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Author(s): Flávia A. C. Silva and Antônio R. Panizzi
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Cotton Balls as an Oviposition Substrate for Laboratory Rearing of Phytophagous Stink Bugs (Heteroptera: Pentatomidae)

FLÁVIA A. C. SILVA[1] AND ANTÔNIO R. PANIZZI[2]

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ABSTRACT Studies were conducted to test the suitability of absorbent cotton as an oviposition substrate for phytophagous stink bugs (Heteroptera: Pentatomidae). In confined boxes, >80% of total egg masses of *Euschistus heros* (F.), *Dichelops melacanthus* (Dallas), and *Thyanta perditor* (F.) were laid on cotton; for *Piezodorus guildinii* (Westwood) and *Chinavia impicticornis* (Stål), >60%. However, *Nezara viridula* (L.) and *Edessa meditabunda* (F.) did not oviposit on cotton balls, but instead on the box walls, on filter paper, or on food. Newly hatched nymphs successfully (>95%) left the eggshells despite the presence of cotton fibers around the egg masses.

KEY WORDS stink bug, egg deposition, soybean, cotton, insect rearing

One of the critical issues when rearing phytophagous stink bugs is to provide a suitable ovipositional substrate. Heteropterans oviposit on paper towels suspended inside cages or on draped strips of cheesecloth (Shearer and Jones 1996, Bundy and McPherson 2000). Bugs also oviposit on artificial substrates such as plastic structures that mimic soybean, *Glycine max* (L.) Merrill, leaflets by laying eggs on the lower (abaxial) surface (Panizzi et al. 2000).

More recently, an ovipositional substrate has been developed for the southern green stink bug, *Nezara viridula* (L.), that consists of cheesecloth (Fisher, Pittsburgh, PA) stretched tightly over wooden embroidery rings and treated with extracts from soybean plants (Panizzi et al. 2004). These extracts, particularly those from soybean pods extracted using methanol, yielded the best results, and they are under investigation to identify the chemicals eliciting positive responses.

While keeping a colony of the Neotropical brown stink bug, *Euschistus heros* (F.) in the laboratory, we noticed that females preferred to oviposit on the wet cotton used to provide water. From this casual observation, we decided to test commercial cotton (dry) as an ovipositional substrate for several stink bug species, in routine rearing. The rationale for testing dry cotton as an ovipositional substrate was its convenience, acceptability by the bugs, and potential use of a material that is clean and disposable.

Materials and Methods

Insect Colonies. Adults were field-collected by using a sweep net on wild [siberian motherwort, *Leonurus sibiricus* L., hairy beggarticks, *Bidens pilosa* L.] and cultivated [soybean; corn, *Zea mays* (L.)] host plants during December 2005–January 2006 in Londrina, PR, Brazil (latitude 23° 18′ S). Colonies of seven stink bug species [*Euschistus heros* (F.), *Dichelops melacanthus* (Dallas), *Chinavia impicticornis* (Stål), *Thyanta perditor* (F.), *Piezodorus guildinii* (Westwood), *Edessa meditabunda* (F.), and *Nezara viridula* (L.)] were established in the laboratory at the Embrapa National Soybean Research Center facilities. They were fed fresh green bean, *Phaseolus vulgaris* L., pods; raw shelled peanuts, *Arachis hypogaea* L.; mature soybean seeds; and fruits (berries) of privet, *Ligustrum lucidum* Aiton, which were replaced every 2 d. Several pairs (n = 20–30) of each species were placed in transparent plastic containers (20 by 20 by 24 cm; three containers/species) covered with a meshed lid. With two exceptions, the colonies were maintained without introduction of feral insects for 6 mo, to provide healthy laboratory-reared adults for the test. The exceptions were the colonies of *E. meditabunda* and of *C. impicticornis* to which field-collected adults were added because of high mortality of nymphs.

Oviposition Site. Ten pairs of each species were selected from the rearing colonies and placed individually in a translucent plastic box (11 by 11 by 3.5 cm) lined with filter paper and covered with a lid. Food (green bean pod, mature seeds of soybean, and raw shelled peanut) and a ball (5 cm in diameter) of commercial absorbent cotton (Cremer S.A., Blumenau, SC, Brazil) were added. The boxes were observed daily for 16 d. Food was replaced every

[1] Universidade Federal do Paraná, Departamento de Zoologia, Caixa Postal 19020 Curitiba 81531-990, PR, Brazil.

[2] Corresponding author: Embrapa Soja, Laboratório de Entomologia, Caixa Postal 231, Londrina 86001-970, PR, Brazil (e-mail: panizzi@cnpso.embrapa.br).

