

The effects of relative humidity on *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) egg hatch, nymph survival, and adult reproduction

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The brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), originally from South Korea, Japan, and eastern China, was discovered recently in the USA (Hoebeke & Carter 2003; Hamilton 2009) and has since spread throughout most of eastern and western North America (Rice et al. 2014). As of Jun 2019, only 1 limited population has been detected in Lake County, Florida, USA (Penca & Hodges 2018). However, 36 interceptions were recorded at Florida Agricultural Inspection Stations prior to Feb 2016 (S. Halbert, Florida Division of Agriculture and Consumer Services, Division of Plant Industry, personal communication). The potential for *H. halys* to establish in other areas of Florida depends on various environmental factors, including atmospheric moisture. We tested 15 to 90% RH at 25 °C on egg hatch, nymph survival, and adult reproduction to determine if *H. halys* could establish in Florida.

The current project was conducted in the quarantine laboratory at the University of Florida, Entomology and Nematology Department, Gainesville, Florida, USA, using an *H. halys* colony established with about 95 egg clutches obtained from a colony maintained for several yr at the USDA, ARS Crop Protection and Management Research Laboratory, Tifton, Georgia, USA. Rearing procedures were adapted from Medal et al. (2012), and maintained in the laboratory at 25 ± 3 °C, 55 ± 3% RH, and a 16:8 h (L:D) photoperiod. The insects were reared for 1 generation in Gainesville before either eggs, nymphs, or adults were placed into an 18 cm diam by 7.5 cm high plastic container for the tests. Containers were placed inside a reach-in environmental chamber maintained at 25 ± 3 °C with a 16:8 h (L:D) photoperiod provided by 32 W and 3,500 K lights mounted on a stand in front of the chambers (Ecolux XL Starcoat®, GE Lighting, Cleveland, Ohio, USA) (Niva & Takeda 2003). Lights were turned on at 4:00 A.M. and off at 8:00 P.M. The RH was maintained at 15%, 35%, 55%, 75%, or 90% provided by glycerol-water solutions of 390:8, 350:58, 302:119, 230:211, and 135:330, respectively. Salt solutions could have been used to maintain RH levels at constant temperatures (Winston & Bates 1960) but glycerol solutions are relatively independent of temperature (Soderstrom et al. 1990; Forney & Brandl 1992). A constant flow of humidified air was pushed from a flask containing a glycerol solution into the test container by an aquarium pump (Graystone Creations, Cleveland, Georgia, USA) (0.014 MPa air pressure and 0.00015 m³ per s air vol). Hobo software (Onset®, Cape Cod, Massachusetts, USA) was used to record temperature and RH inside the containers.

The stink bug stages to be tested were removed randomly from the colony and exposed to each RH in the test container. A single clutch containing 25 to 28 eggs was used for each egg hatch test. To further randomize the eggs, single clutches were divided into 5 approximately equal samples, and single samples were placed into the test container. The number of eggs that hatched and survived to third, fourth, fifth instar or adults were recorded after 7 d. A more precise nymph survival test was conducted by removing 6 second instar nymphs from the colony and placing them in a test container where they were fed peanuts and beans, then observed daily until they died or eclosed as adults. To test the effect of RH on reproduction, 2 males and 2 females were taken from the colony, marked with different dye colors (Testors®, Vernon Hills, Illinois, USA), placed in a test container, then exposed to 1 of the 5 RH treatments for 10 d. Adult survival and number of eggs oviposited were recorded. All tests were repeated 6 times and data analyzed by 1-way ANOVA with means compared by Tukey-Kramer HSD (RStudio 2012). Differences were considered significant at $P \leq 0.05$.

