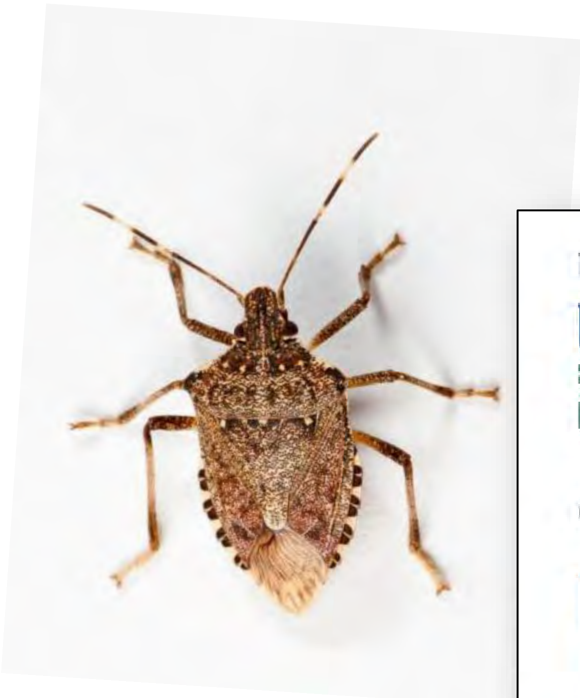
2.1. Development of Monitoring Tools for BMSB



Funding

USDA

United States Department of Agriculture

National Institute of Food and Agriculture

Specialty Crop Research Initiative
Grant #2011-01413-30937

Collaborating Institutions

University of Delaware

ARS

Penn State 1855

The State University of New Jersey Rutgers

Northeastern IPM Center

Washington State University

Cornell University

University of Maryland

Virginia Tech 1872

OSU Oregon State University

NC State University

StopBMSB.org

2.1. Development of Monitoring Tools for BMSB

Summer = Yellow Fall = Orange
Winter = White Spring = Pink

2011-2012				2012-2013				2013-2014				2014-2015				2015-2016				
Year 1				*Year 2*				*Year 3*				*Year 4*				*Year 5*				
S	F	W	S	S	F	W	S	S	F	W	S	S	F	W	S	S	F	W	S	S

OBJECTIVE 2. DEVELOP AND REFINE MONITORING AND MANAGEMENT TOOLS FOR BMSB

OBJ.	Description	Participants	Timeline
2.1	Develop monitoring tools for BMSB		
2.1.1	Trap-based monitoring		
2.1.1.1	Identification of pheromone and other attractants	Khriman, Leskey, Landolt, Lee, Wiman, Shearer, Rondon	
2.1.1.2	Optimization of pheromone and kairomone dispensers for monitoring BMSB	Khrimian, Leskey, Bergh, Krawczyk, Saunders, Rodriguez-Saona, Hamilton, Polk, Wiman, Shearer	
2.1.1.3	Refining utility of light-based traps for BMSB	Leskey, Hamilton, Krawczyk, Jacobs, Wiman, Shearer, Agnello, Jentsch	
2.1.1.4.	Define behavioral characteristics of BMSB and active space of baited traps to develop efficient traps and deployment strategies	Leskey, Bergh, Krawczyk, Saunders, Shearer Wiman	
2.1.2	Assess other types of monitoring tools	Leskey, Wright, Bergh, Krawczyk, Saunders, Wiman, Shearer	

- Tools that provide accurate measurements of presence, abundance, and seasonal activity of BMSB. Growers can make informed management decisions.
- Tactics that reduce the use of broad-spectrum insecticides.

JUNE 2011

SPRAY SCHEDULE - BMSB

* every other row lg. apples, peaches
* every 4th row trellis apples

			1	— ARNS in stone fruit pome fruit + brambles 2	apples - peaches - 3	McHenry Highland Festival* Blueberries Brambles Cherries (4th)
5	apples peaches, plums strawberries (OUTSIDE) 6	cherries ½ potatoes tomatoes vegetables 7	cherries ½ ½ brambles ½ blueberry blackberry 8	apples peaches, plums (INSIDE) 9	blueberries 39/40 44 Brambles 13, 15, 16, 44, 41 10	Early Summer Season rates begin this weekend Crack spray cherries 11
Apples Peaches (OUTSIDE) 12	vegs tomatoes cherries grapes, gooseb plums, apricot 13	Blueberries Brambles 14	apples peaches (INSIDE) 15	crack spray cherries Cherries tomatoes, flowers 16	Blueberries brambles → vegetables 17	Peach Apple (OUTSIDE) 18
Father's Day 19	Brambles, Blueberries grapes, gooseberries (OUTSIDE) 20	Peaches apples (INSIDE) 21	Blueb. (admin) cherries/plums (inside) 22	Bramble (outside) Blueberry (inside) 23	cherries crack spray peach apple (outside) 24	Summer Season rates begin this weekend 25
Brambles Blueberries (outside) (#50/48) 26	[illegible] cherry 27	tomatoes vegs. flowers potatoes 28	Brambles Blueberries (inside) 29	Apples Peaches (OUTSIDE) 30	tomatoes, vegs potatoes, flowers 7/1	wood's edge orchard 7/2