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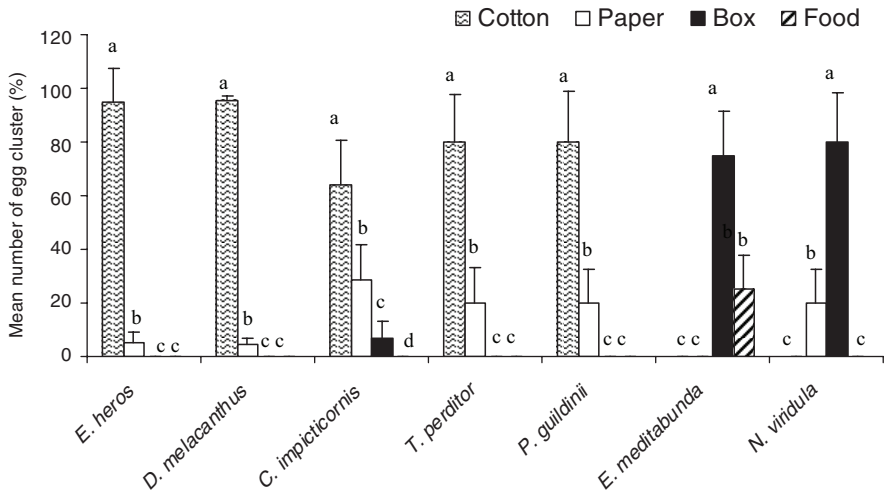

Fig. 1. Oviposition preference between cotton balls, paper, box, and food in the laboratory of seven stink bug species. Means followed by the same letter do not differ significantly, using the Tukey test ($P = 0.05$).

other day, and the cotton ball was changed daily. The number of egg masses laid on the cotton ball, and on filter paper, food, and walls and lid of the plastic box was recorded daily, and the percentages were calculated. All maintenance of adults and testing were conducted in a walk-in environmental chamber [25 ± 1°C, 60 ± 10% RH, and a photoperiod of 14:10 (L:D) h].

Data Analyses. The mean percentages of egg masses laid on cotton balls and on the walls and lid of the plastic box, filter paper or food, by each species, were analyzed with analysis of variance (ANOVA). Means were transformed to arcsine before using the Tukey test for multiple means ($P = 0.05$) comparison, which was performed using the program STATISTICA, version 6.0 (StatSoft 2001).

Results and Discussion

Of the seven species of stink bug, five species (i.e., *E. heros*, *D. melacanthus*, *C. impicticornis*, *T. perditor*, and *P. guildinii*) showed a variable (65–95%) and significant ($P = 0.05$) preference for cotton balls as an ovipositional site (Fig. 1). These same species also oviposited on the filter paper, but only *C. impicticornis* also oviposited on the box. No bugs laid eggs on the food (green bean pod, and mature seeds of soybean and peanut). In general, these stink bug species lay egg clusters of no >25 eggs, with few rows. For example, *E. heros* deposit egg clusters with three to 14 eggs in two to three rows (Villas Bôas and Panizzi 1980); *D. melacanthus* five to 14 eggs in three rows (F.A.C.S. and A.R.P., unpublished data); *C. impicticornis* 12–14 eggs in three to four rows (Grazia et al. 1982); *T. perditor* 11–25 eggs in two rows (Panizzi and Herzog 1984), and *P. guildinii* 11–15 eggs in two rows (Panizzi and Smith 1977).

The remaining two species, *E. meditabunda* and *N. viridula*, did not oviposit on the cotton balls. *E. meditabunda* oviposited preferentially on the box, followed by the food, but they did not oviposit on the filter paper. *N. viridula* oviposited preferentially on the box, followed by the filter paper, but they did not oviposit on the cotton balls or food (Fig. 1). That *E. meditabunda* did not oviposit on cotton balls, despite its small-size egg cluster (12–14 eggs disposed in two rows; Rizzo 1976), may be related to the size of eggs; *E. meditabunda* eggs are larger than those of the other species, which may prevent them from being glued to the irregularly disposed cotton fibers.

The southern green stink bug did not oviposit on the cotton balls, probably because of the shape and size of its egg clusters (usually >100 eggs deposited in five to seven rows; Rizzo 1976); this egg mass requires a more even surface to be properly accommodated, as is the case with soybean leaflets, a natural substrate (Todd 1989), or an artificial substrate made up of stretched cheesecloth (Panizzi et al. 2004).

