Rearing Protocol and Density Trials of the Brown Marmorated Stink Bug (Hemiptera: Pentatomidae) in the Laboratory

Authors: Rosen, Sara J., Cambridge, John E., and Hamilton, George C.

Source: Florida Entomologist, 99(4) : 808-810

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.099.0443

Rearing protocol and density trials of the brown marmorated stink bug (Hemiptera: Pentatomidae) in the laboratory

Sara J. Rosen, John E. Cambridge, and George C. Hamilton*

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), commonly known as the brown marmorated stink bug, is an invasive pest species that was accidentally introduced to the U.S. from eastern Asia in the early to mid-1990s (Hoebeck & Carter 2003). *Halyomorpha halys* is a polyphagous herbivore and a serious crop pest in many agricultural systems (Rice et al. 2014). The economic burden that *H. halys* poses to growers takes the form of increased costs of pesticide applications and yield losses. In 2010 alone, *H. halys* cost US$37.5 million of damage to mid-Atlantic apple growers (Anonymous 2011). In addition to causing agricultural pressures, *H. halys* is known to be an urban nuisance pest when it enters the home for overwintering purposes (Inkley 2012; Cambridge et al. 2015). Research done on the suppression of *H. halys* has focused on insecticide susceptibilities and natural enemies (Leskey et al. 2012). The study designs used to investigate these topics require large sample sizes and highlight the need for effective colony rearing protocols.

The following procedures provide a framework for rearing *H. halys* in a laboratory setting. Also, a study was conducted to investigate the impact of adult density on the number of egg masses laid per female and female survival for the purpose of optimizing egg mass production.

This rearing protocol describes the method used to sustain roughly 1,000 individuals of *H. halys* across all life stages in a laboratory colony. The insects were housed in 29.2 × 25.4 × 26.6 cm polyethylene bug dorms (BioQuip, Rancho Dominguez, California). The dimensions of the bug dorms used for this study are slightly larger than the 30 × 23 × 10 cm dimensions for cages used in another study (Medal et al. 2012). Each dorm was modified from its original shape to a triangular prism and fitted with a plastic foam top. Each bug dorm contained a paper towel sheet to line the bottom. Tap water was supplied in a 60 mL disposable cup covered with a lid in which a 1 cm diameter incision was made so that a cotton roll could be inserted. Bug dorms were then placed inside one of two 53.34 × 57.15 × 11.76 cm Plexiglass cages (NJAES Machine Shop, Rutgers University, New Brunswick, New Jersey) (Fig. 1). A humidifier (Sunbeam, USA) was attached to each larger cage and connected to a light timer that turned on for 30 min every 8 h. This system maintained the relative humidity at 65 to 70%. The temperature of the room was set to 26 ± 2 °C. We found that the bug dorms used for this study and by Nielsen et al. (2008) were most likely more efficient than the cages used in other studies. *Halyomorpha halys* females laid eggs mostly on the netting sleeve of the bug dorms, allowing for easy removal of egg masses by peeling. The bug dorms could also be stacked without preventing air circulation, unlike in other studies (Medal et al. 2012).

