

Cannibalistic Oophagy in *Halyomorpha halys* (Hemiptera: Pentatomidae) Laboratory Colonies¹

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Abstract *Halyomorpha halys* (Stål), commonly known as the brown marmorated stink bug, is an invasive and economically damaging insect pest in U.S. agriculture. To date, *H. halys* has been found in 42 U.S. states and two Canadian provinces. The stink bug feeds on more than 300 plant species, many of which are important agricultural crops including apples, peaches, persimmons, blackberries, sweet corn, field corn, and soybeans. As a species that has the potential to cause significant economic damage, having *H. halys* colonies maintained in a laboratory setting for research purposes is critical. While cannibalism occurs in both predatory and phytophagous insects, only two phytophagous pentatomids have been reported to be cannibalistic. After observing cannibalistic oophagy within our *H. halys* colony, we sought to identify the effects of this behavior on the hatch rate of eggs. Laboratory-reared *H. halys* egg masses were exposed to either second- or fourth-instar *H. halys* nymphs for varying lengths of time, after which the proportion of eggs hatched was determined. Both the number of days an egg mass was exposed to nymphs and the age of the nymphs affected egg mortality and hatch rate. This knowledge is useful to researchers attempting to maintain healthy, stable populations of *H. halys* in laboratory colonies and aids in developing a more successful rearing protocol. Those managing a *H. halys* colony should be aware that cannibalism may occur in *H. halys* and take appropriate measures to minimize the impact of this behavior.

Key Words insect rearing, pentatomids, insect colony health, bug-days, brown marmorated stink bug

Halyomorpha halys (Stål), the brown marmorated stink bug, has become a major agricultural pest in the United States (Rice et al. 2014) and has been found in Europe as well (Haye et al. 2014). *Halyomorpha halys* was first detected in Minnesota in 2010 (Koch 2014). By 2015 this invasive phytophagous insect had been found in 42 U.S. states and two Canadian provinces (Stop BMSB 2013). This species can feed on 300+ plant species including many important agricultural crops such as apples, sweet corn, field corn, and soybeans (Cira and Hutchison 2014; Nielsen and Hamilton 2009). Because of the economic damage caused by *H. halys*, it is advantageous to have laboratory colonies for research purposes, particularly in states where established populations do not yet exist, such as Minnesota. A healthy colony can be disrupted by many factors, however, including cannibalism. Although

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cannibalism has been shown to exist in both predatory and phytophagous insects, there are few published reports regarding cannibalism in phytophagous insects (Richardson et al. 2010). Historically, only two phytophagous pentatomids, *Nezara viridula* (L.), and *Parastrachia japonensis*, had been reported as cannibalistic (Stam et al. 1987; Tachikawa and Schaefer 1985). More recently, Medal et al. (2012) observed cannibalism in a laboratory colony of *H. halys* in Florida. However, the frequency and impact of this behavior was not quantified.

Upon observing cannibalism of eggs in our laboratory colony, we sought to quantify the effect of this behavior on the health of the colony. Our objectives were, therefore, designed to determine if the rate of egg hatch would significantly decrease as the length of exposure to nymphs increased and whether cannibalistic oophagy was higher in older (fourth instar) or younger (second instar) *H. halys* nymphs.

Materials and Methods

Halyomorpha halys cannibalism trials were arranged in a completely random design and were conducted from 7 January 2014 to 12 June 2014. Egg masses were collected from an *H. halys* colony maintained at $26^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and 16L:8D. The numbers of viable eggs per egg mass were determined via visual inspection at 8 $\times$ magnification, with viability defined as an egg exhibiting no signs of cannibalistic activity or abnormalities (no egg discoloration, indentations, or presence of stylet sheaths). Each egg mass was randomly assigned to be exposed to either *H. halys* second instars, fourth instars, or no nymphs. A total of 92 egg masses were utilized in this experiment; 62 of them were exposed to nymphs while the remaining 30 were used for the uninfested control. A half sheet of filter paper (Fisher Scientific, Pittsburgh, PA) measuring 4.5 cm in diameter was placed in the bottom of a Petri dish (VWR, Radnor, PA; 100 $\times$ 15 mm) and one egg mass was placed in each dish. The filter paper was moistened daily, and half sheets were used in order to keep the Petri dishes at the ideal moisture level. Micro-centrifuge tubes (Dot Scientific Inc., Burton, MI) were filled with water, plugged with a cotton ball, and placed in each Petri dish to ensure the nymphs had access to water and to further control humidity in the dishes. Second and fourth instars were placed in the dishes at a density between 4–8 insects for a period of between 1–5 d. To ensure all nymphs and egg masses were exposed to the same conditions, the dishes were placed in a Percival growth chamber programmed at 25°C 16L:8D (Percival Scientific, Perry, IA).

