Laboratory rearing of *Bagrada hilaris* (Hemiptera: Pentatomidae) under quarantine conditions in Florida, USA

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Bagrada hilaris (Burmeister) (Hemiptera: Pentatomidae) is an invasive stink bug from Africa that was discovered in California in 2008 and has since established in Arizona, Nevada, Utah, New Mexico, and Texas (Reed et al. 2013). *Bagrada hilaris* infests plants in the family Brassicaceae, including important crops like broccoli, cabbage, kale, and cauliflower, *Brassica oleracea* L. (Brassicaceae). Feeding by *B. hilaris* causes wilting, desiccation, and discoloration of the plants (Palumbo et al. 2016). This pest preferentially feeds on young plants, damaging the meristem, and later causing deformation or lack of formation of reproductive heads in broccoli and cauliflower (Palumbo & Natwick 2010). On average, the injury caused by *B. hilaris* has been 10 to 25%, although losses as high as 70% in direct seeded fields were reported (Reed et al. 2013). In 2017, fresh market green cabbage was considered the tenth most valuable crop for Florida, and severe economic losses could result from the establishment of *B. hilaris* (FDACS 2018).

Bagrada hilaris is 1 of 3 pests regulated under the Federally Recognized State Managed Phytosanitary Program (USDA 2015). Currently, *B. hilaris* is not established in Florida, but has been intercepted at agricultural interdiction stations on multiple occasions, indicating that pathways exist for its arrival to the state (S. Halbert, personal communication). Therefore, research is needed on the potential for *B. hilaris* to establish in Florida, and methods have been developed to maintain a colony under quarantine conditions.

In previous research, a non-quarantine colony was maintained at 25 °C, with a 14:10 h (L:D) photoperiod and undefined RH (Taylor et al. 2015). In another colony, *B. hilaris* had the greatest survival at 24 to 35 °C (Reed et al. 2017), although optimal survival of a different colony occurred at 22 to 25 °C and 54% RH (Singh & Malik 1993). Our colony was maintained by placing 4 dehumidifiers around the rearing room to provide 50 to 65% RH. The temperature was kept at 23 to 27 °C and monitored using 2 HOBO data loggers (Onset Computer Corporation, Bourne, Massachusetts, USA; https://www.onsetcomp.com/). A fluorescent light (two 40 watt, 1.2 meter cool white lamps) was installed above the cages and a 14:10 h (L:D) photoperiod was maintained.

Bagrada hilaris has been reared successfully on potted plants or plant cuttings of Indian mustard, *Brassica juncea* (L.) Czern. (Brassicaceae) (Singh & Malik 1993); mesa pepperwort, *Lepidium alyssoides* A. Gray (Brassicaceae) (Taylor et al. 2014); cabbage, *Brassica oleracea* var. *capitata* L. (Brassicaceae) (Verma et al. 1993); and broccoli, *Brassica oleracea* var. *italica* Plenck (Brassicaceae) (Huang et al. 2014). In our colony, insects were fed a mixed diet of cabbage, *B. oleracea* var. *capitata*, and kale, *Brassica oleracea* var. *sabellica* L. (Brassicaceae), and reared in custom-made cages.

The stink bugs were reared in custom-made cages; the largest ones were made from 1-gal wide-mouth polyethylene terephthalate jars (25.6 cm H × 14.7 cm diam) (ULINE, Pleasant Prairie, Wisconsin, USA; https://www.uline.com). Four 5 cm diam holes were cut into each jar at an equal distance around the sides and covered with off-white 150 × 150 mesh nylon netting secured with hot glue. Smaller cages were constructed from 6.7 cm high × 11.4 cm diam cylindrical plastic restaurant-style containers (Tupperware Corporation, Orlando, Florida, USA; www.tupperwear.com). Four 5 cm diam holes were cut into each lid and covered with nylon mesh that was secured with hot glue. For additional security, all cages were placed inside a BugDorm-2120F Insect Tent (BD2120F, MegaView Science Co., Ltd., Taichung, Taiwan; www.bugdorm.com).

