



MARMORATING

A day in the life of BMSB rearing at USDA ARS Beltsville

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Brown Marmorated Stink Bug Working Group Meeting
Winchester, Virginia
3 December 2014

BMSB rearing: overview

OUTLINE OF TALK

- Uses of culture and origin
- Materials used
- Daily Tasks
- Weekly Tasks
- Productivity and “gearing up” the rearing





BMSB research: Uses for culture so far

- 🌿 Pheromone discovery: male volatile emission
(Khrimian et al. 2014*; Weber et al. 2014*)
- 🌿 Attraction of adults and nymphs to volatiles
(Weber & Khrimian ongoing)
- 🌿 Field parasitism of egg masses in different habitats
(Greenstone; Cornelius; Herlihy; ongoing)
- 🌿 Repellency experiments with adults in lab (Zhang et al. ongoing)
- 🌿 Genomics and transcriptomics with RNAi using inbred F9 adults
(Sparks et al. 2014*, Gundersen-Rindal et al. ongoing)
- 🌿 Establishment of other research colonies
(Del Fosse et al. ongoing)

* PUBLICATIONS:

Khrimian, Ashot, Aijun Zhang, Donald C. Weber, Hsiao-Yung Ho, Jeffrey R. Aldrich, Karl E. Vermillion, Maxime A. Siegler, Shyam Shirali, Filadelfo Guzman, and Tracy C. Leskey. 2014. Discovery of the aggregation pheromone of the brown marmorated stink bug (*Halyomorpha halys*) through the creation of stereoisomeric libraries of 1-bisabolen-3-ols. *Journal of Natural Products* 77:1708-1717.

Sparks, Michael E., Shelby, Kent S., Kuhar, Daniel, & Gundersen-Rindal, Dawn E. (2014). Transcriptome of the Invasive Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae). *PloS One* 9(11), e111646

Weber, Donald C., Tracy C. Leskey, Guillermo Cabrera Walsh, and Ashot Khrimian. 2014. Synergy of aggregation pheromone with methyl (E,E,Z)-2,4,6-decatrienoate in attraction of *Halyomorpha halys* (Hemiptera: Pentatomidae). *Journal of Economic Entomology* 107:1061–1068.

BMSB rearing: overview



Origin of Beltsville culture

Established by Jeff Aldrich in 2007

from adults collected in Allentown, Pennsylvania

Supplemented annually (2008-2011) with ~20 field-collected adults from Beltsville, Maryland

No field supplementation since 2011

Other Pentatomid cultures:

Euschistus heros (Brazil; under APHIS permit)

Nezara viridula (Georgia, USA; under APHIS permit)

Murgantia histrionica (Maryland source)

Materials Used

Percival chambers

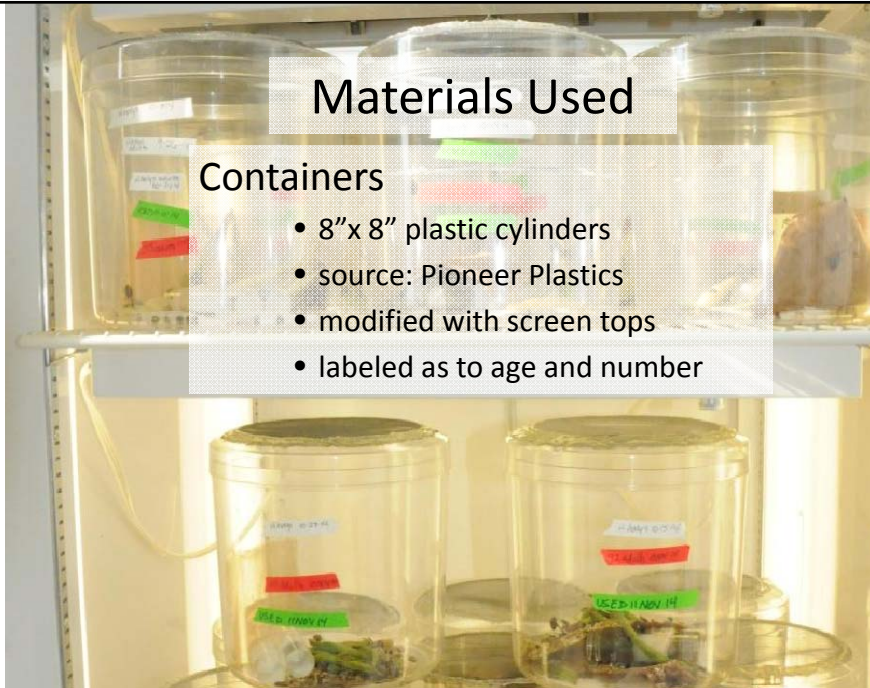
- Temperature 25°C
- ~50% humidity
- 16:8 Light/dark cycle