2.1.1.1. Identification of Pheromone and Other Attractants

Published Manuscripts

Leskey T.C., B.D. Short., B.B. Butler, and S.E. Wright. 2012. Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) in mid-Atlantic tree fruit orchards in the United States: case studies of commercial management. Psyche. Article ID 535062, DOI:10.1155/2012/535062.

Weber, D.C., T.C. Leskey, G.C. Walsh, and A Khrimian. 2014; Synergy of aggregation pheromone with methyl (*E,E,Z*)-2,4,6-decatrienoate in attraction of brown marmorated stink bug, *Halyomorpha halys* (Stål). Journal of Economic Entomology 07:1061-1068

Khrimian A, A. Zhang, D.C. Weber, H.-Y. Ho, J.R. Aldrich, K.E. Vermillion, M.A. Siegler, S. Shirali, F. Guzman, and T.C. Leskey. 2014. Discovery of the aggregation pheromone of the brown marmorated stink bug *(Halyomorpha halys*) through the creation of stereo isomeric libraries of 1-bisabolen-3-ols. Journal of Natural Products 77: 1708-1717.

Leskey, T.C., A. Agnello, J. C. Bergh, G. P. Dively, G. C. Hamilton, P. Jentsch, A. Khrimian, G. Krawczyk, T. P. Kuhar; D. Lee, W. R. Morrison III, D. F. Polk, C. Rodriguez-Saona, P. W. Shearer, B. D. Short, P. M. Shrewsbury, J. F. Walgenbach; D. C. Weber, C. Welty, J. Whalen, N. Wiman and F. Zaman. 2015. Attraction of the Invasive *Halyomorpha halys* (Hemiptera: Pentatomidae) to Traps Baited with Semiochemicals Stimuli across the United States. Environmental Entomology (in press).