There was no significant difference in the percentage of egg hatch for whole and divided clutches for each RH (Fig. 1). However, significantly more eggs hatched at 55%, 75%, and 90% RH than at 15% and 35%. At 55%, 75%, and 90% RH, mean hatch was 85%, 81%, and 88% for whole clutches, and 83%, 87%, and 92% for divided clutches, respectively. Survival of nymphs from divided clutches was greater than from whole clutches at 55% and 75% RH, but not at 15%, 35%, or 90%. At 55% and 75% RH, respective mean nymph survival was 72% and 68% for whole clutches, and 75% and 77% for divided clutches, respectively.

Survival of the 6 second instar nymphs to the third instar was highest at 55%, 75%, and 90% RH, averaging 6.0, 5.5, and 5.1, respectively (Fig. 2). At these same relative humidities, mean survival to the fourth instar was 5.8, 4.8, and 4.6, and to the fifth instar was 4.5, 2.8, and 3.0, respectively. Second instar nymphs survived to the adult stage only under 55%, 75%, and 90% RH, and averaged 2.5, 1.3, and 0.8, respectively.

On the average, significantly more egg clutches (12.0 ± 0.3) were laid at 55% RH compared with 35%, 75% at 4.0 ± 0.2 each, whereas 90% RH produced only a mean of 1.0 ± 0.2 . No eggs were laid at 15% RH. The respective mean numbers of egg clutches oviposited at 35%, 55%, 75%, and 90% RH were 4.0 ± 0.2 , 12.0 ± 0.3 , 4.0 ± 0.2 , and 1.0 ± 0.2 .

Clearly, *H. halys* could establish and proliferate within the range of RH that occurs in Florida, exhibiting a wide host range that would

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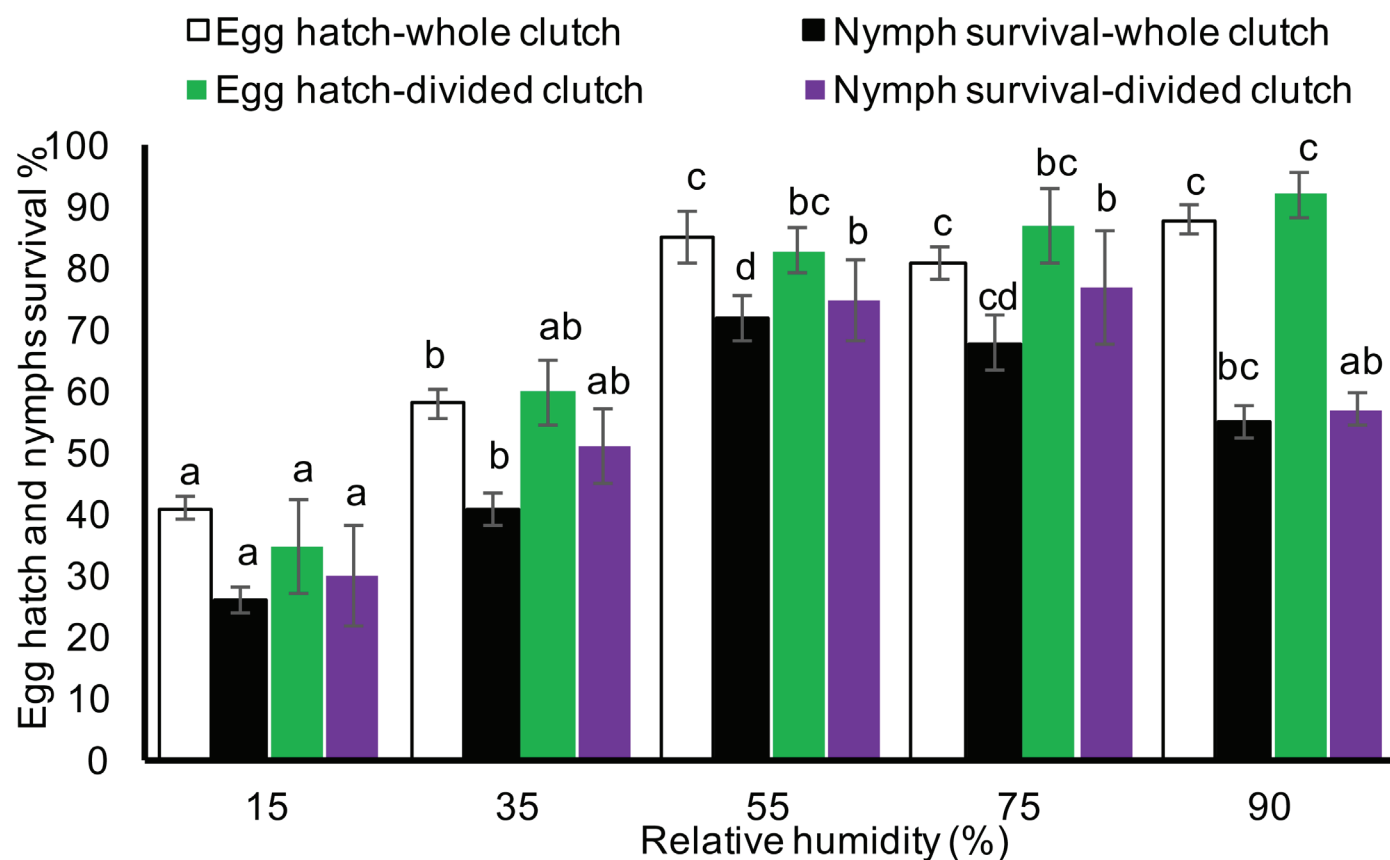


