Have we got the right ratio?

Attraction of brown marmorated stink bug to different pheromone ratios, and to different ratios of mixed pheromone and (E,E,Z)-methyl-2,4,6-decatrienoate (MDT)

Brown Marmorated Stink Bug Workshop
30 November 2016, somewhere west of Winchester, Virginia

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USDA Agricultural Research Service

Invasive Insect Biocontrol & Behavior Lab & Appalachian Fruit Research Lab

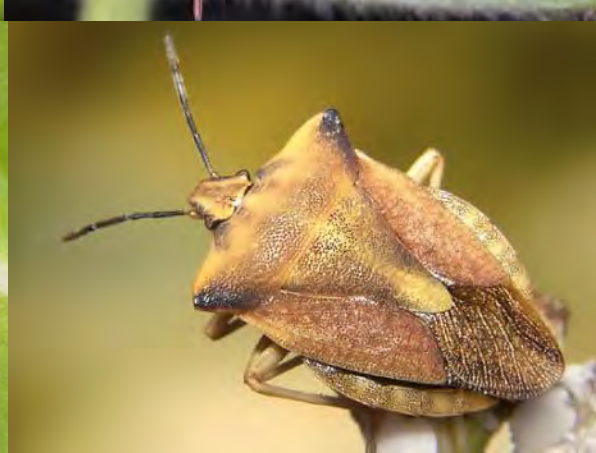
Beltville, Maryland, and Kearneysville, West Virgina



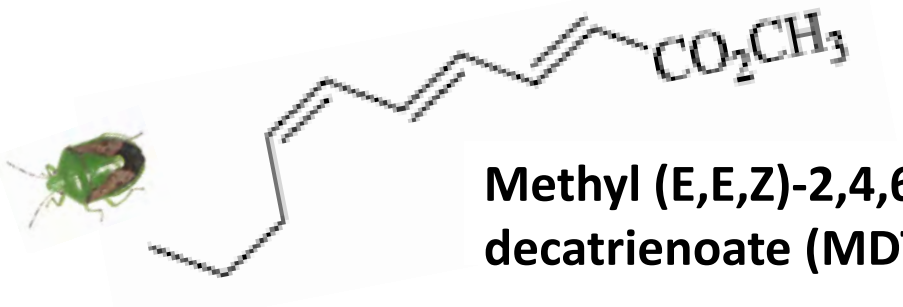

©Terry Thorn







- Discovery by Sugie *et al.* 1996 of the aggregation pheromone of *Plautia stali*, the brown-winged green stink bug of Asia:



Methyl (E,E,Z)-2,4,6-decatrienoate (MDT)

- Japanese trapping revealed MDT attraction of two other species: *Glaucias subpunctatus and Halyomorpha halys* (BMSB), the latter mainly in the late season.

- BMSB was first identified in the USA in 2001, having been introduced several years earlier in PA.

- Synthetic chemistry and trapping with of BMSB with MDT and its stereoisomers in USA.

Sugie et al. 1996; Funayama 2008; Khrimian 2005; Khrimian et al. 2007, Leskey et al. 2012

Methyl (E,E,Z)-2,4,6-decatrienoate (MDT)

Halyomorpha halys

Brown marmorated stink bug

Asian native

responsive to MDT

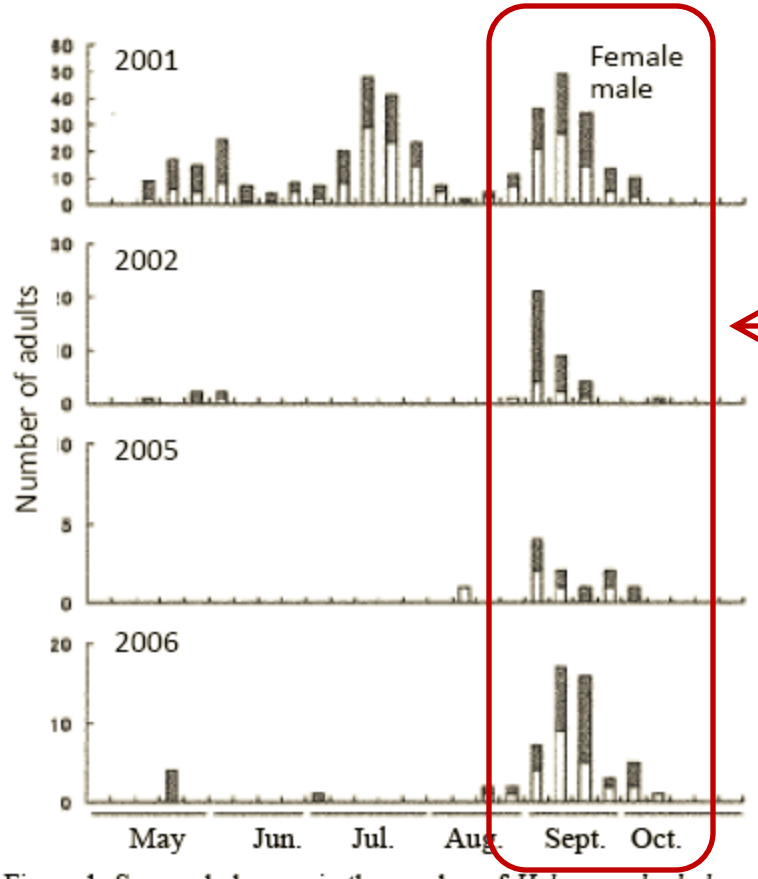
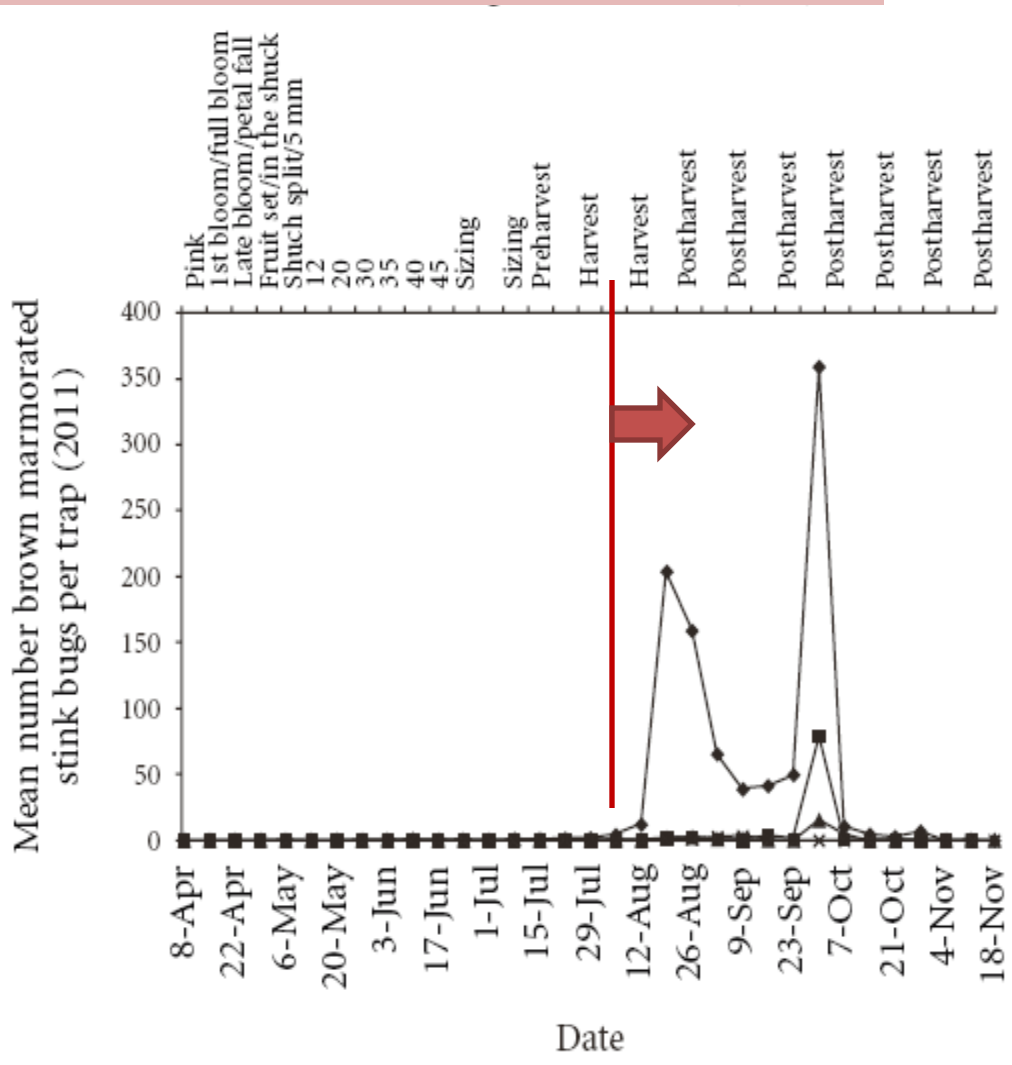