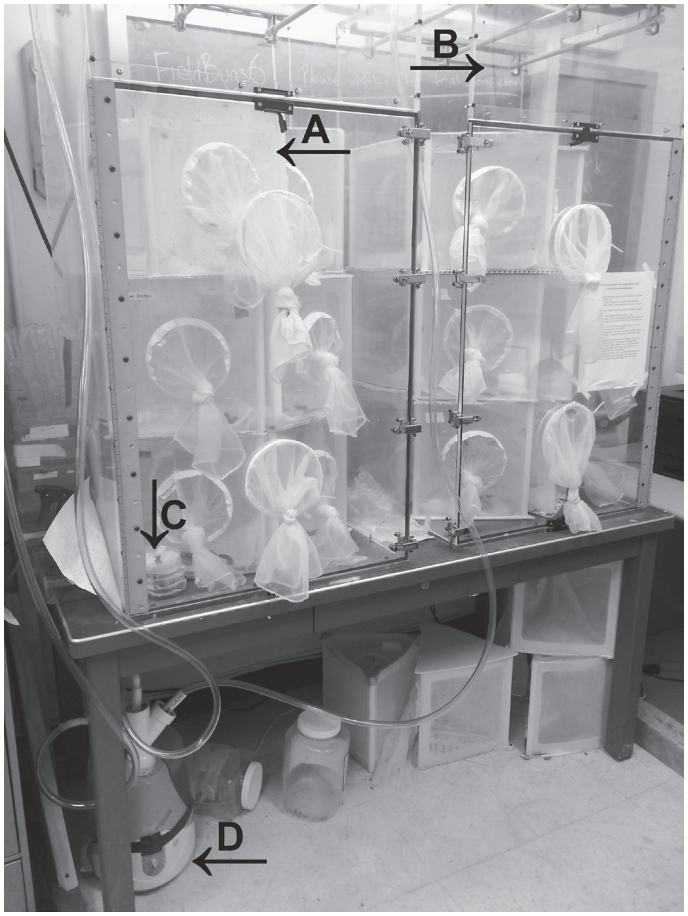

Fig. 1. Bug dorms. **A.** The large cage can house up to 12 bug dorms, with each stack containing 3 dorms. **B.** The large insect cage with humidifier attached. **C.** Stacks of Petri dishes. **D.** Humidifier.

All food and water were replaced on Monday, Wednesday, and Friday. This schedule was found to be much more cost effective and efficient compared with other studies in which food and water were replaced every other day (Medal et al. 2012). Food was administered by placing 2 prewashed organic baby carrots, 3 prewashed organic

Department of Entomology, Rutgers University, New Brunswick, New Jersey 08901, USA; E-mail: sjrosen27@gmail.com (S. J. R.); john.cambridge000@gmail.com (J. E. C.), hamilton@aesop.rutgers.edu (G. C. H.)
*Corresponding Author, E-mail: sjrosen27@gmail.com (S. J. R.)

green beans, and approximately 20 organic sunflower seeds into the dorm. The food provided to *H. halys* in this protocol is consistent with the types of food used in other studies (Funayama 2006; Li et al. 2007; Medal et al. 2012; Haye et al. 2014). Funayama (2006) used carrots, soybeans, and peanuts as food for this insect. We used green beans and sunflower seeds instead of soybeans and peanuts and found these items to be suitable. We have successfully used carrots, green beans, and sunflower seeds for over 12 yr to promote *H. halys* survival and oviposition.

Exuvia and old sunflower seeds were removed with a handheld mini-vacuum (Sharper Image, San Francisco, California) and discarded. If mold was present, all dorm materials were removed and replaced. This maintenance schedule was chosen because less intensive care consistently resulted in mold growth on the paper towel linings, which then spread to other dorms. Colony maintenance was always done between 8:00 AM and 6:00 PM. In addition to sunlight through laboratory windows, the colony was exposed to a 15 watt white GE fluorescent light (GE, Indonesia) on a 16:8 h L:D cycle. Wild-caught individuals were incorporated into the colony throughout the year when possible to maintain genetic diversity.

The 2nd and 3rd instars were housed together and maintained at a density of approximately 55 individuals per bug dorm. The 4th and 5th instars were housed together and maintained at a density of approximately 35 individuals per dorm. Adults were maintained at a density of approximately 10 individuals per bug dorm with a 1:1 ratio of males to females. Once an insect molted, it was moved into a bug dorm corresponding to that life stage. The 1st and 2nd instars always represented the largest proportion of total colony individuals.

All egg masses were collected from the bug dorms on maintenance days. The majority of eggs were found on the lid, netting sleeve, and paper towel lining. The eggs were removed from these surfaces by peeling them off gently with fingers. Peeling the egg masses off caused them to break apart less often than during other methods of removal. Once collected, egg masses were placed into an 8 cm diameter Petri dish (BioQuip, Rancho Dominguez, California) lined with filter paper (Fisher Scientific, USA) on the bottom. Water-proof tape (Fisher Scientific, USA) was placed on the filter paper so that a 3 mL glass vial (BioQuip, Rancho Dominguez, California) filled with tap water and a cotton roll could rest on top and not soak the filter paper. Once the items inside of the Petri dish were secured, the lid was replaced to maintain humidity. The water in these Petri dishes was refilled on maintenance days. The Petri dishes were labeled with the dates that the eggs were collected and discarded if no hatch had occurred after 14 d. All Petri dishes were stacked and placed inside of the large cage. Once the nymphs hatched, the 1st instars were kept inside the Petri dish without food until they molted into 2nd instars. The 2nd instars were removed from the Petri dish and placed into a bug dorm containing 2nd and 3rd instars and reared to the adult stage.