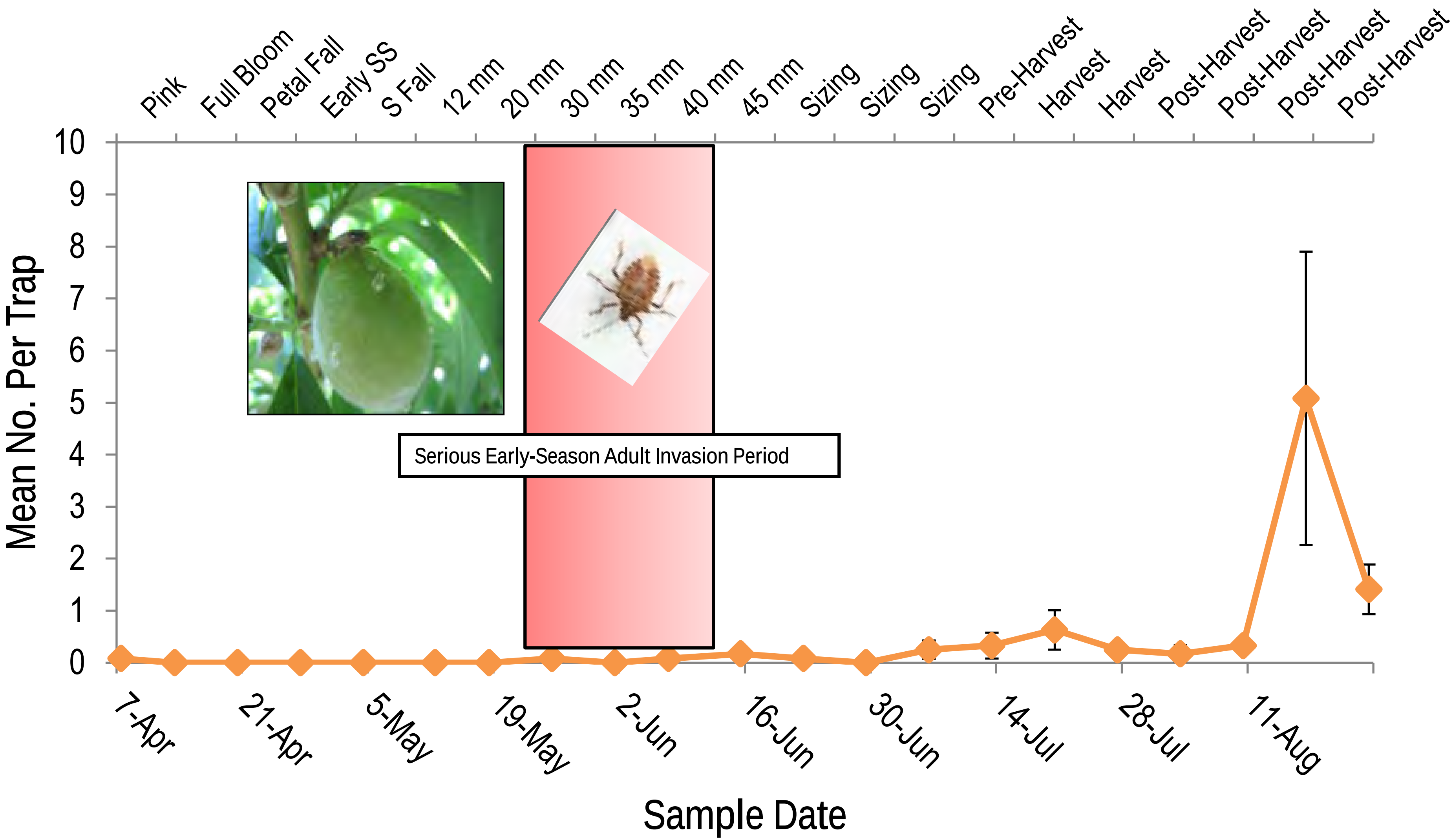
Leskey, T.C., A. Khrimian, D.C. Weber, J.C. Aldrich, B.D. Short, D.-H. Lee and W.R. Morrison III. 2015. Behavioral responses of the invasive *Halyomorpha halys* (Stål) to traps baited with stereo isomeric mixtures of 10, 11-epoxy-1-bisabolen-3-ol. Journal