Figure 1: Seasonal changes in the number of *Halyomorpha halys* adults captured in traps baited with synthetic aggregation pheromone of *Plautia crossota stali* in a coppice in Akita Prefecture in 2001, 2002, 2005 and 2006. Bars: total number of adults captured in 6 traps.

... but in most years, almost exclusively late in the season

Structure & synthesis from Khrimian 2005, Tetrahedron 61:3651–3657;
Field data from Funayama 2008, Applied Entomology and Zoology 52: 69-75.

Captures using black pyramid traps baited with MDT (50mg) in VA & MD apple orchards, 2011



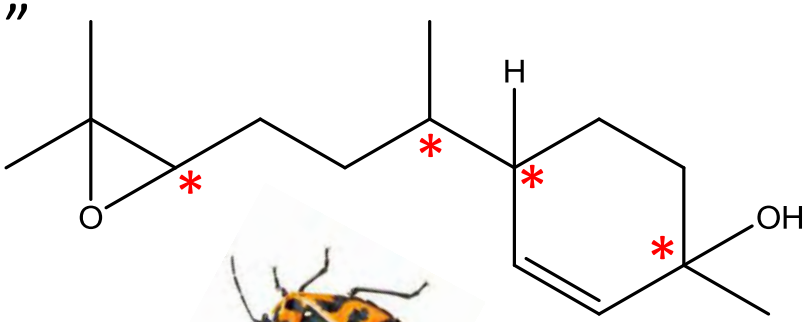
Halyomorpha halys
Brown marmorated stink bug

Asian native responsive to MDT

but usually only after harvest of apple crop, in late season

Leskey et al. 2012, Psyche 2012: 535062, Figure 5b. Also shown by Khrimian et al. 2007, J.Ag.Food Chem.56: 197-203.

- Harlequin bug (*Murgantia histrionica*) aggregation pheromone discovered and partially characterized by UC Riverside researchers; dubbed “murgantiol” =10,11-epoxy-1-bisabolen-3-ol with undefined stereochemistry (chiral centers: carbons 3,6,7,10)

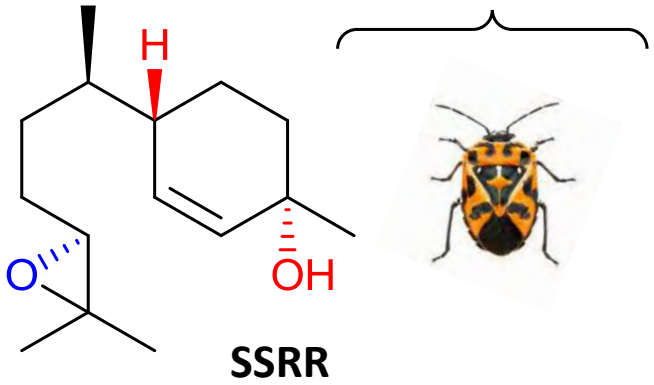


- Male brown marmorated stink bug, *Halyomorpna halys*, found to produce 10,11-epoxy-1-bisabolenol(s), but with undetermined stereostructures and biological activities
  (Dr. Hsiao-Yung Ho, Taiwan Academy of Science)

Zahn et al. 2008; Ho unpublished 2009

- Hsiao-Yung Ho shared this information on BMSB emissions with Jeff Aldrich at IIBBL, and Khrimian *et al.* (2014a,b), through very involved analytical and synthetic stereochemistry, including a “library” of 10,11-epoxy-1-bisabolen-3-ols, identified the specific components of both harlequin bug and BMSB aggregation pheromones!

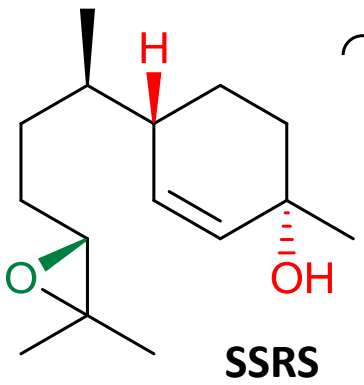
Murgantia histrionica pheromone



SSRR

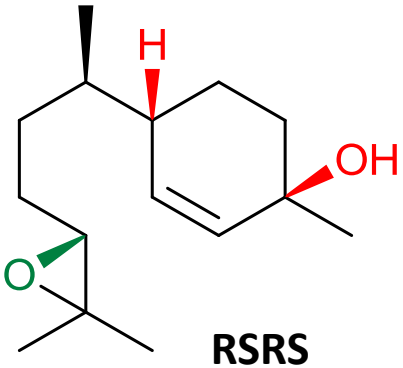
(1 : 1.4)

Halyomorpha halys pheromone



SSRS

(3.5 : 1)



RSRS

Back to ratios (=mixology), doses, and purity !

- ratio between pheromone components
  - most stink bug pheromones have at least two components (two each for BMSB & Hq bug)
  - stereoisomer synthesis is challenging
  - purity an issue (biological, economic)
- ratio between pheromone & other attractants
  - BMSB and Hq bug examples here too: BMSB with MDT (*Plautia stali* pheromone), and Hq bug with plant volatiles
  - optimal mix may change seasonally
  - economic issues for trapping, etc.