Fig 1. Mean (\pm SE) percent egg hatch and nymphal survival of *Halyomorpha halys* from whole and divided egg clutches exposed to 15% to 90% RH. Means with the same letter are not significantly different (Tukey-Kramer test, $P \leq 0.05$).

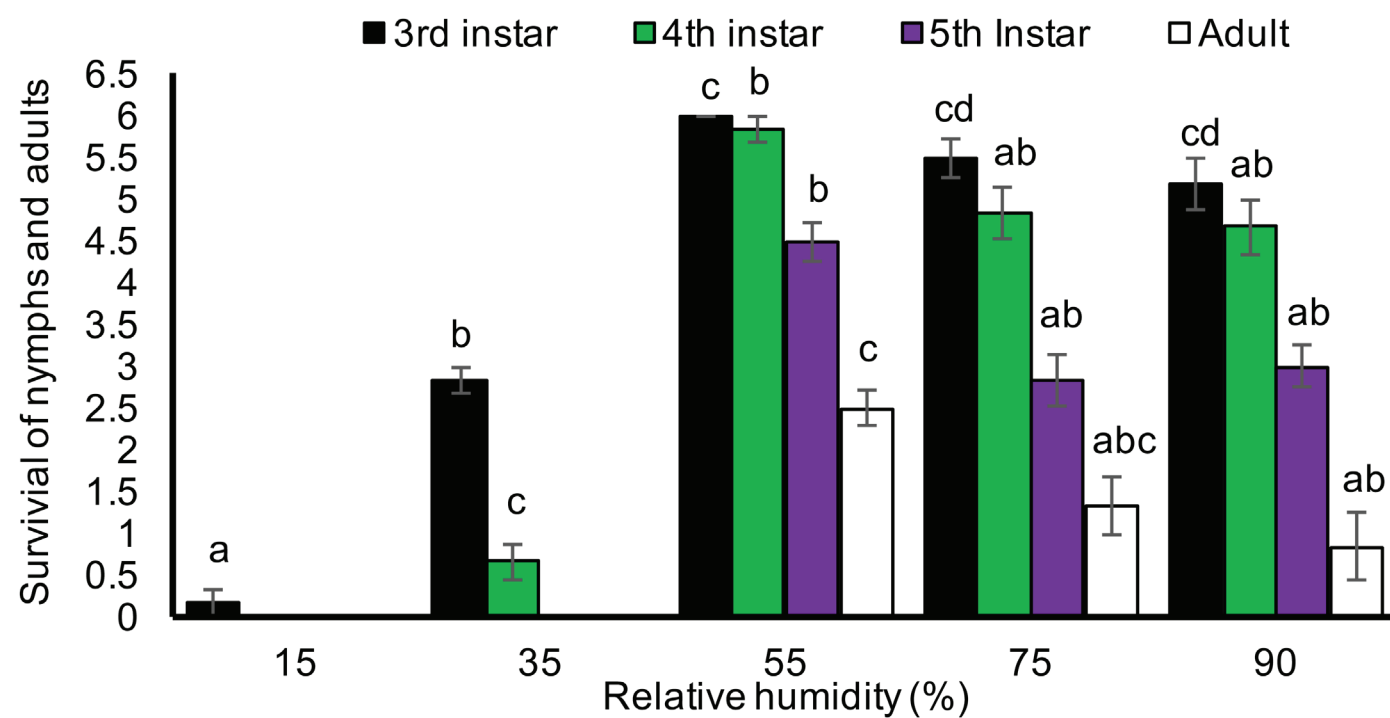


Fig 2. Mean survival (\pm SE) of 6 second instar nymphs to the third, fourth, and fifth instar, and adult stage of *Halyomorpha halys* exposed to 15% to 90% RH. Means with the same letter are not significantly different (Tukey-Kramer test, $P \leq 0.05$).

include fruit, vegetables, and ornamental plants (Hoebeke & Carter 2003; Leskey et al. 2012; Rice et al. 2014). Establishment of this pentatomid also would depend on other environmental factors (Niva & Takeda 2003), the seasonal availability of suitable hosts (Wallner et al. 2014; Acebes-Doria et al. 2016), and biotic factors, such as parasitoids, predators, and competitors. Moreover, adult *H. halys* overwinter in structures and other places that protect them from inclement weather. Therefore, it is probable that this highly mobile and adaptable pest will eventually become established in Florida.

The authors thank Joseph E. Eger, formerly affiliated with Dow AgroSciences, Indianapolis, Indiana, USA, for providing research guidance, and Julio Medal, previously employed by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida, USA, for guidance in rearing *H. halys*. We also thank Jennifer Carr, Nick Goltz, and Remy Powell from the University of Florida, Biosecurity Research and Extension Laboratory, Gainesville, Florida, USA, for assistance in collecting data.