To measure the effect of adult density in bug dorms on egg mass production and female adult mortality, 5th instars were collected, separated individually into 1 L polyethylene cubic containers (8.89 × 8.89 × 8.26 cm; BioQuip, Rancho Dominguez, California), and then stacked inside a large cage. Each container was outfitted with the same materials as the bug dorms but using smaller quantities. On maintenance days, small containers were maintained in the same fashion as the bug dorms.

When the isolated 5th instars eclosed, the adults were sexed and transferred into a bug dorm. If there were not enough adults to start a new trial, individuals were kept in isolation until sufficient numbers of individuals had eclosed. Each trial consisted of an equal number of males (M) and females (F) per dorm. The densities tested were 1F:1M, 2F:2M, and 5F:5M per dorm. All trials were replicated 3 times. Trial dorms were set up and maintained as described for rearing. The number of egg masses laid and female survival were recorded for each trial dorm on maintenance days.

Data were analyzed using a generalized linear model that used density to investigate the mean number of egg masses or the mean percentage of survival of females. The tests were performed in SAS 9.4 using the genmod procedures (SAS Institute 2014). There was a difference between total egg production associated with density. The 5F:5M density dorms had fewer eggs produced than either 1F:1M ($P = 0.032$) or 2F:2M ($P = 0.055$) (Fig. 2). There was also a significant difference between lifespan of a female after initial egg production associated with density. The females reared at 1F:1M density had significantly longer lifespans than those reared at 2F:2M ($P < 0.001$) and 5F:5M ($P < 0.001$). These findings suggest that higher densities reduce egg mass production and lead to shorter adult female longevity in a laboratory colony setting.

Other laboratories can easily adopt the rearing protocol described in this study. This procedure requires 2 h of labor per maintenance day, an initial setup cost of under US$1,000, roughly US$30 per wk, and a 0.76 × 1.52 m table space with access to electricity making this protocol space, cost, and time efficient. We believe that the findings of this study should be substantiated with further research involving a larger colony size. Raising the adult stink bugs at a 1F:1M ratio might not be as cost and space efficient as rearing them at a higher density. However, if one desires to obtain the greatest egg production or longevity per female, the lowest rearing density would be most advised.

The results of this study should be incorporated into the development of future rearing protocols for *H. halys*. However, these should be validated further before assuming they will work with much larger colonies.

We would like to thank Rutgers University for allowing the use of their facilities as well as funding this research. We would also like to thank Laurie Francoeur, Tooba Mohammed, Ryan Fredericks, Gabe Batzli, and Nidhi Agrawal for their continual support and assistance throughout this project.

Summary

The following procedures provide a framework for rearing *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). The insects were cultured in slightly modified, commercially available bug dorms. A diet of organic beans, organic carrots, organic sunflowers seeds, and water

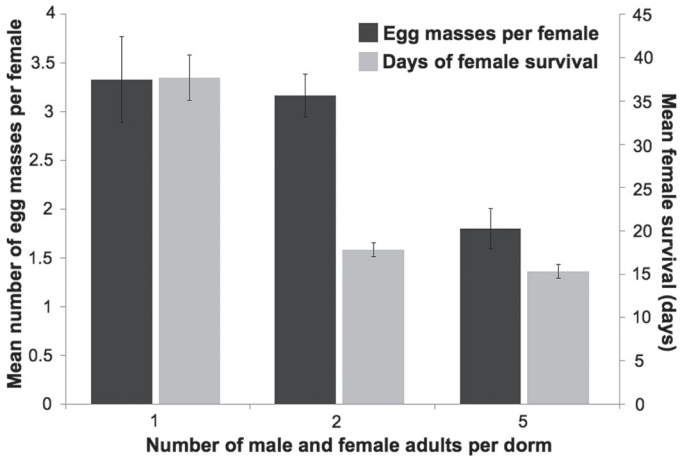

Fig. 2. Mean (± SD) number of egg masses laid per female at densities of 1:1, 2:2, and 5:5 (female to male) per cage and mean (± SD) survival of females from first egg mass laid at densities of 1:1, 2:2, and 5:5 per cage

was replaced on the maintenance days of Monday, Wednesday, and Friday to maximize freshness of the food. Egg masses were collected and bug dorms were cleaned on maintenance days as well. Adult density affected the number of egg masses laid per female and female survival in this protocol. Densities greater than 2 mating pairs per bug dorm significantly reduced egg mass production per female and led to shortened adult female longevity.

