**N O T E**

# Meridic Diet for *Halyomorpha halys* (Heteroptera: Pentatomidae)[1]

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*Halyomorpha halys* (Stal) (Heteroptera:Pentatomidae), the brown marmorated stink bug, was first recorded in the U.S. in 1996 in Allentown, PA, and has since spread to 38 states and the District of Columbia (Leskey et al. 2012, J. Econ. Entomol. 105(5): 1726 - 1735). The brown marmorated stink bug is a native to northeast Asia, Japan, and Korea where it has been a serious fruit pest since postWorld War II. It was not realized that the insect was invasive until 2001 when the population exploded (Hoebeke and Carter 2003, Am. Pro. Entomol. Soc. Wash. 105: 225 - 237). This phytophagous pentatomid causes considerable damage to various trees, vegetables, and leguminous crops. Brown marmorated stink bug is highly mobile and often switches hosts, moving from plants with early-ripening fruits to those with late-ripening fruits. The wide range of host plants and seasonal migration among various hosts make the study of its life cycle biology and ecology difficult (Funayama 2004, App. Entomol. And Zool. 39(4): 617 - 623). The insect feeds via a stylet inserted into plant tissues that renders the fruit unmarketable as a fresh product and also becomes a disturbing pest for homeowners when it is attracted in very large numbers to houses in search of protected, overwintering sites. Beginning in 2006, commercial fruit growers reported damaged fruit in eastern Pennsylvania and western New Jersey orchards. The year 2010 marked a huge surge in its population density in the midAtlantic, both in homes as a vexatious pest and a detrimental herbivore in orchard systems, where it caused more that $37 million in crop losses (Martinson et al. 2013, Ann. Entomol. Soc. Am. 106(1): 47 - 52).

The female typically lays an egg mass containing 28 eggs which hatch in 3 - 7 days (Nielsen and Hamilton 2009, J. Econ. Entomol. 102: 1133 - 1140). The nymphs pass through 5 stadia (substages), with a molt between each, before reaching the adult stage (Nielsen et al. 2008, Environ. Entomol. 27: 348 - 355; Takahashi 1930, Pg. 617 - 620 *In* Insect Pests on Fruit Trees). Because of the relatively recent introduction of this

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pest into the U. S., available information on nutritional requirements for adult and nymphal stages is deficient.

Laboratory rearing of insects for research, especially damaging insect pests such as the brown marmorated stink bug, is crucial to the study of their life cycles, host preferences, and management tactics including susceptibility to potential biological control agents (Medal et al. 2012, Florida Entomol. 95(3): 800 - 802). Lack of an artificial diet has been a major obstacle in laboratory studies of *H. halys.* Bioassays of insecticide efficacy and resistance are facilitated with this type of uniform diet. Currently, in the U.S., brown marmorated stink bug laboratory colonies are raised on a variety of fruits, nuts, and vegetables. The diets vary, but consist primarily of green beans, carrots, soybeans, sunflower seeds, and apples, with other seasonal fruits and vegetable added (BMSB Working Group 2011). This diet for rearing has many drawbacks, most notably the labor intensity and expense of maintaining the fresh fruits and vegetables, the rapid deteriorations of the food at normal laboratory temperature, and the lack of a standardized rearing protocol. An insect's diet affects its development during both immature and adult stages. Adult emergence, female size, preoviposition egg production, and nymphal development times are affected during the immature stage; whereas, effects on postoviposition, egg production, diet ingestion, sexual acceptance, or longevity (survival) occur during adulthood (Cangussu and Zuoloto 1997, Revista Brasileira de Biologia. 57: 611 - 618). For insect rearing, the use of meridic diets has many advantages over diets containing host plant materials because production is easier, quality is uniform, and rearing can take place throughout the year. The aim of the present study was to develop a meridic diet for optimum growth and development of this insect. The reduction in rearing costs and the simplicity of producing the diet will be economically advantageous for future research of the *H. halys.* The in-house colony of brown marmorated stink bug was established in 2011 using insects obtained from the Pennsylvania State University research station in Bigglerville, PA, Pittsburgh, PA, and the USDA-ARS Appalachian Fruit Research Station in Kearneysville, WV. The insects were reared in 19-L plastic buckets, insect-rearing containers (Plastican, Inc., Leominster, MA) with netted tops and mesh sleeves. The colonies were placed in an environmental chamber, maintained at a temperature of 25°C, approx. 60% RH, and a photoperiod of 16:8 (L:D)h. The colonies were fed a diet of carrots, green beans, tomatoes, sunflower seeds, blueberries, grapes, and corn. Water-soaked sponges and cotton balls soaked in a 1:3 honey-water solution were placed on the bottom of the containers. Containers were checked daily for new egg masses which were removed and placed in 250-ml plastic deli containers (Fabri-Kal, Kalamazoo, MI). The lids of the deli containers were fitted with a 6.5 cm circle of netting material to allow for air circulation. All containers were kept in an environmental chamber set at 25°C, 60% RH, and a photoperiod of 16:8 (L:D)h. Nymphs remained in these containers until their 5$^{th}$ molt when they were individually transferred to the adult colonies. Nymph stages were fed a diet similar to the above fresh fruit and vegetable diet except for eggs masses and 1$^{st}$ instars that received only a moistened cotton ball. Food in these containers was replenished 3 times a week to prevent mold and rotting. Under these conditions, egg hatch to adult eclosion typically takes 538 DD base 14.17°C with another 148 DD for female maturation (Neilson and Hamilton 2009, Ann. Entomol. Soc. Am. 102: 608 - 616).