Materials Used

Containers

- 8"x 8" plastic cylinders
- source: Pioneer Plastics
- modified with screen tops
- labeled as to age and number



Materials Used

Rain-X[®]

spray and wipe ~3cm around rim of cylinder



Materials used

Fitting out containers:

- lined with 150mm filter paper
- 2 water vials with cotton plugs, attached to one another with a rubber band to prevent rolling.
- Acrylic mesh for egg laying.
- Seed paper.
- Organic green beans from MOM's supermarket or Trader Joes.



Organic green beans (purchased commercially)

- Triple-rinsed and then allowed to dry
- Replaced every 2-3 days
- Have also used field-grown beans and commercial snow peas



Seed Paper

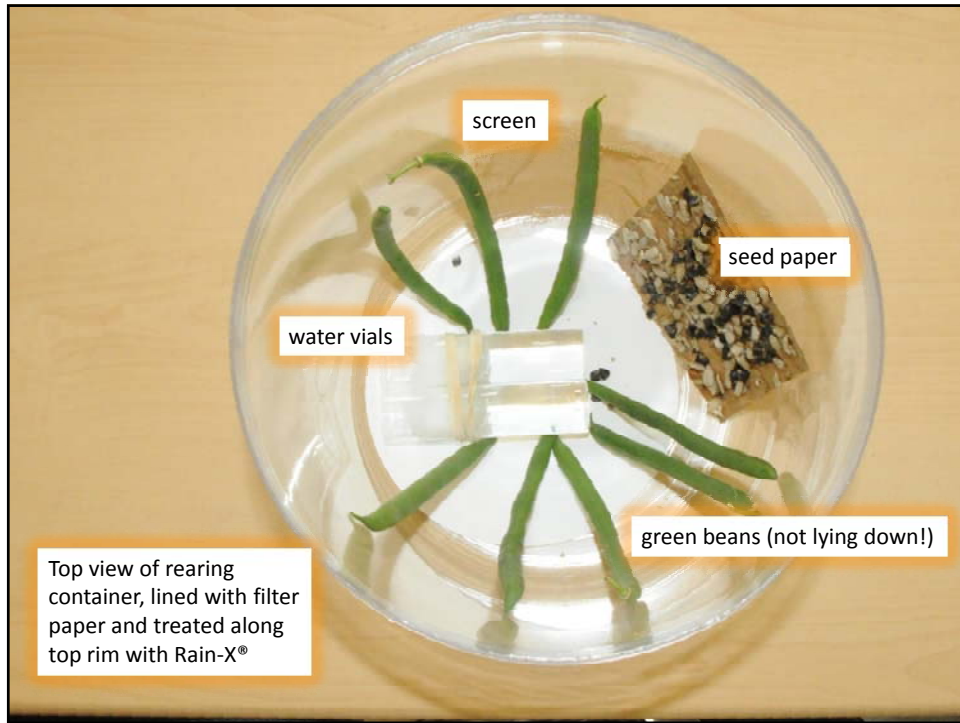
- 2:1 hulled sunflower to buckwheat seed ratio.
- Glued to brown craft paper with Gold Harvest organic wheat wallpaper paste.