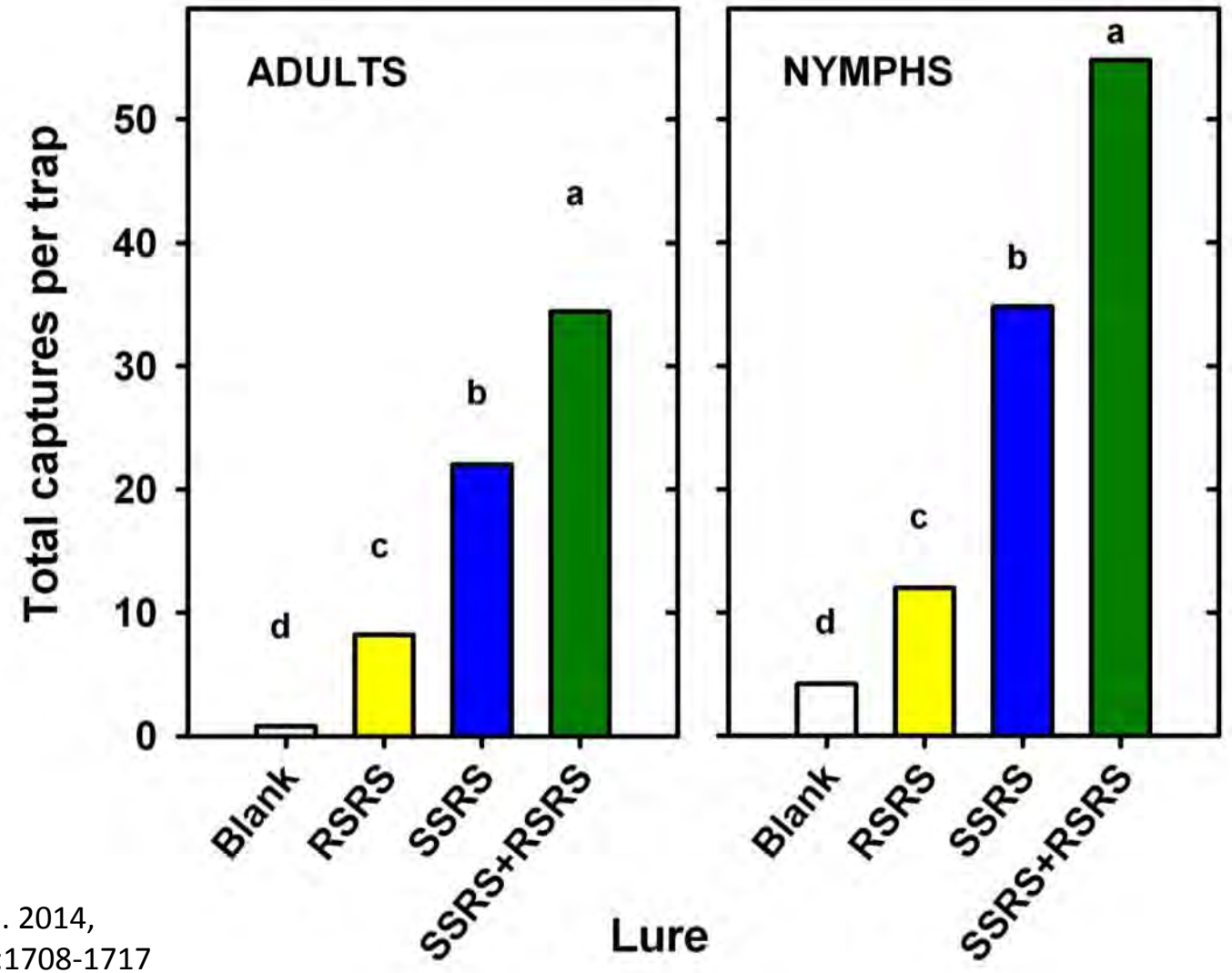
Back to ratios doses, and purity! – our bugs

- issue: individual stereoisomers are expensive; better to use a mixture – but only *if* it works!
- economical synthesis from *R*-citronellal produces 8 isomers – for each species, that's 6 that are not part of pheromone
- synthesis also produces a different ratio than that emitted by either species:
  - for BMSB, SSRS:RSRS is 1:1.7 instead of 3.5:1 !
  - for Hq bug, SSRS:SSRR is 1:1 instead of 1.4:1 .

H. halys captures in pyramid traps with pheromone components

loading: RSRS, 4mg; SSRS, 4mg; SSRS+RSRS, 4mg+1.1mg

5 randomized blocks, 8 June through 30 July 2013, Beltsville, Maryland



Khrimian et al. 2014,
J.Nat.Prod.77:1708-1717

Purity: How important for attraction?

For BMSB, mixed-isomer preparations with equivalent principal component, (3*S*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol, attract comparable numbers of adult (equal male/female) and nymphal bugs in the field

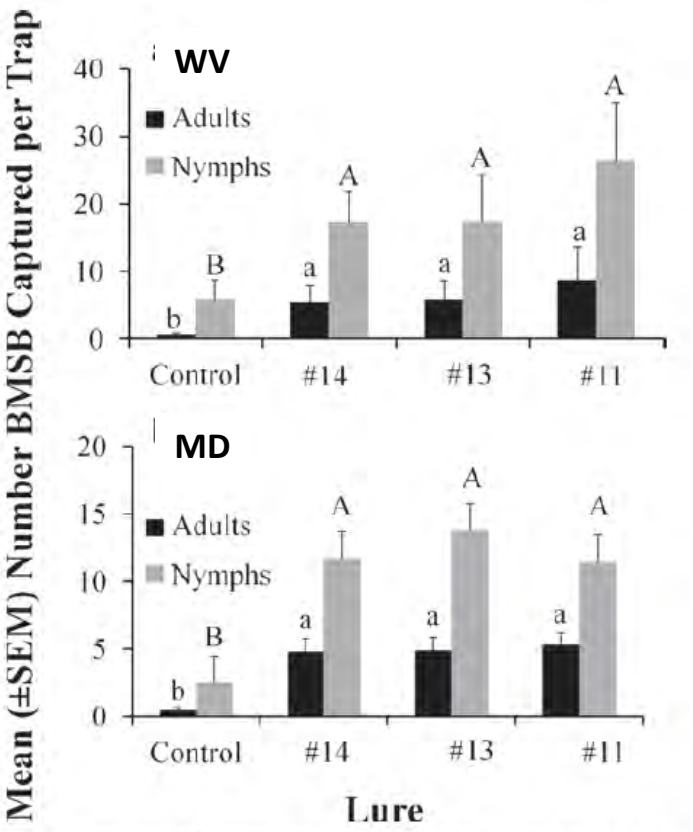


Fig. 4 Purity trial showing mean catches of *Halyomorpha halys* (± SE) in a West Virginia and b Maryland from 12 Aug—4 Sep 2013 for lures that were synthesized using racemic citronellal and were minimally purified by one or no chromatographic separations. Bars with shared

Leskey et al. 2015, J. Chem. Ecol 41: 418-429

Halyomorpha halys
Brown marmorated stink bug

With... **knowledge of seasonally-limited attraction of MDT**
and... **new availability of quantities of BMSB pheromone**
as... **an isomer mixture which we knew had comparable attractiveness to pure isomers ...**

We set up a simple factorial experiment:


BMSB pheromone (mixed isomer with ~2mg of SSRS)


MDT (66mg)