of Chemical Ecology 41:418–429.

One Attractant Available Prior to 2012

- Methyl (2E, 4E, 6Z)-decatrieonate is an attractant produced by the Asian stink bug, *Plautia stali.*

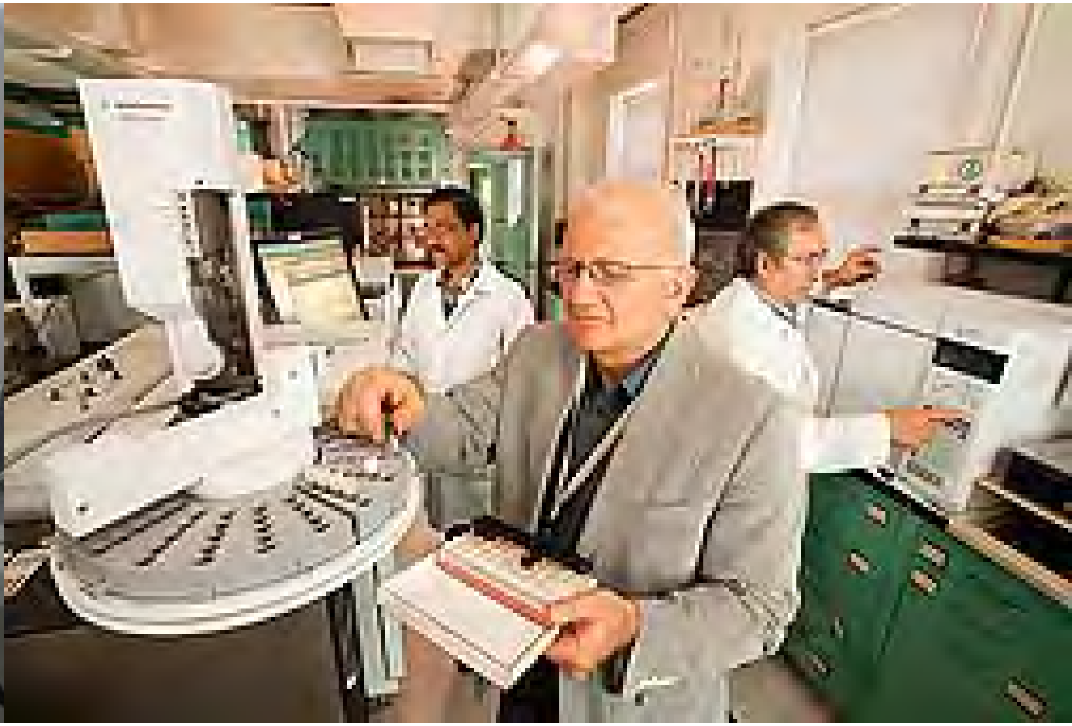
- Cross attractive to BMSB and other pentatomids.