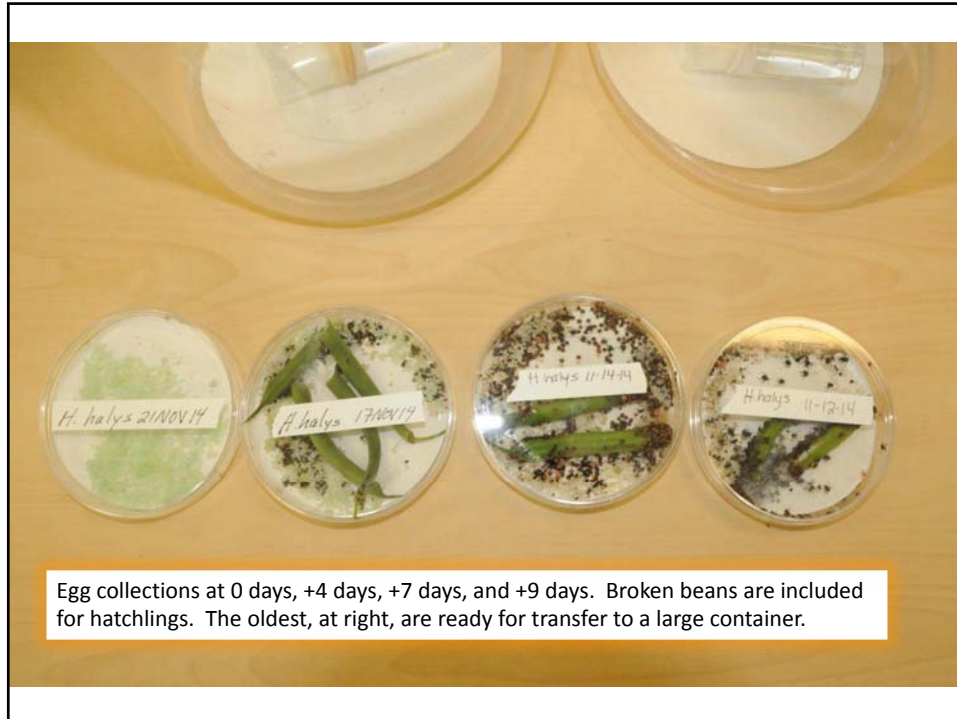


Daily and weekly tasks:
*see also the supply list and
protocols at end below*



Transferring adults to a new rearing container



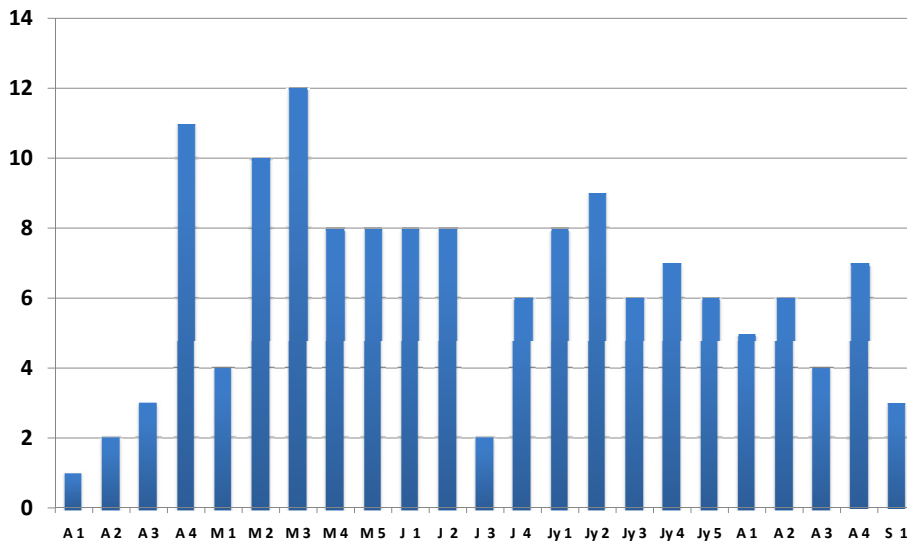


BMSB production for 2014 field season

~50-70 adults/container (containers are split when large nymphs or new adults to achieve this range of numbers per container)