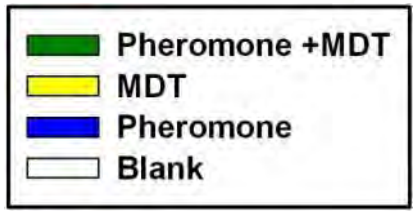

Both

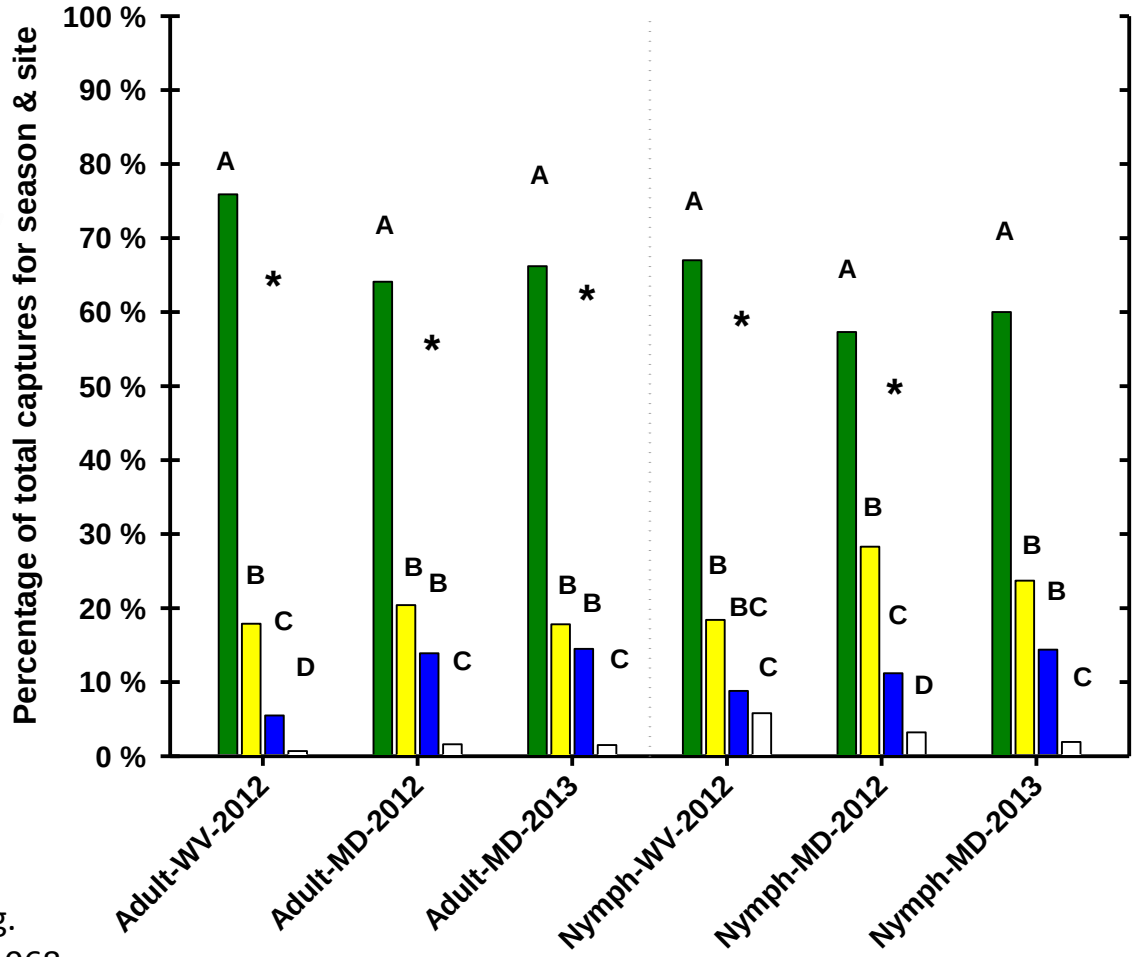

Neither

(black pyramid traps, MD & WV, randomized complete blocks, season-long 2012 & in MD 2013)

Use of pheromone together with MDT produces synergistic attraction ...

Halyomorpha halys
Brown marmorated stink bug



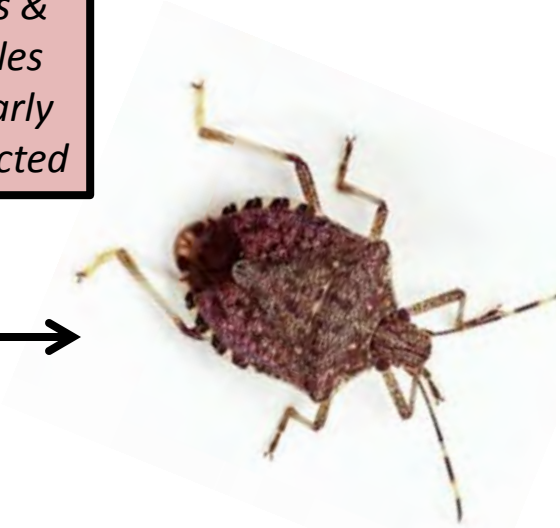


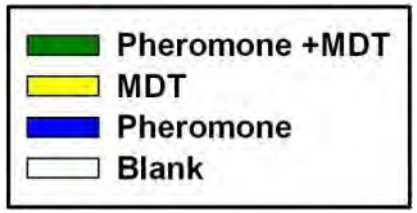
pheromone is mixed-isomer synthetic lure with ~2mg of SSRS-murgantiol; MDT loaded 60-66mg.
Weber et al. 2014, J.Econ.Entomol. 107: 1061–1068.

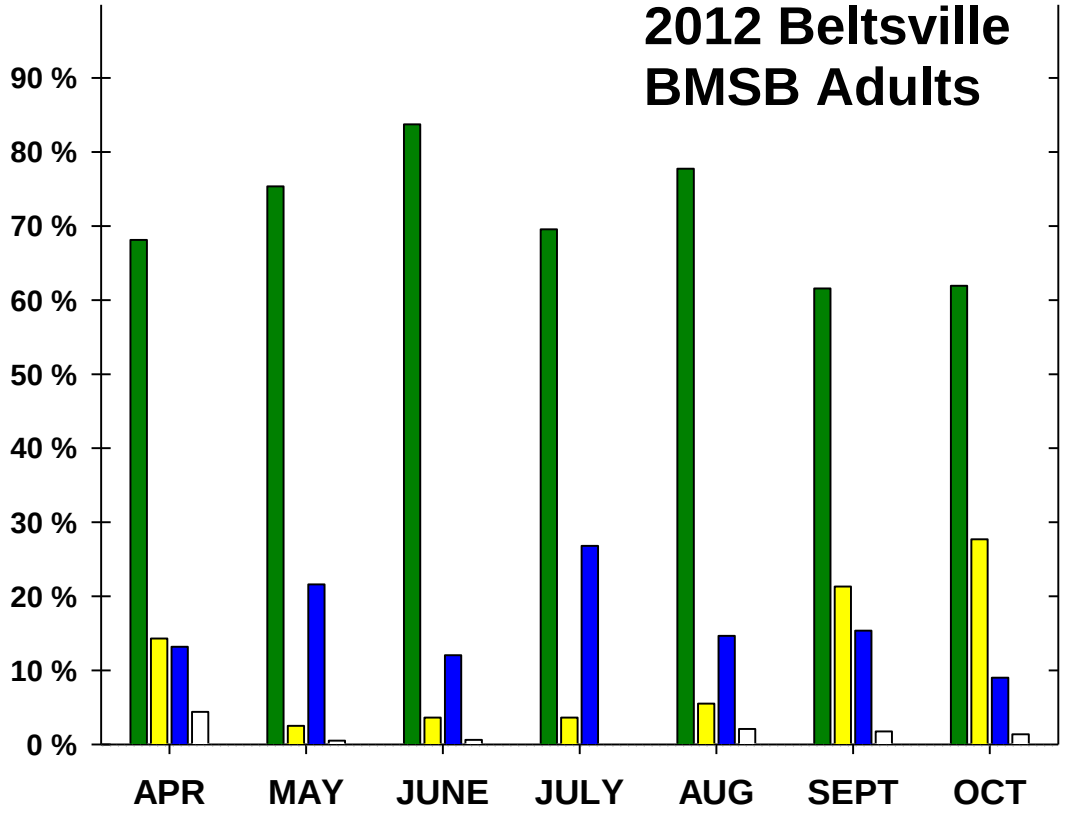
Combined lure is superior over the entire season for adults