Our colony was established with 214 insects collected on broccoli at the University of Arizona Agricultural Center in Yuma, Arizona, USA, in Jun 2016. The insects were maintained in the Florida Department of Agriculture and Consumer Services, Division of Plant Industry maximum security quarantine facility in Gainesville, Florida, USA. For colony maintenance, each of the large cages were lined with a folded paper towel, and 6 dental wicks (Dynarex, Orangeburg, New York, USA; www.dynarex.com), 6 cotton balls (Publix, Lakeland, Florida, USA; www.publix.com), and 2 KimTech Science™ Kimwipes™ (KimTech, Roswell, Georgia, USA; www.kcprofessional.com) were added to each as oviposition substrates. A maximum of 15 pairs of males and females were contained in each large cage and provided with an approximately 180 cm^2 kale leaf that was suspended from the top of the cage using an insect pin and 3 approximately 40 cm^2 pieces of cabbage. The food and oviposition substrates were replaced every 3 to 4 d. A maximum of 100 eggs were extracted randomly from the substrates and placed onto a paper towel in the bottom of each small cage. Nymphs emerged from the eggs and were given half of a kale leaf and 2 pieces of cabbage (about 40 cm^2 each). After reaching the third instar, nymphs were collected randomly and transferred to large cages with a maximum of 50 insects per cage. Following eclosion, the adults were sexed, sorted into new large cages with a 25:25 M:F ratio, and then returned in large cages to the insect tents.

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Maintenance of the *B. hilaris* colony would be more efficient if the females oviposited on a substrate from which the eggs could be removed easily. In the field, females oviposit single eggs or small egg masses beneath the soil surface (Taylor et al. 2014) but it would be difficult to use soil as an oviposition substrate in the laboratory. Consequently, alternative oviposition substrates have been tested, including suspended cheesecloth strips (Taylor et al. 2015), wool (Atwal 1959), and paper towels (Reed et al. 2017). The purpose of this study was to evaluate artificial oviposition substrates for maintaining a *B. hilaris* colony in a quarantine facility.

For both no-choice and choice tests, 3 oviposition substrates were evaluated in a randomized block design: i.e., dental wicks, cotton balls, and Kimwipes™. In the no-choice test, 9 large adult cages were prepared, each containing food, 10 pairs of unmated male and female adult *B. hilaris*, and 1 of the oviposition substrates (Fig. 1). For this no-choice test, 3 cages of each substrate were tested for replication. Choice tests were performed similarly, but with 3 large adult cages testing all 3 oviposition substrates within the same cage. Standard rearing procedures were followed and cages were inspected visually for eggs until the last female died. In the choice test, 1 large adult cage was prepared as in the no-choice test but contained equal amounts of all 3 substrates (Fig. 1). Data was analyzed with R (R Project 2013) in RStudio (RStudio Team 2015) using an ANOVA and a Tukey HSD procedure in the *Agricolae* package to test the effect of substrate type on the number of eggs oviposited by female *B. hilaris* (Mendiburu 2020; Hothorn et al. 2008).

For the no-choice test, there was a significant effect of substrate type on the mean number of eggs oviposited by female *B. hilaris* (F = 7.981; df = 2; P < 0.001). There was no significant difference between eggs oviposited on the cotton balls (n = 1,211) and the dental wicks (n = 1,029), but the number of eggs oviposited on cotton balls and dental wicks both were significantly greater than on Kimwipes™ (n = 320) (P < 0.001, P = 0.008, respectively) (Fig. 2). When given no choice, both cotton balls and dental wicks were adequate artificial oviposition substrates for *B. hilaris*.

For the choice test, there was a significant effect of substrate on the mean number of eggs oviposited by female *B. hilaris* (F = 28.61; df = 2; P < 0.001). The number of eggs oviposited on the cotton balls (n = 1,573) was significantly greater than on the dental wicks (n = 141) and the Kimwipes™ (n = 13) (P < 0.001), but there was no difference between the number of eggs on dental wicks and Kimwipes™ (P = 0.829). Based on this and the results from the no-choice tests, cotton balls were preferred by *B. hilaris* as an artificial oviposition substrate.

The authors thank Brianna Whitman and Cleveland Ivey for assistance with colony maintenance and data collection. The authors thank the Florida Department of Agriculture and Consumer Services, Division of Plant Industry for allocation of space in their maximum security quarantine facility, and Phillip Lake and David Davidson for assistance with quarantine procedures. Funding for the development of *B. hilaris* rearing protocols under quarantine conditions was provided by USDA-APHIS-PPQ Plant Protection Act Agreement No. AP18PPQS&T00C098 and AP19PPQFO000C460. Partial funding also was provided by USDA-National Institute of Food and Agriculture project number 2017-70006-27149.

Summary

There is a risk that *Bagrada hilaris* will be transported from the southwestern USA and establish in Florida where it could infest *Brassi-*

Fig. 1. Large cages for no-choice and choice tests for evaluating the preference of *Bagrada hilaris* for 3 artificial oviposition substrates: dental wicks, cotton balls, or Kimwipes.