Serious Limitations For Season-Long Monitoring

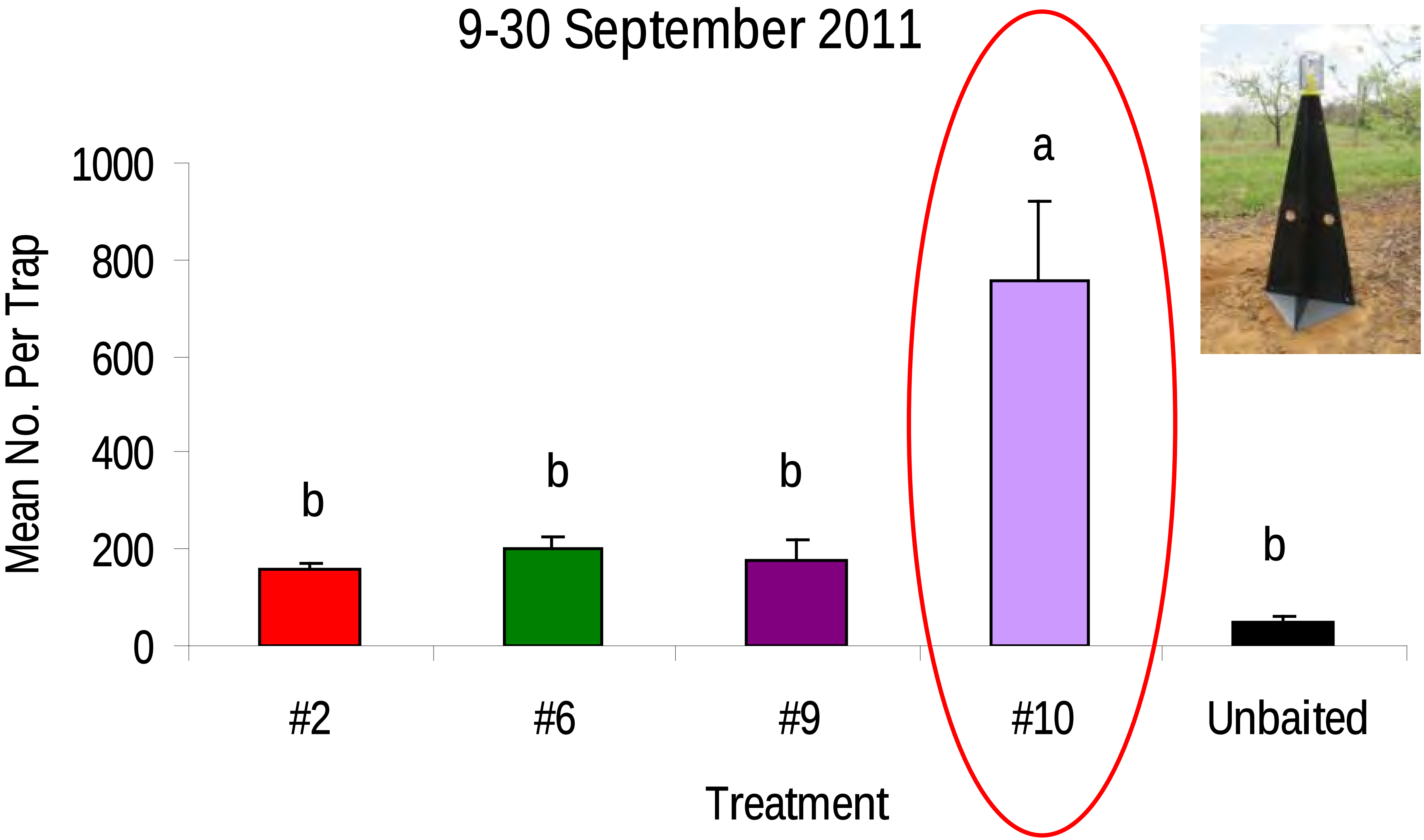


Leskey et al. 2012

Identification and Commercialization of BMSB Aggregation Pheromone