Summary

Eggs, nymphs, and adults of *Halyomorpha halys* were exposed to 15%, 35%, 55%, 75%, or 90% RH provided by glycerol-water solutions. Although egg hatch and nymphal survival occurred at all concentrations, they were highest at 55% to 90% RH. Nymphs exposed at the second instar reached the adult stage only at 55% to 90% RH. Females laid the greatest number of egg clutches at 55% RH. Therefore, we believe that *H. halys* could survive in Florida at 55% to 90% RH, but would be most prolific at 55%.

Key Words: insect rearing; relative humidity; brown marmorated stink bug; hatching

Sumario

Los huevos, las ninfas y los adultos de *Halyomorpha halys* fueron expuestos al 15%, 35%, 55%, 75% o 90% de HR proporcionado por las soluciones de agua y glicerol. Aunque la eclosión de huevos y la sobrevivencia de las ninfas se sucedieron en todas las concentraciones, ellas fueron más altas con una HR del 55% al 90%. Las ninfas expuestas en el segundo estadio alcanzaron el estadio adulto solo con 55% a 90% de

HR. Las hembras pusieron el mayor número de grupos de huevos a 55% de HR. Por lo tanto, creemos que *H. halys* podría sobrevivir en Florida con 55% a 90% HR, pero sería más prolífico con 55%.

Palabras Clave: cría de insectos; humedad relativa; chinche marrón apestoso; eclosión

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