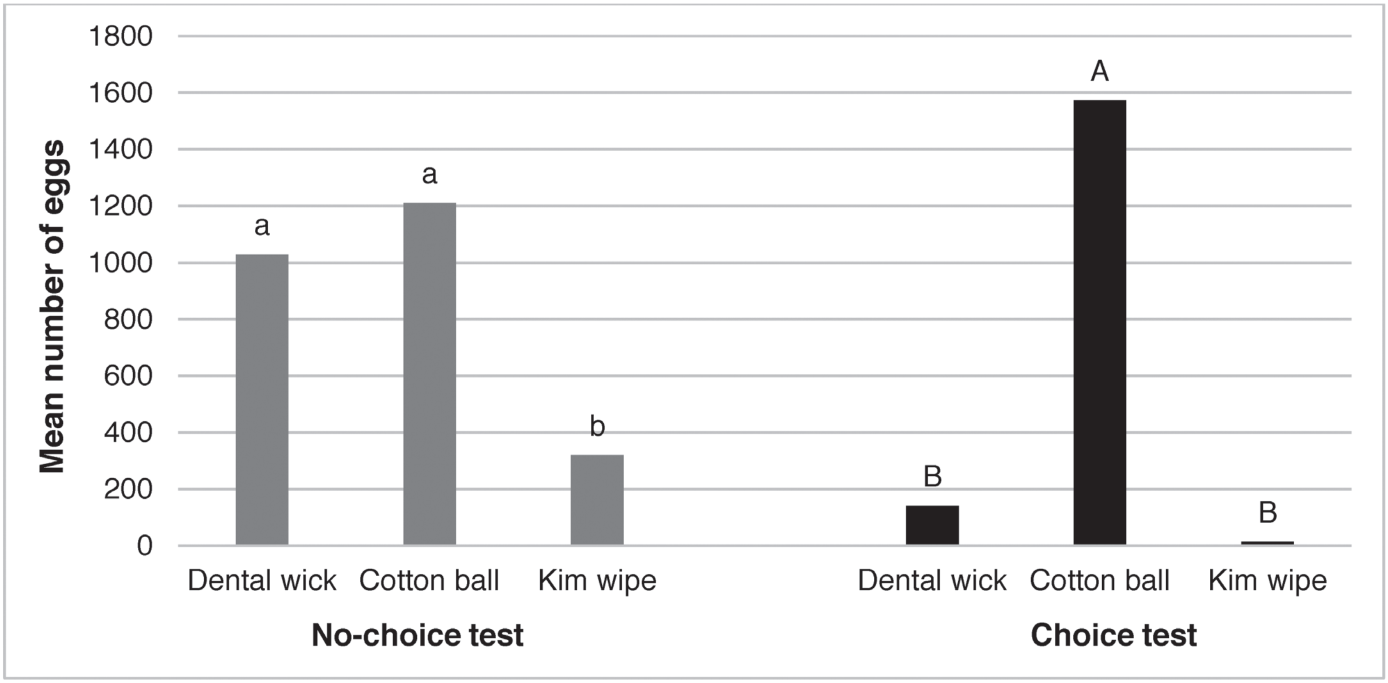

Fig. 2. Mean number of eggs oviposited by 10 *Bagrada hilaris* females on dental wicks, cotton balls, or Kimwipes in no-choice and choice tests. There was a significant effect of substrate type on the number of eggs oviposited in both the no-choice ($F = 7.981$; df = 2; $P < 0.001$) and choice ($F = 28.61$; df = 2; $P < 0.001$) tests.

ca crops. Therefore, new rearing methods were developed to maintain a colony of *B. hilaris* under quarantine conditions to conduct research on the potential for this pest to become established in Florida. Dental wicks, cotton balls, and Kimwipes™ were evaluated as artificial oviposition substrates. Cotton balls were the preferred oviposition substrate by *B. hilaris*.

Key Words: oviposition substrates; insect rearing; stink bug; risk assessment

Sumario

Existe el riesgo de que *Bagrada hilaris* sea transportada desde el suroeste de los EE. UU. y se establezca en Florida donde podría infestar los cultivos de *Brassica*. Se desarrollaron nuevos métodos de cría de *B. hilaris* en condiciones de cuarentena para investigar el potencial que *B. hilaris* se establezca en Florida. Se evaluaron, las mechas dentales, las bolas de algodón, y los Kimwipes™ como sustratos artificiales de oviposición. Las bolas de algodón fueron el sustrato preferido por *B. hilaris*.

Palabras Clave: sustratos de oviposición; cría de insectos; chinches; evaluación de riesgos

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