Our meridic diet was developed using a combination of the *Lygus hesperus* (Knight) (Miridae:Hemiptera) diet (48 g) and the tobacco hornworm (*Manduca sexta* [L.]) (Lepidoptera: Sphingidae) diet (20 g) to which frozen blueberries (100 g), frozen edamane (100 g), pinto beans (40 g), agar (10 g), and distilled water (550 ml) were added. All commercial diets were purchased from Bio-Serv Corporation (Frenchtown, NJ). All mixing bowls, utensils, and supporting equipment were sterilized with a 10% Clorox solution immediately before use. The pinto beans were soaked overnight in water, boiled until fully cooked, and the water was drained. The blueberries and edamane were mixed in a blender along with the cooked pinto beans, the weighed *M. sexta* diet and *L. hesperus* diet, and 250 ml of distilled water. The agar was mixed with 300 ml of distilled water in a saucepan, heated to 80°C, and then added to the contents of the blender. Eight milliliters of a 10% formaldehyde solution was added to the mixture to inhibit mold in the diet. The diet was blended for 1 - 2 min. at high speed. Hot diet was poured into a plastic squeeze bottle and dispensed into 32-mL transparent plastic cups (Solo Cup a diversion of Dart Container Corp, Mason, MI) to cover the bottom to approx. 2 cm thick. Lids were placed on the cups, which were stored in the refrigerator. The diet solidified to a gel-like consistency that could be easily consumed by the BMSB piercing and sucking stylet mouthparts. The quantity made was sufficient for approx. 150 cups. Although the amount of diet in each individual cup is more than is needed for a week of insect development, reducing the amount resulted in the diet desiccating.

Two 1$^{st}$ instar nymphs were placed into each diet cup. These nymphs were transferred to diet cups weekly. Nymphs were reared to maturity in diet cups and compared with nymphs of the same age reared on the standard fresh fruit and vegetable diet. Live weights of the adults were taken within 24 h of the final molt into adulthood. Insect mortality was lessened when 2$^{nd}$ instars were used for transferring into diet cups. First instars are reported to not eat until the first molt. (Leskey and Hamilton, 2012, BMSB NEIPM Working Group Web Page).

Twenty-four adults (12 females, 12 males) newly emerged from overwintering sites were acquired from USDA-ARS Appalachian Fruit Research Station in Kearneysville, WV. These were placed in each of two 19-L plastic buckets, insect-rearing containers (as described previously). Adults were maintained under laboratory conditions at 25°C, 60% RH and a photoperiod of 16:8 (L:D)h. One group was reared on the standard fresh fruit and vegetable diet, and the other group was fed exclusively the meridic diet. The meridic diet used in the rearing containers was poured into 10-cm plastic petri dishes (VWR International, Radnor, PA) to 3 cm thick. Three diet dishes were placed in the rearing container twice a week. Both rearing containers were supplied with water-soaked sponges. Numbers of eggs were enumerated and raised to maturity from both containers. After 75 d, adults were weighed and their pronotums were measured on the anterior widest section of their dorsal side to determine growth and nutritional health (Funayama 2006, App. Entomol. And Zool. 41(3): 415 - 418). Adults that died before the 75 d time period were weighed, and pronotum width was measured.

The mean weight of females reared on meridic diet from 2$^{nd}$ instars ($n$ = 20) was 146 ± 22.2 mg, and mean pronotum width was 9.46 ± 0.510 mm. Females reared on the standard fresh fruit and vegetable diet from 2$^{nd}$ instar ($n$ = 21) had a mean weight of 142 ± 19.8 mg and mean pronotum width of 9.31 ± 0.49 mm. Males reared on meridic diet from 2$^{nd}$ instar ($n$ = 18) had a mean weight of 79 ± 16 mg and mean pronotum width of 8.42 ± 0.76 mm. Males reared on the standard fresh fruit and vegetable diet ($n$ = 17) had a mean weight of 82.8 ± 15 mg and mean pronotum width of 8.68 ± 0.76 mm. Nymphs and adults showed normal feeding behavior on our meridic diet. All nymphs reared on the meridic diet took longer to develop than the nymphs reared on the fresh fruit and vegetable diet with a maturity delay time of approx. 83 DD (7 days under laboratory conditions).