Key Words: *Halyomorpha halys*; rearing protocol; density-dependent factor

Sumario

Los siguientes procedimientos proveen un marco para la cría de *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). Se criaron los insectos en los dormitorios ligeramente modificados de los comercialmente disponibles para insecto. Una dieta de granos orgánicos, zanahorias orgánicas, semillas de girasol orgánicas y agua fue reemplazado en los días de mantenimiento de los lunes, miércoles y viernes para maximizar la frescura de los alimentos. Se recogieron las masas de huevos y se limpiaron los dormitorios de los insectos en los días de mantenimiento también. La densidad de adultos afectó el número de masas de huevos puestos por hembra y la sobrevivencia de las hembras en este protocolo. Densidades de más de 2 parejas de apareamiento por dormitorio de insecto redujeron significativamente la producción en masa de huevos por hembra y produjo una reducción en la longevidad de las adultas hembras.

Palabras Clave: *Halyomorpha halys*; cría de protocolo; actor dependiente de la densidad

References Cited

Anonymous. 2011. Brown marmorated stink bug causes $37 million in losses to mid-Atlantic apple growers. American/Western Fruit Grower. http://growingproduce.com/article/21057/brown-marmorated-stink-bug-causes-37-million-in-losses-to-mid-atlantic-apple-growers (last accessed 17 Apr 2016).

Cambridge J, Payenski A, Hamilton GC. 2015. The distribution of overwintering brown marmorated stink bugs (Hemiptera: Pentatomidae) in college dormitories. Florida Entomologist 98: 1257–1259.

Funayama K. 2006. A new rearing method using carrots as food for the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae). Applied Entomology and Zoology 41: 415–418.

Haye T, Abdallah S, Gariepy T. 2014. Phenology, life table analysis and temperature requirements of the invasive brown marmorated stink bug, *Halyomorpha halys*, in Europe. Journal of Pest Science 87: 407–418.

Hoebeck ER, Carter ME. 2003. *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. Proceedings of the Entomological Society of Washington 105: 225–237.

Inkley DB. 2012. Characteristics of home invasion by the brown marmorated stink bug (Hemiptera: Pentatomidae). Journal of Entomological Science 47: 125–130.

Leskey TC, Short BD, Butler BR, Wright SE. 2012. Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in mid-Atlantic tree fruit orchards in the United States: case studies of commercial management. Psyche, doi: 10.1155/2012/535062.

Li X, Yin XY, Ma L, Liu YH, Fu J, Liu XF. 2007. *Halyomorpha halys* behaviors and their application for control. Journal of Northwest A & F University (Natural Science Edition) 35: 139–145.

Medal J, Smith T, Fox A, Santa Cruz A, Poplin A, Hodges A. 2012. Rearing the brown marmorated stink bug *Halyomorpha halys* (Heteroptera: Pentatomidae). Florida Entomologist 95: 800–802.

Nielsen AL, Hamilton GC, Matadha D. 2008. Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). Environmental Entomology 27: 348–355.

Rice KB, Bergh JC, Bergmann EJ, Biddinger D, Dieckhoff C, Dively G, Fraser H, Gariepy T, Hamilton G, Haye T, Herbert A, Hoelmer K, Hooks C, Jones A, Krawczyk G, Kuhar T, Mitchell W, Nielsen A, Pfeiffer D, Raupp M, Rodriguez-Saona C, Shearer P, Shrewsbury P, Venugopal D, Whalen J, Wiman N, Leskey TC, Tooker J. 2014. Biology, ecology, and management of brown marmorated stink bug (*Halyomorpha halys*). Journal of Integrated Pest Management 5: 1–13.

SAS Institute. 2014. Base SAS 9.4 Procedures Guide: Statistical Procedures. SAS Institute, Cary, North Carolina.