Several egg clusters were placed together on cotton balls by *E. heros* (Fig. 2A), cotton fibers were found around the eggs (Fig. 2B), and several fibers fused to the bottom of the eggs (Fig. 2C); yet, newly hatched nymphs easily managed to move atop the eggshells (Fig. 2D). Preliminary tests indicate that survivorship of nymphs is not affected by the fibers (95.7%; on soybean leaflets, 97.0%).

Bundy and McPherson (2000) used draped strips of cheesecloth, to obtain egg clusters of several species of stink bugs in the laboratory. Strips of cheesecloth are apparently a suitable substrate for egg deposition; however, these authors mention that egg clusters were loosely arranged in a somewhat irregular pattern on cheesecloth strips. Addi-

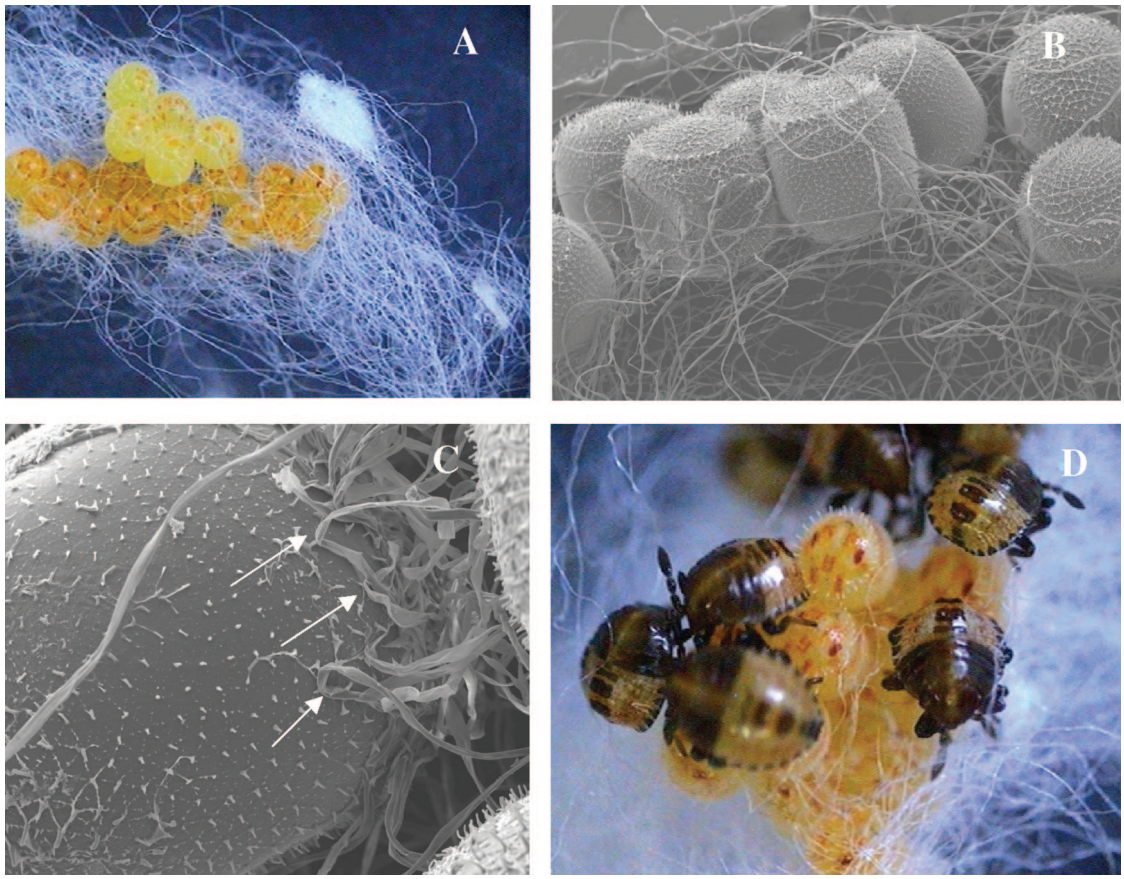

Fig. 2. Eggs of *E. heros* laid on cotton balls. (A) Egg clusters atop cotton fibers (20×). (B) Scanning electron microscopy of cotton fibers below and around eggs (40×). (C) Enlarged view of cotton fibers glued to the egg bottom (note arrows) (150×). (D) Newly hatched nymphs emerging from egg shells (20×).

tional studies are needed to compare cotton balls with cheesecloth (whether stretched or not) as an ovipositional substrate for the majority of the species tested.

These results demonstrate that cotton balls acquired commercially can be used as a suitable substrate for oviposition by several species of phytophagous stink bugs associated with soybean and probably by other species that lay clusters with few eggs. We have used this substrate routinely in our laboratory, and we suggest it can be a useful tool in maintaining laboratory colonies of stink bugs.

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