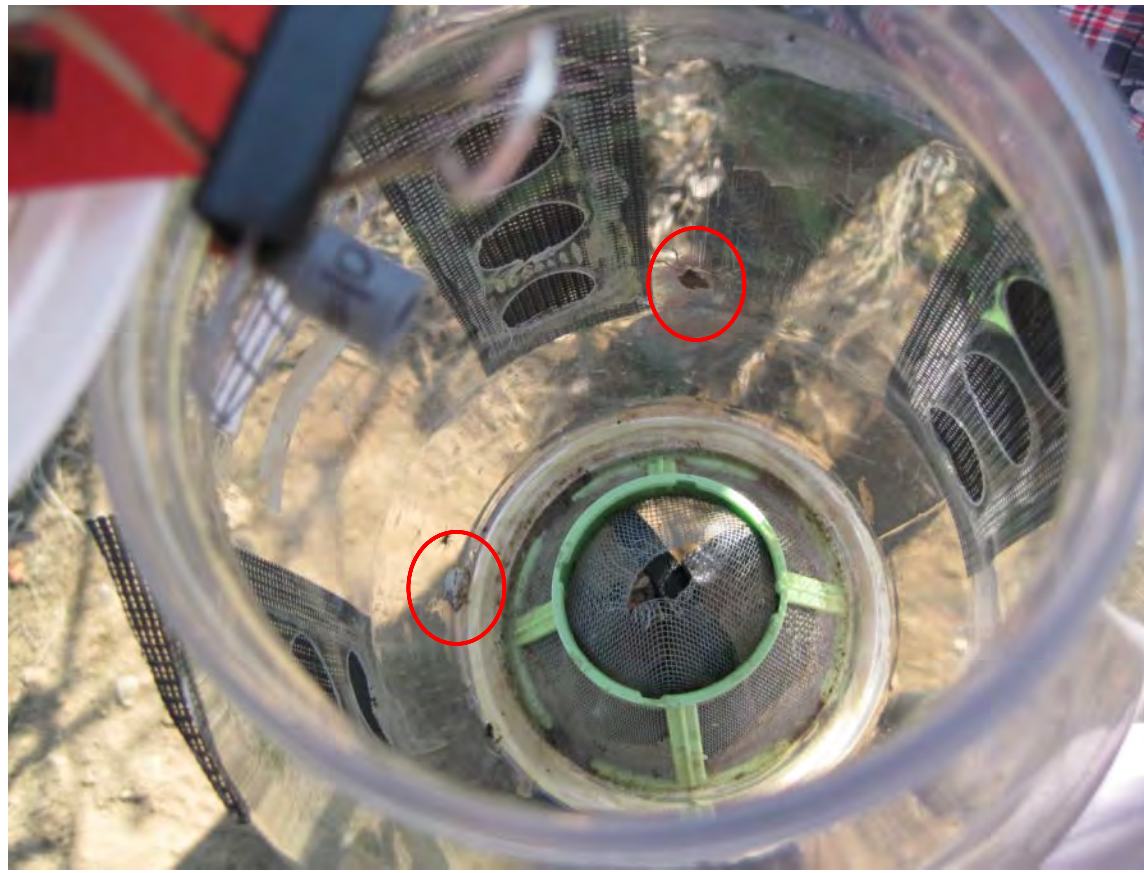


BMSB Aggregation Pheromone Breakthrough

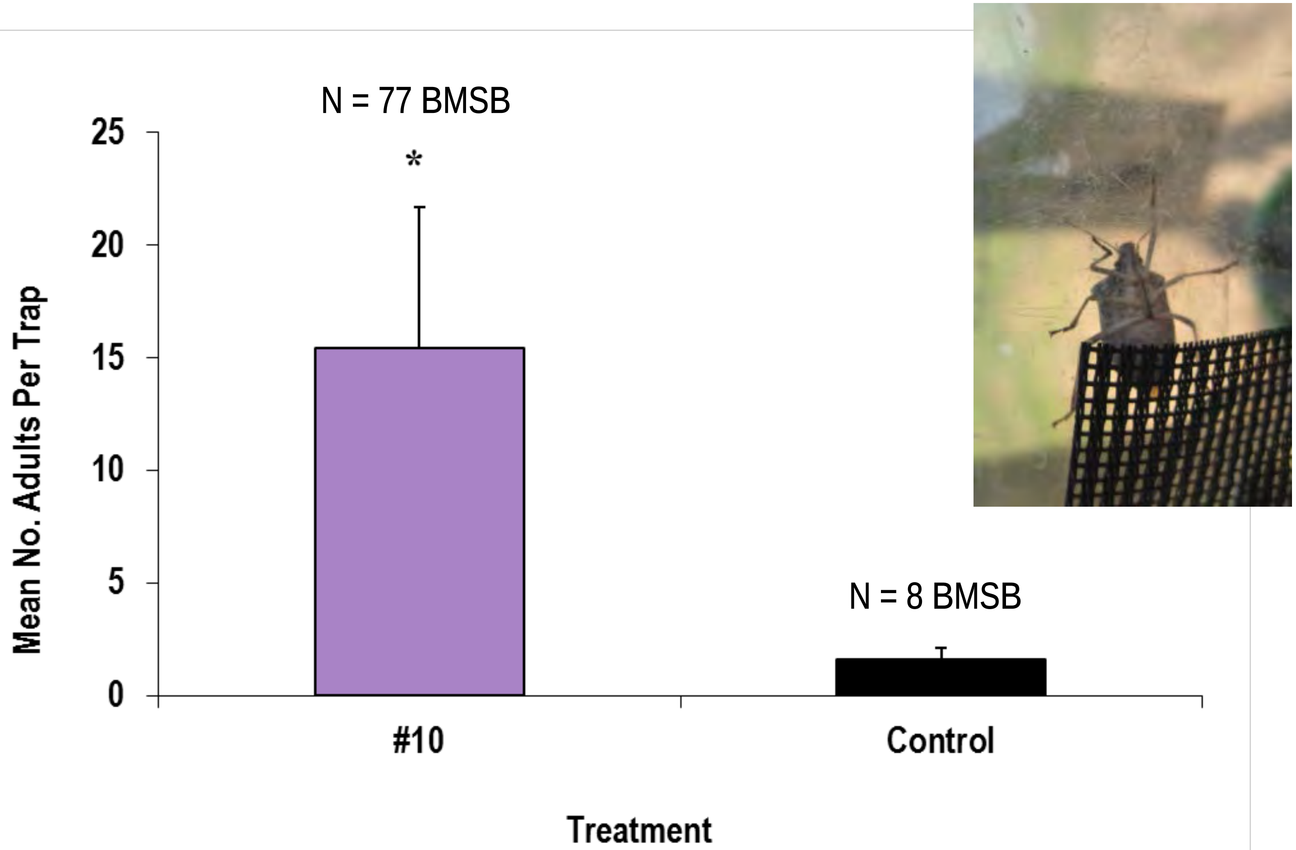


Is #10 Attractive in the Early Season?