After egg masses hatched, the proportion of successfully hatched first-instar nymphs (first-instar nymphs hatched divided by total viable eggs) were recorded. The numbers of second- or fourth-instar nymphs still alive on the last day of each trial were also recorded. For comparison and to standardize a measure of nymphal exposure, we calculated cumulative bug-days (e.g., Ruppel 1983) using the following formula:

$$\text{Bug days} = \left[\frac{(\alpha_1 + \alpha_2)}{2} \right] \text{No. days of nymphal exposure,}$$

where α_1 is the number of nymphs alive at beginning of the trial and α_2 is the number of nymphs alive at the end of the trial. Cumulative bug days are utilized in

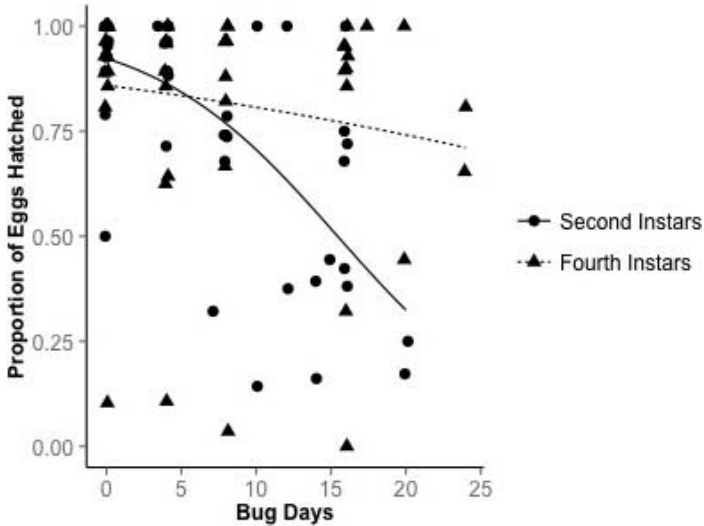


Fig. 1. Proportion of *H. halys* eggs, hatched after exposure to either *H. halys* second- or fourth-instar nymphs for a varying amount of time, expressed as bug-days.

order to measure the pressure across variable numbers of bugs and variable lengths of time, thus accounting for nymphal mortality (Ruppel 1983).

In addition, to determine the relationship between egg hatch and bug exposure, proportion egg hatch was regressed against bug-days. All statistics were run using R version 3.2.0 (R Core Team 2014) in RStudio version 0.98.1102 (RStudio 2014) and graphing was done in ggplot2 1.0.1 (Wickham 2009). A fully crossed generalized linear model was fit to untransformed data with hatch rate as the response variable and nymph age and bug-days as predictor variables.

Results and Discussion

Halyomorpha halys is an additional cannibalistic phytophagous pentatomid, with second instars tending to cannibalize conspecific eggs at a higher rate than do fourth instars (Fig. 1). Generalized linear model results revealed both main effects, bug-days ($Z = -13.71$, $df = 88$, $P < 0.0001$), and nymph age ($Z = -4.02$, $df = 88$, $P < 0.0001$) as significant. The interaction between bug days and nymph age was also significant ($Z = 8.12$, $df = 88$, $P < 0.0001$) and supports the pattern shown in Fig. 1.

Even considering the phytophagous physiology of *H. halys*, cannibalism is feasible because it provides the predator with a nutritious meal as well as reducing competition for potential mates and sustenance (Thomas and Manica 2003). With regard to the difference in feeding by age, Taylor et al. (2014) reported an endosymbiotic bacterial film that is spread on the chorion of eggs by females on which first instars feed. First instars feed on the chorion in order to obtain the

Table 1. Mean ($\pm$ SEM) proportion of *H. halys* nymphs alive at the end of each trial period (sample size; number of egg masses).*

Stage	1-d Trial	2-d Trial	3-d Trial	4-d Trial	5-d Trial
2nd instars	0.97 $\pm$ 0.05 (9)	0.97 $\pm$ 0.06 (8)	1.00 $\pm$ 0.00 (1)	0.72 $\pm$ 0.15 (9)	0.83 $\pm$ 0.22(3)
4th instars	1.00 $\pm$ 0.00 (12)	1.00 $\pm$ 0.00 (6)	1.00 $\pm$ 0.00 (2)	1.00 $\pm$ 0.00 (9)	0.92 $\pm$ 0.16(3)

* The mean number of eggs per egg mass in this study was 26.47 ± 2.76 .

beneficial gut bacteria and, if prevented from doing so, will experience developmental delays (Kenyon et al. 2015). It is possible that younger nymphs, including second instars, continue to be attracted to feeding on the endosymbiotic bacteria and perhaps inadvertently feed on the embryo during the process of stylet probing and feeding.