- March ~ 200 adults in culture
(~6 containers)
- July ~ 2,000 adults in culture
(30-35 containers)
- September ~ 500 adults in culture
(8-10 containers)

**Number of nymphal containers set up per week
April - September 2014**



Halyomorpha halys, Brown marmorated stink bug

Supplies and Standard Operating Procedures for Rearing

Invasive Insect Biocontrol & Behavior Lab, November 2014

for Brown Marmorated Stink Bug Workshop, Winchester, Virginia, 3 December 2014

Supplies:

- 7-15/16" X 7-1/2" hard plastic round containers (Pioneer Plastics, part # 289C)
<https://www.pioneerplastics.com/shop/round-containers/289c/>
- 100 X 22 mm petri dishes
- Whatman filter papers
[http://www.coleparmer.com/Category/Whatman_Filter_Papers/1289?referred_id=20965&mkwid=\[distribution\]\[uniq_id\]&pcrid=\[creative_id\]&kw=\[keyword_text\]&mt=\[matchtype\]&pdv=c&gclid=CLCM4f7epcICFcv7AodDw0AgQ](http://www.coleparmer.com/Category/Whatman_Filter_Papers/1289?referred_id=20965&mkwid=[distribution][uniq_id]&pcrid=[creative_id]&kw=[keyword_text]&mt=[matchtype]&pdv=c&gclid=CLCM4f7epcICFcv7AodDw0AgQ)
 - 150 mm (Whatman, catalog # 1001-150)
 - 90 mm (Whatman, catalog # 1001-90)
- Seed (hulled sunflower and intact buckwheat) <http://meyerseedco.com/>
- Organic green beans
<http://momsorganicmarket.com/location-and-stores?location-id=5> or
<http://www.traderjoes.com/>
- Amber Lumite fine mesh screen (32x32 mesh per inch)
<http://www.bioquip.com/search/DispProduct.asp?pid=7250P>
- Large cotton balls, non-sterile
<http://www.uscottonsite.com/branded-products-products.html> (catalog # 79111000)
- Vials: 8 dram
- Model paint brushes
- Parafilm "M" (4" X 250',
<http://www.parafilm.com/FloraTapeHome.aspx?Menu=2>)
- Indelible markers
- Tape (packing, Scotch)
- Rain X ® original glass treatment, 3.5 oz

Comparable products are available from multiple vendors. Mention of commercial products is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture.

Set up container

1. Set up as needed by checking eggs in chamber P1
2. Make sure container of lid has had screening installed, and Rain-X treatment
3. Place 150 mm filter paper on bottom of container
4. Place two vials with water-soaked cotton, rubber banded together, on top of the filter paper
5. Place one pre-cut seed sheet in container, and peice of plastic screen
6. Place 5 - 8 green beans in container
7. Place eggs or neonates in container

Maintain container

1. Check every day
2. Change beans every day in adult containers and every-other-day in the nymphal containers
3. Change filter paper on bottom of container as needed
4. Change seed sheets every as needed (at any sign of mold)
5. Change from seed sheet to seed mesh pouch when bugs start laying eggs

Harvesting eggs

1. This is done every Monday and Friday
2. Put 90 mm filter paper in bottom half of 100mm petri dish
3. Put a piece of tape on the lid of the petri dish and label "*H. halys*" with date
4. Remove fine-mesh screen, fine-mesh screen seed pouch, beans, and 150 mm filter paper and peel off the light blue-green eggs into the weighed petri dish
5. Replace filter paper and beans with new, and water vials as needed

Invasive Insect Biocontrol & Behavior Lab, November 2014

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