Halyomorpha halys
Brown marmorated stink bug

males & females similarly attracted



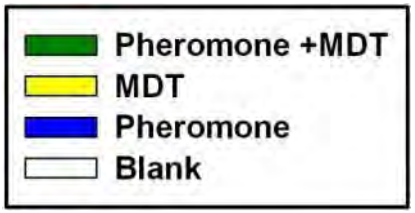


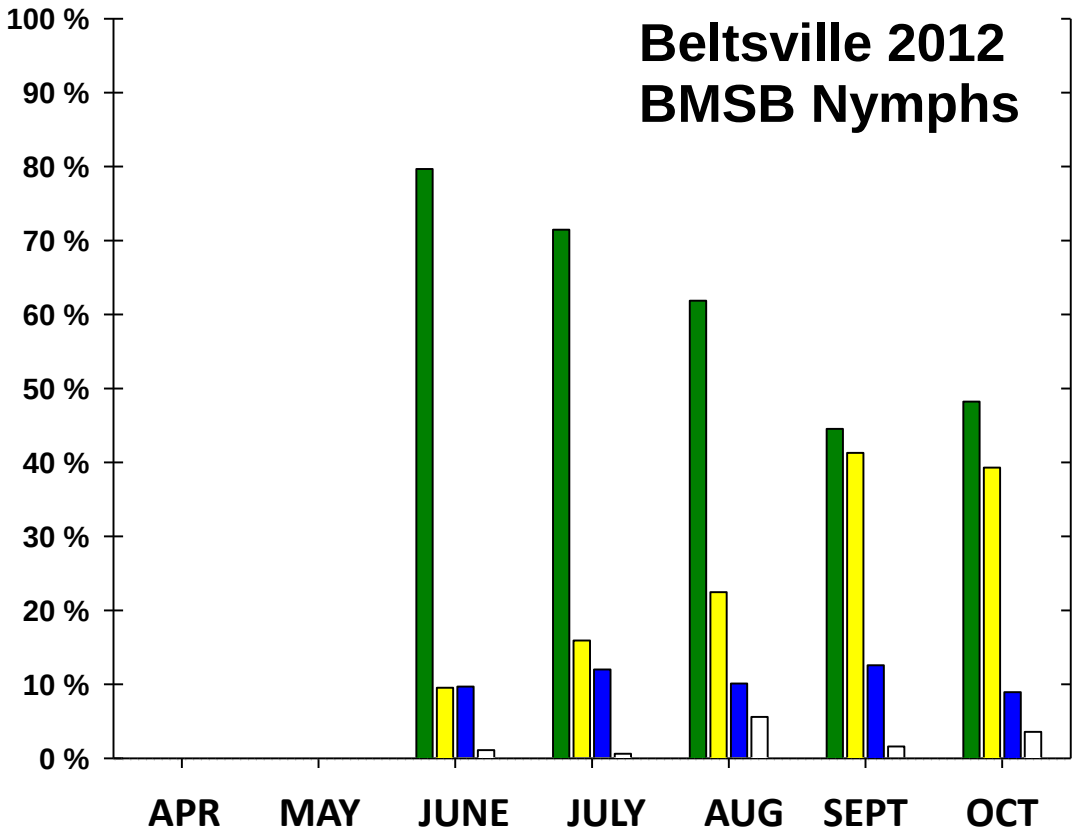


Weber et al. 2014, J.Econ.Entomol. 107: 1061–1068

Halyomorpha halys
Brown marmorated stink bug

... and also provides superior season-long attraction in nymphs

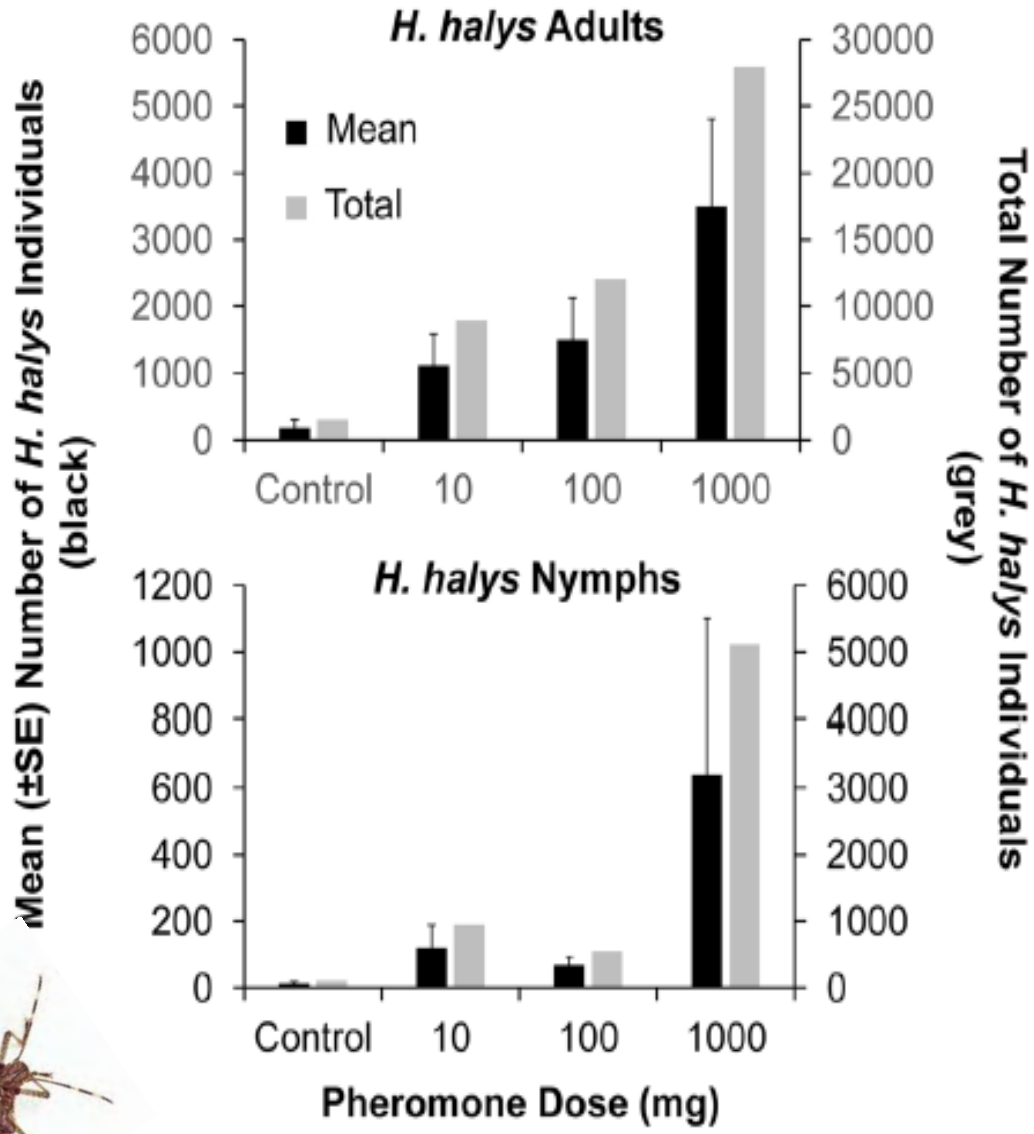




Weber et al. 2014, J.Econ.Entomol. 107: 1061–1068

H. halys attraction to mixed-isomer pheromone lures of 0, 10, 100, and 1000mg (!), with 66mg MDT

August 2013, soybean, West Virginia



pheromone is mixed-isomer synthetic lure
Morrison et al. 2016, J. Pest Sci. 89: 81-96.

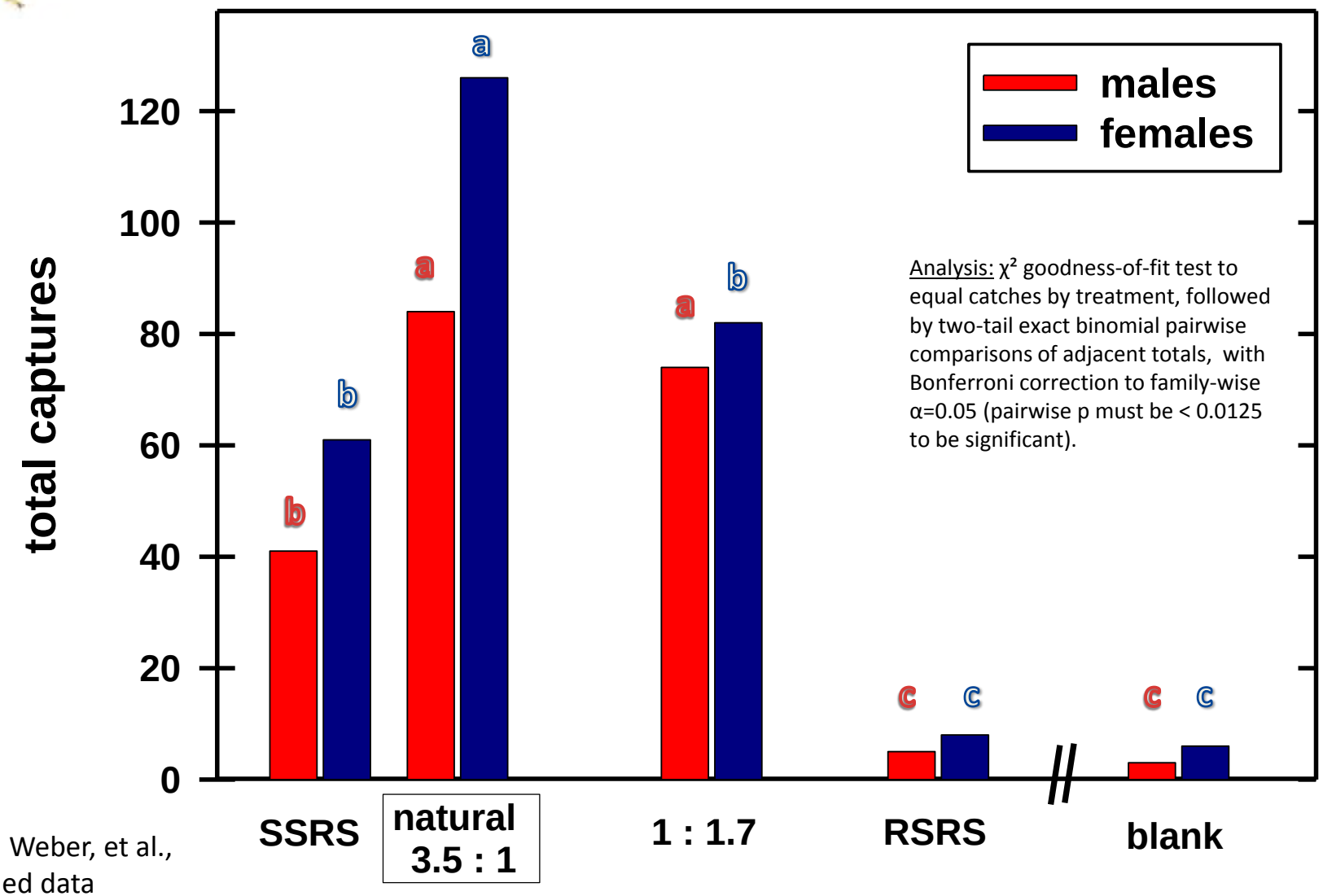
Month-long Trial of
Ratio of two BMSB pheromone components
2016 West Virginia only