Our data reveal that both cumulative bug-days and nymph age are strong predictors of the rate of cannibalism. While the results of this experiment were highly significant, variation in the data is notable (Fig. 1). We measured the proportion of nymphs alive at the end of each trial period and found the mean proportion of second instars alive at the end of each trial period was 0.97 ± 0.05 for 1-d trials, 0.97 ± 0.06 for 2-d trials, 1.00 ± 0.00 for 3-d trials, 0.72 ± 0.15 for 4-d trials, and 0.83 ± 0.22 for 5-d trials (Table 1). For fourth instars, the mean proportion of nymphs alive at the end of each trial period was 1.00 ± 0.00 for 1-d trials, 1.00 ± 0.00 for 2-d trials, 1.00 ± 0.00 for 3-d trials, 1.00 ± 0.00 for 4-d trials, and 0.92 ± 0.16 for 5-d trials (Table 1). The mean proportion of dishes in which 100% of second-instar *H. halys* nymphs lived throughout the duration of the entire trial period was 0.89 ± 0.10 for 1-d trials, 0.88 ± 0.12 for 2-d trials, 1.00 ± 0.00 for 3-d trials, 0.44 ± 0.17 for 4-d trials, and 0.67 ± 0.27 for 5-d trials (Table 2). For fourth instars, the mean proportion of nymphs alive at the end of each trial period was 1.00 ± 0.00 for 1-d trials, 1.00 ± 0.00 for 2-d trials, 1.00 ± 0.00 for 3-d trials, 1.00 ± 0.00 for 4-d trials, and 0.67 ± 0.27 for 5-d trials (Table 2). These results show that mortality of nymphs during the experiment, which could affect hatch rates,

Table 2. Mean ($\pm$ SEM) proportion of dishes in which 100% of *H. halys* nymphs were alive at the end of each trial period (sample size; number of egg masses).

Stage	1-d Trial	2-d Trial	3-d Trial	4-d Trial	5-d Trial
2nd instars	0.89 $\pm$ 0.10 (9)	0.88 $\pm$ 0.12 (8)	1.00 $\pm$ 0.00 (1)	0.44 $\pm$ 0.17 (1)	0.67 $\pm$ 0.27 (3)
4th instars	1.00 $\pm$ 0.00 (12)	1.00 $\pm$ 0.00 (6)	1.00 $\pm$ 0.00 (2)	1.00 $\pm$ 0.00 (9)	0.67 $\pm$ 0.27 (3)

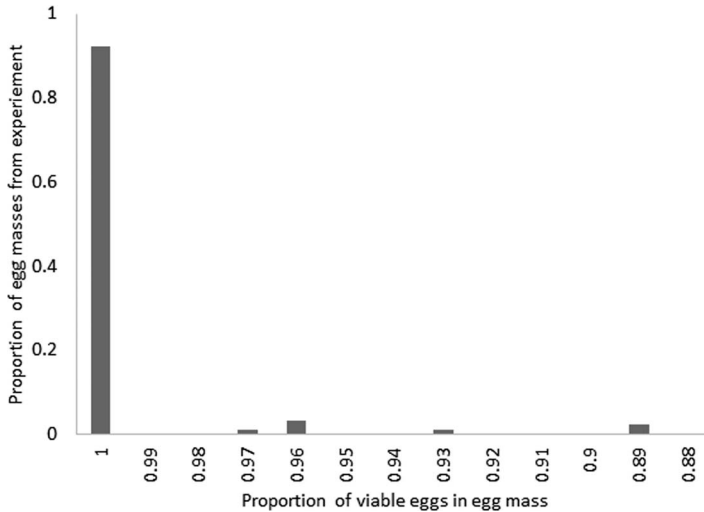


Fig. 2. Total number of *H. halys* egg masses, out of the 92 total used in this study, which had various proportions of viable eggs in the mass at the start of the experiment.

did occur. The amount of nymphal mortality was higher for second instars across different lengths of time and could have affected feeding and subsequent hatch rate. Other potential sources of variability include the inconsistencies in sublethal nymphal health and pretrial feeding status.

We also measured potential variability in the health of egg masses going into the experiment. The vast majority of eggs masses used (84 out of the total 92, or 93.5%) contained no unviable eggs (Fig. 2). Those that did contain unviable eggs ranged from 89–97% viability (Fig. 2). While the unviable eggs were not considered when calculating hatch rate, it is possible that nymphs could feed on already unviable eggs rather than viable eggs, which would alter our measures of nymphal feeding.

For optimum colony production, cannibalism of *H. halys* eggs via *H. halys* nymphs can be minimized in several ways. Cannibalism can be instigated by food shortages, poor food quality, or specific nutrient deprivation, meaning cages containing insects should be consistently stocked with an ample food supply (Cooper et al. 2014; Pires et al. 2011). Additionally, crowding of insects can induce cannibalism even when ample food is available, indicating that insects should not be reared at high densities per cage (Fox 1975). Most importantly, *H. halys* nymphs should be kept in age-specific cages, given our results demonstrating that second instars have a higher tendency to cannibalize conspecific eggs than do fourth instars. Therefore, it is especially important to remove egg masses from cages with younger nymphs. Although this research did not specifically study the cannibalism of *H. halys* eggs by *H. halys* adults, it would be prudent to also remove eggs from

cages containing older *H. halys* nymphs or adults as quickly as possible after they are deposited.

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