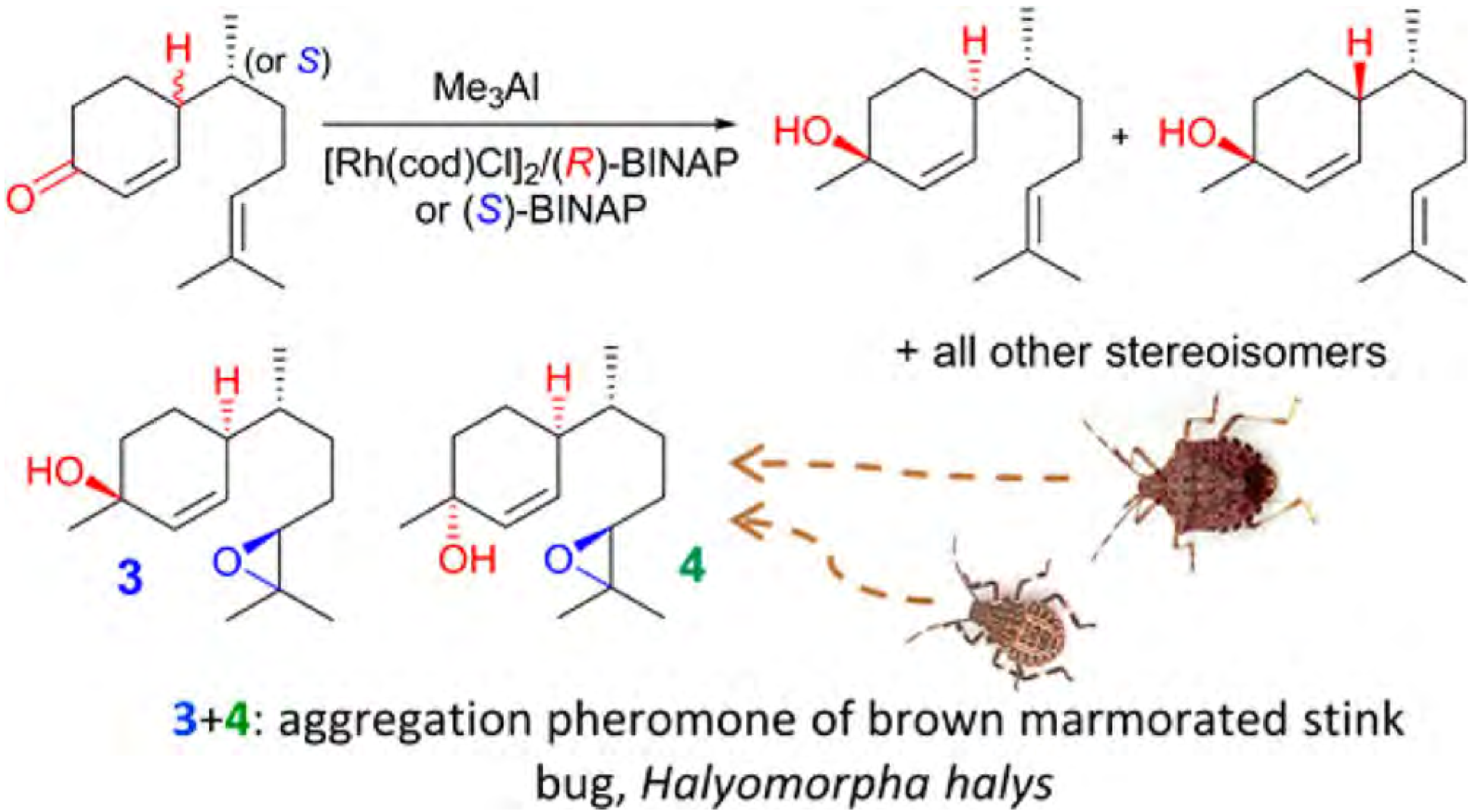
Pre-Trial (March 20-April 17, 2012)