- 5 treatments, pheromone only (no MDT):
  - pure SSRS (major component)
  - 3.5:1 SSRS:RSRS (natural ratio of synthetic isomers)
  - 1:1.7 SSRS:RSRS (ratio in established synthesis)
  - pure RSRS (minor component)
  - blank
- 4 RCB, full-sized black pyramid traps, collected & re-randomized weekly

Halyomorpha halys
Brown marmorated stink bug

Captures at pyramid traps, Bardane WV with 4mg blends with varied isomer ratio

5-way field choice test 18 Aug – 16 Sept 2016

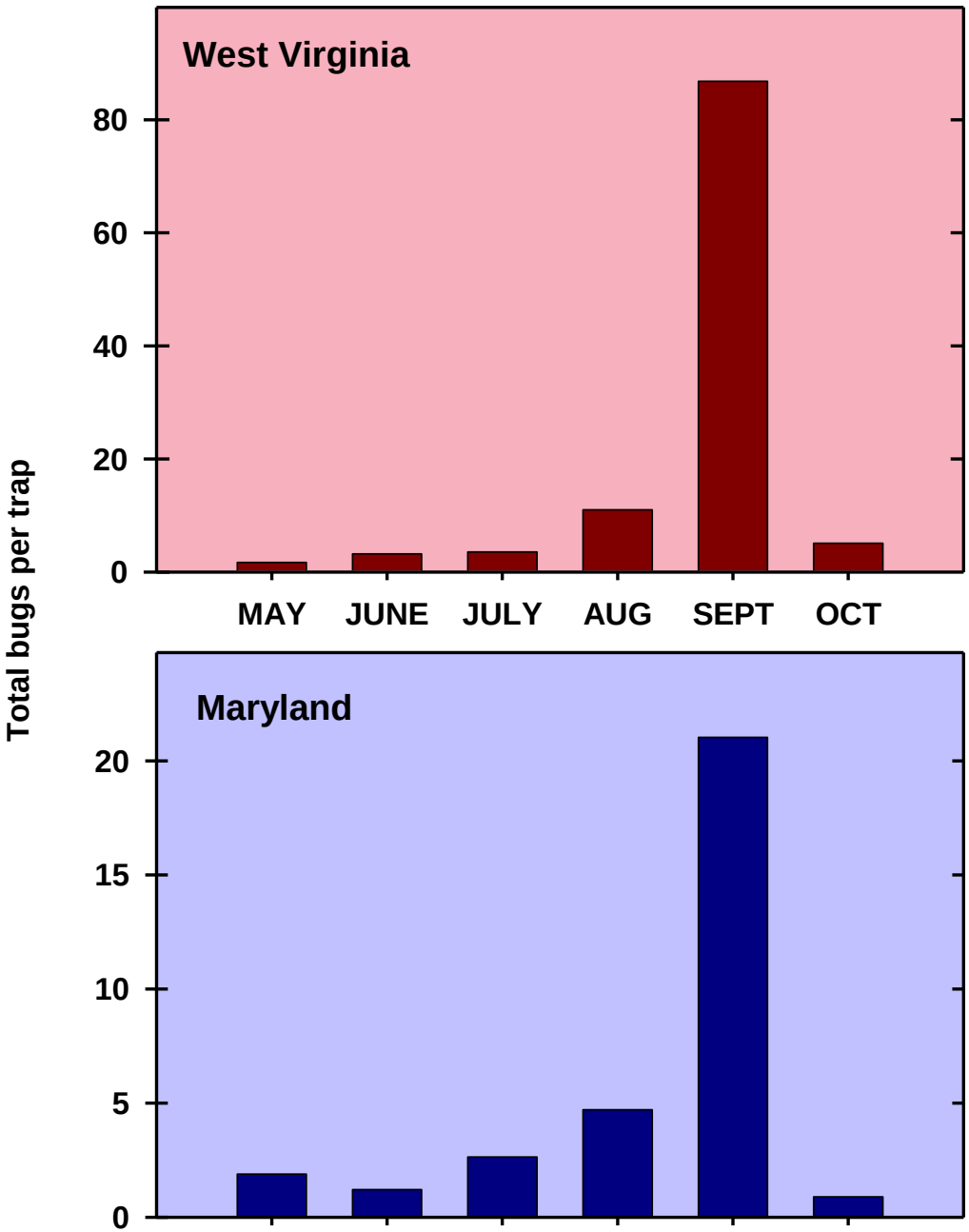


Morrison, Weber, et al., unpublished data

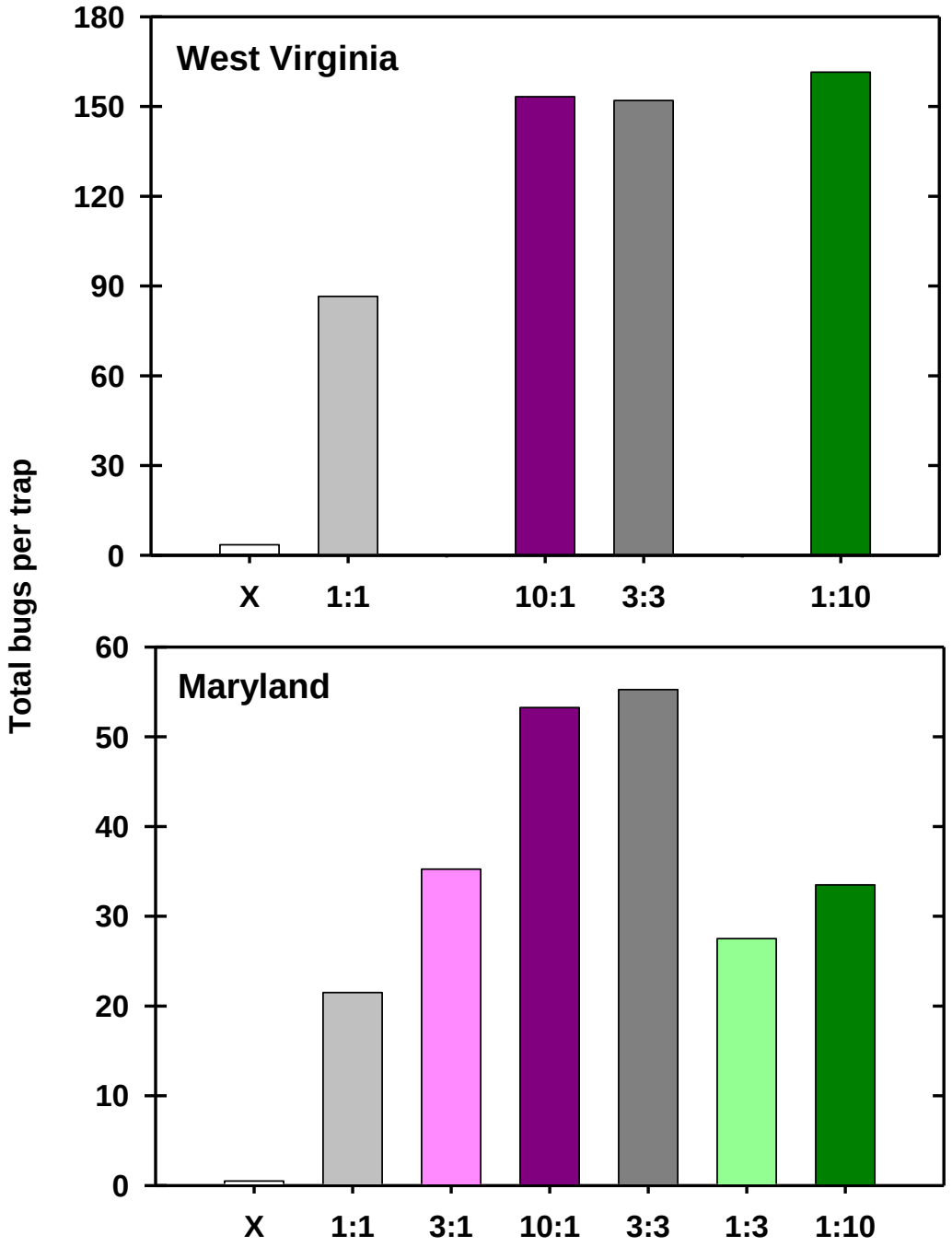
Season-long Trial of Ratio of BMSB pheromone and MDT 2016 Maryland & West Virginia

