ significantly using the Student's  $t$  test ( $P < 0.05$ ).

<sup>2</sup>Means in each column followed by the same letter do not differ significantly using the Tukey test ( $P < 0.05$ ).

<sup>3</sup>Number of egg masses laid on each substrate in brackets.

<sup>4</sup>Statistical comparison was not performed because only 1 value was obtained.

## SUMMARY

Laboratory studies indicated that the Neotropical brown stink bug, *Euschistus heros* (F.) (Heteroptera: Pentatomidae) prefers cotton ball over polyester veil and cheesecloth as ovipositional substrate. In dual and multiple choice tests cotton balls were greatly preferred as ovipositional substrates over soybean leaflets or bean pods, and cotton balls are as acceptable as a living soybean plant. The use of cotton balls greatly simplifies routine maintenance of laboratory colonies.

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