The adult females in the colony fed only the meridic diet had a mean weight of 159 ± 34.1 mg and pronotum width of 9.50 ± 0.52 mm. The adult females maintained on the standard fruit and vegetable diet had a mean weight of 139 ± 42.9 mg and pronotum width of 9.38 ± 0.53 mm. The adult males fed the meridic diet had a mean weight of 84.9 ± 14 mg; whereas, the adult males fed the standard fruit and vegetable diet had a mean weight of 81.8 ± 15 mg. The pronotum mean width was 8.50 ± 0.14 mm for the meridic fed males and 8.69 ± 0.15 mm for the males fed the standard diet. Weights of males and females and widths of male and female pronotums were statistically analyzed with a one-way analysis of variance (ANOVA) and Tukey's studentized range (HSD) test at $P \leq 0.05$. All analyses were done using SAS V.9.1 (2002 - 2003). There were no significant differences in weight or length of pronotum in the males or females with either diet (Fig. 1).

Females on the meridic diet laid a total of 20 egg masses. The females fed the standard fresh fruit and vegetable diet laid 21 egg masses. Egg masses in both colonies averaged 28 eggs consistent with previous studies and had ovarioles in multiples of 7 (Nielsen et al. 2008, Environ. Entomol. 27: 348 - 355). Survivorship between colonies was comparable with the first male in each colony dying 1 month after the initiation of the colony, whereas 7 adults from the standard diet colony and 8 adults from the

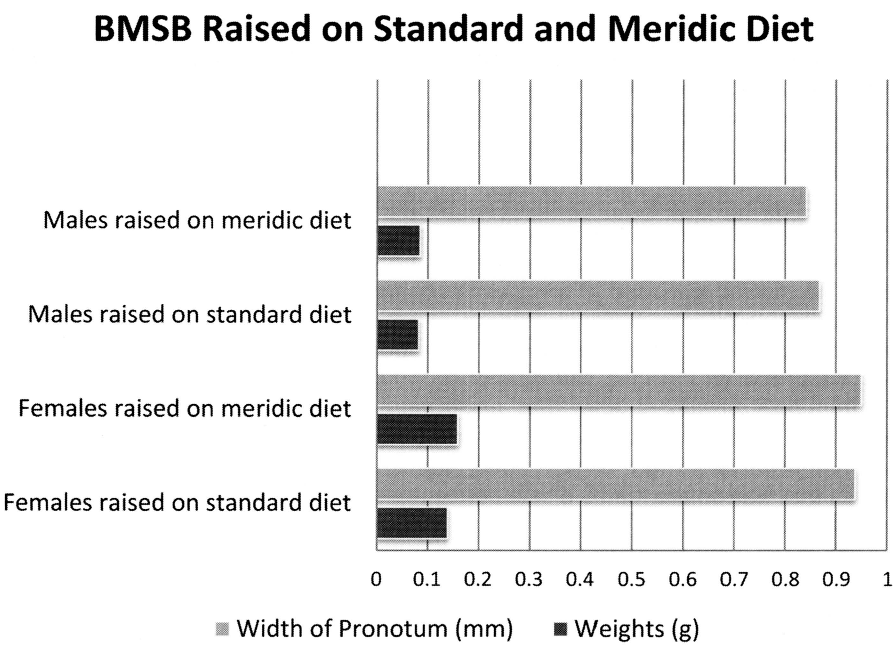


**Fig. 1. Weights (g) and widths of pronotums (mm) of male and female brown marmorated stink bugs (BMSB) raised on standard and meridic diets. There were no significant differences in weight or length of pronotum with males or females fed either diet.**

meridic diet colony survived until the termination of the experiment at the life expectancy threshold of the insect. Currently, most laboratory colony-reared brown marmorated stink bugs have a high mortality rate with egg-to-adult survival ranging from 50 - 70% (BMSB Working group 2011).

Despite the delayed development time of the nymphs on the meridic diet, the total nymphal mortality was equivalent to that of those reared on the standard diet. The nutritional health of both the adults fed solely on the meridic diet for 75 d and the nymphs reared to adults on the meridic diet, as defined by weight and width of pronotum, did not differ significantly. This work demonstrated for the first time that the adult brown marmorated stink bugs could survive with no abnormalities, lack of fecundity, or nutritional decline on an artificial diet as well as a diet of fresh fruits and vegetables. The ease of making large amounts of this diet in a short period of time combined with the success in rearing and maintaining adult brown marmorated stink bugs indicates that this is a good starting point for further research into the rearing of larger quantities of the insect on artificial diets. Although this is not an exhaustive evaluation of continuing generations reared on this diet, the long rearing time of brown marmorated stink bug and the importance of developing a meridic diet make this study relevant as a defined base diet to further determine the nutritional requirements of brown marmorated stink bug and to explore the significance of these individual nutrients in brown marmorated stink bug adult fecundity.

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