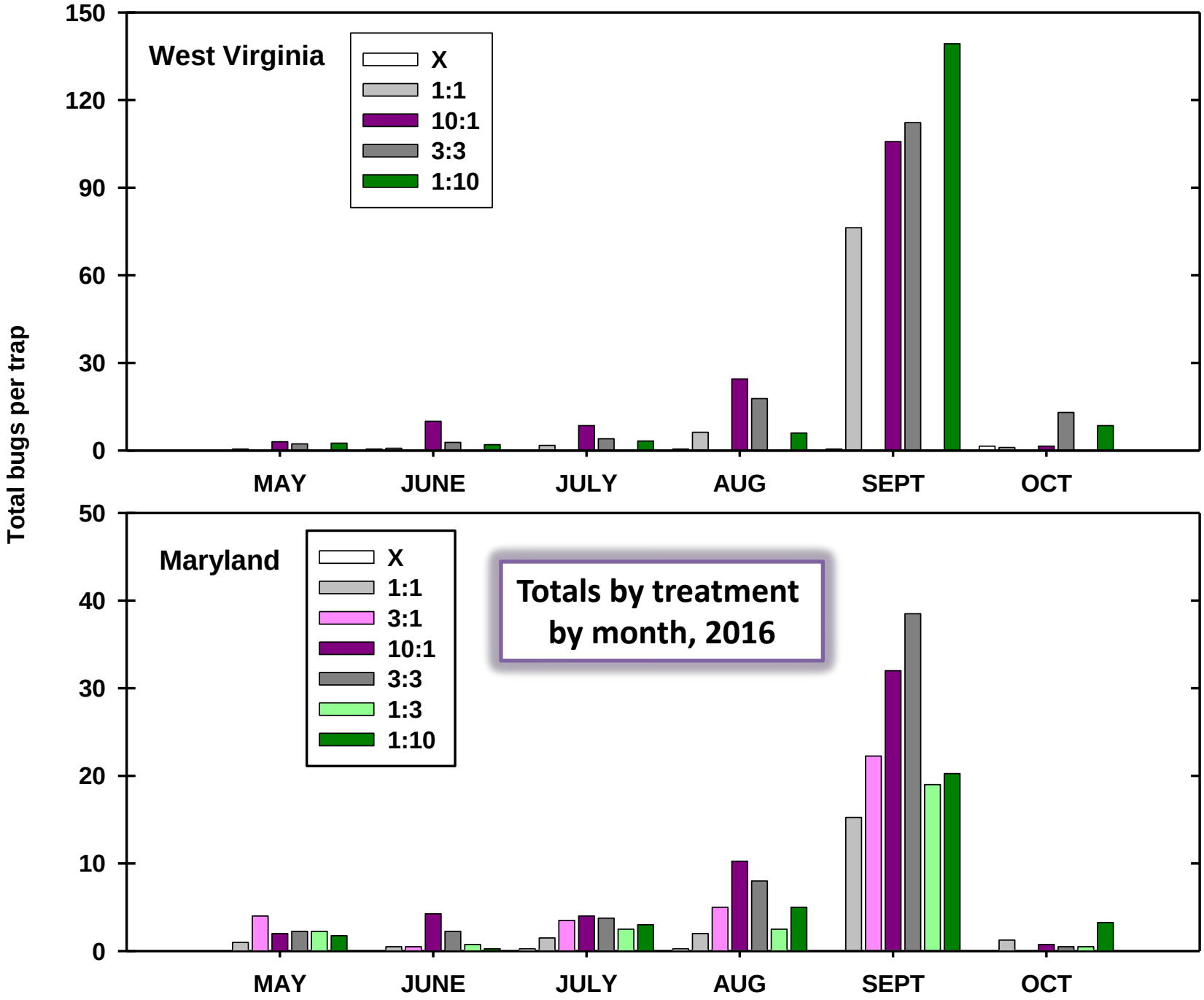
- **Default starting point was one mixed pheromone lure (#20) which contains ~2mg of SSRS-murgantiol, plus 66mg (*E,E,Z*)-2,4,6-MDT = "1:1"**
- **7 treatments in Maryland, 5 in West Virginia:**
  - **1:1, (1:3), (3:1), 3:3, 1:10, 10:1, and blank**
  - **May to October, 4 RCB, full-sized black pyramid traps, collected & re-randomized weekly**
  - **to see if 1:1 starting point was best for season-long capture, or would additional pheromone or MDT result in higher captures?**

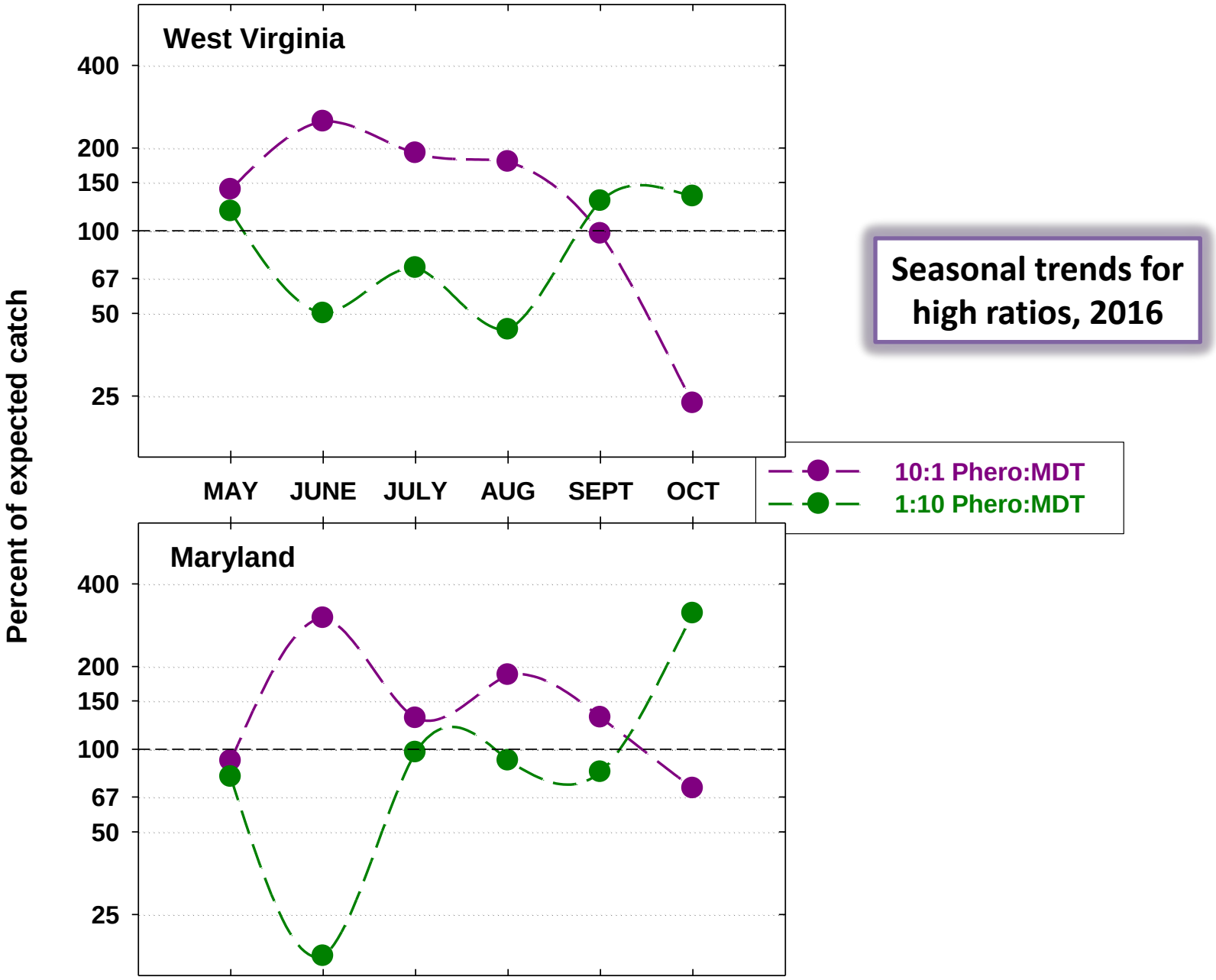
Totals for all traps 2016



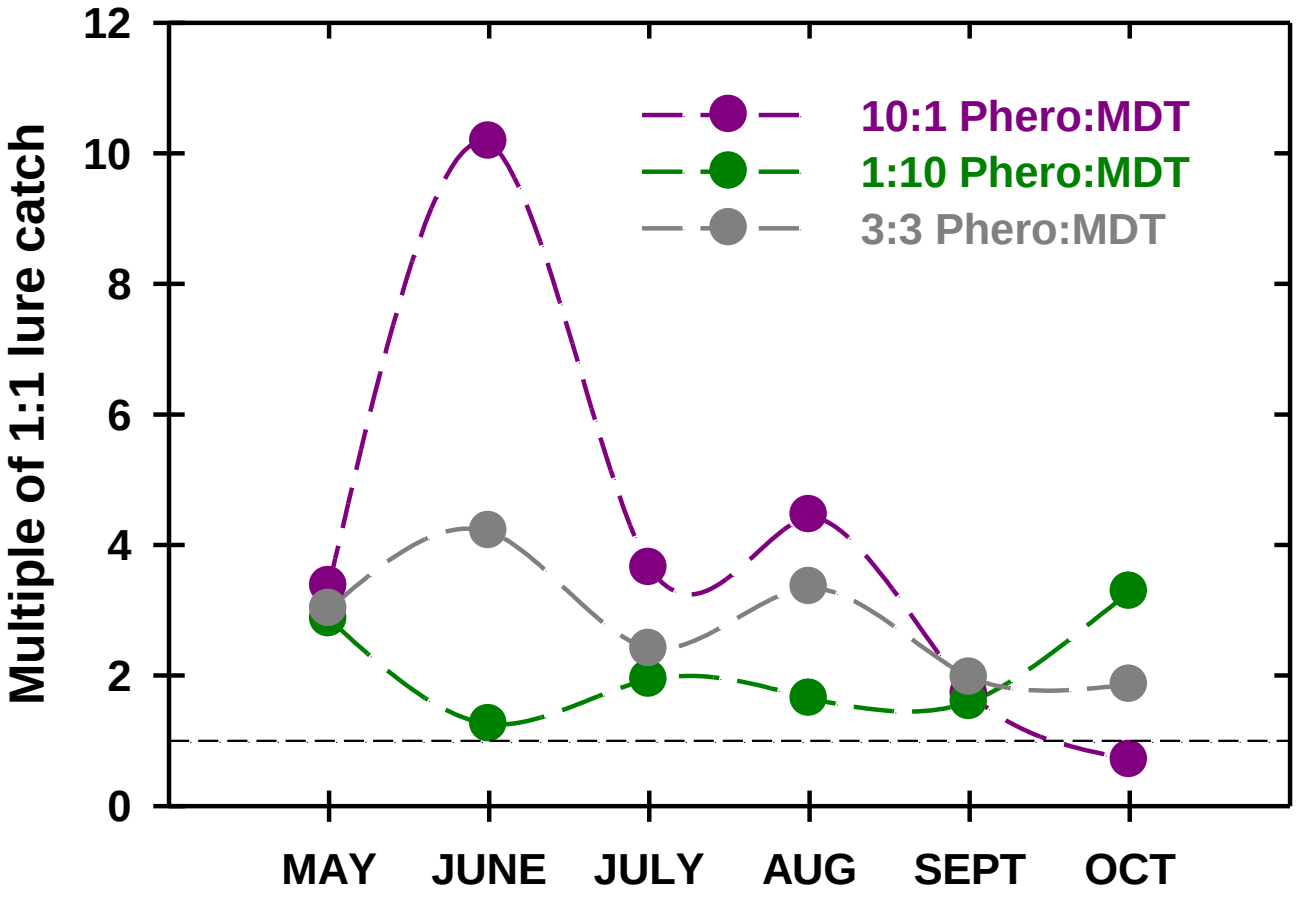
Totals by treatment 2016




West Virginia
X
1:1
10:1
3:3
1:10
150
120
90
60
30
0
MAY
JUNE
JULY
AUG
SEPT
OCT
Total bugs per trap
Maryland
X
1:1
3:1
10:1
3:3
1:3
1:10
Totals by treatment by month, 2016
50
40
30
20
10
0
MAY
JUNE
JULY
AUG
SEPT
OCT


West Virginia
Maryland
Percent of expected catch
400
200
150
100
67
50
25
MAY
JUNE
JULY
AUG
SEPT
OCT
10:1 Phero:MDT
1:10 Phero:MDT
Seasonal trends for high ratios, 2016

Added catch from increase in either or both components, compared to catch from current "1:1" lure



combined West Virginia & Maryland monthly means

Conclusions from BMSB Semiochemical Ratio trials 2016

- **As a season-long lure, the *current combination* (“1:1”) of mixed pheromone lure with ~2mg of SSRS-murgantiol, plus 66mg (*E,E,Z*)-2,4,6-MDT, is *reliably attractive throughout the season* (note which AI’s are quantified here).**
- **For *increased attraction* in all months of BMSB activity, dose should be *increased in both components*, for example, to “3:3”. However, attraction is not very sensitive to the exact ratio (“more gives more”).**
- ***Deviations from current ratio are not critical*. As expected from the experience with the two attractants alone, high ratios of pheromone:MDT tend to attract more in mid-season, whereas low ratios tend to attract more in late season.**
- ***Exact pheromone ratio* (SSRS:RSRS) *is not critical for attraction.* The somewhat higher attractiveness of the natural stereoisomeric ratio to adult females, compared to the synthetic stereoisomeric mix (#20 or similar), may be easily compensated for, by increasing overall dose of the mixed pheromone.**


Have we got the right ratio?


Have we got the right ratio?
pretty much, yes!

Many thanks ....

- **Megan Herlihy, Torri Hancock, McKenzie Allen, Liz Fread, Emma Thrift, Treva Rowley, Mike Athanas, Jeff Aldrich, Ed Clark, Nate Erwin, Fil Guzman, Shyam Shiraly, and others!**
- **USDA NIFA SCRI**
- **USDA ARS**

photos by Alex Surcica, Wil Hershberger, and someone in Japan