Early Season Attraction
Documented for BMSB March 20-April 17, 2012



Two-Component BMSB Aggregation Pheromone Identified



Khrimian et al. 2014

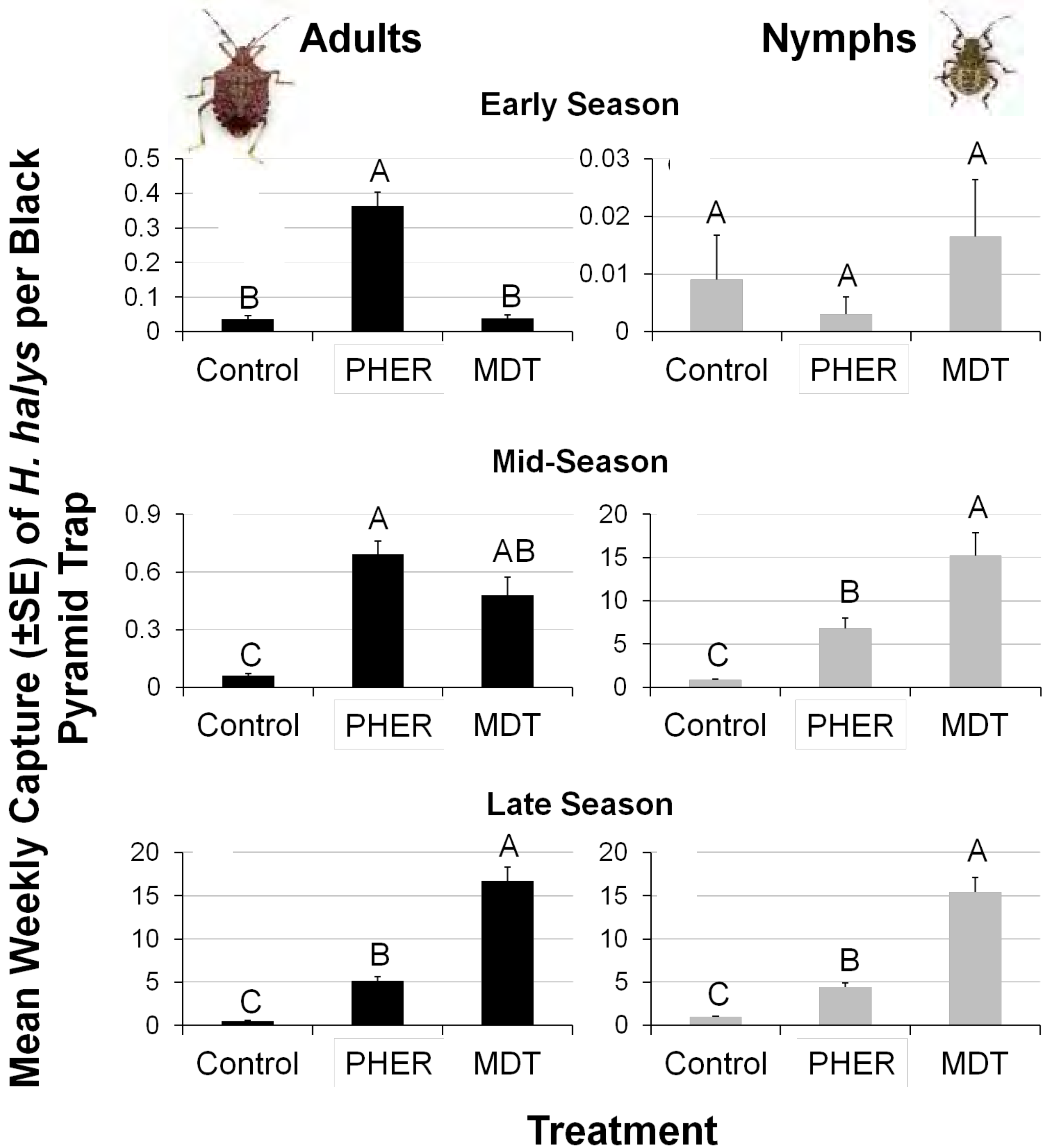
Broad Validation Across The Country

- Is BMSB attracted to the pheromone in the early season?
- Is BMSB attracted to the pheromone season-long?
- How attractive is this stimulus relative to MDT and unbaited traps?
- Traps evaluated in over 12 states across the country.

General Protocol

- Black pyramid traps
- Three odor treatments
  - 1) BMSB Pheromone (10 mg)
  - 2) MDT (119 mg) 10X greater
  - 3) unbaited control
- Traps are deployed between wild host habitat and agricultural production areas.
- Traps were deployed in mid-April and left in place season-long.

2012 Summary Results

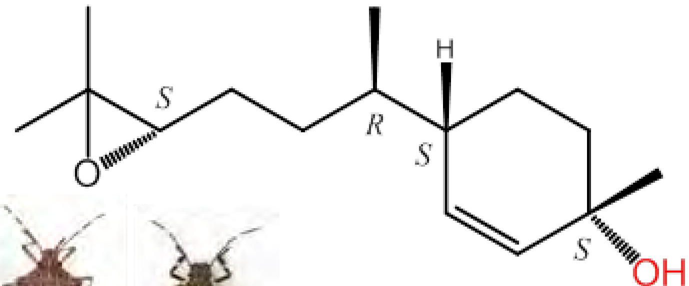


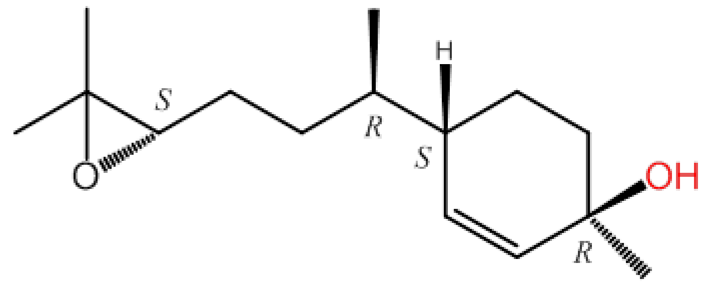
Leskey et al. 2015a

Two-Component BMSB Aggregation Pheromone and Synergist

Main component of BMSB aggregation pheromone (3*S*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol

Minor component of BMSB aggregation pheromone (3*R*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol





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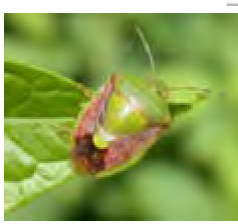
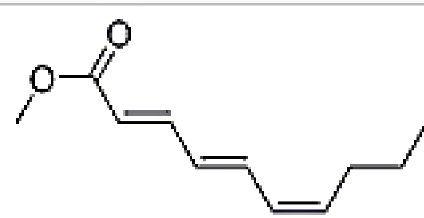
Methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate (MDT) acts as a synergist for BMSB pheromone





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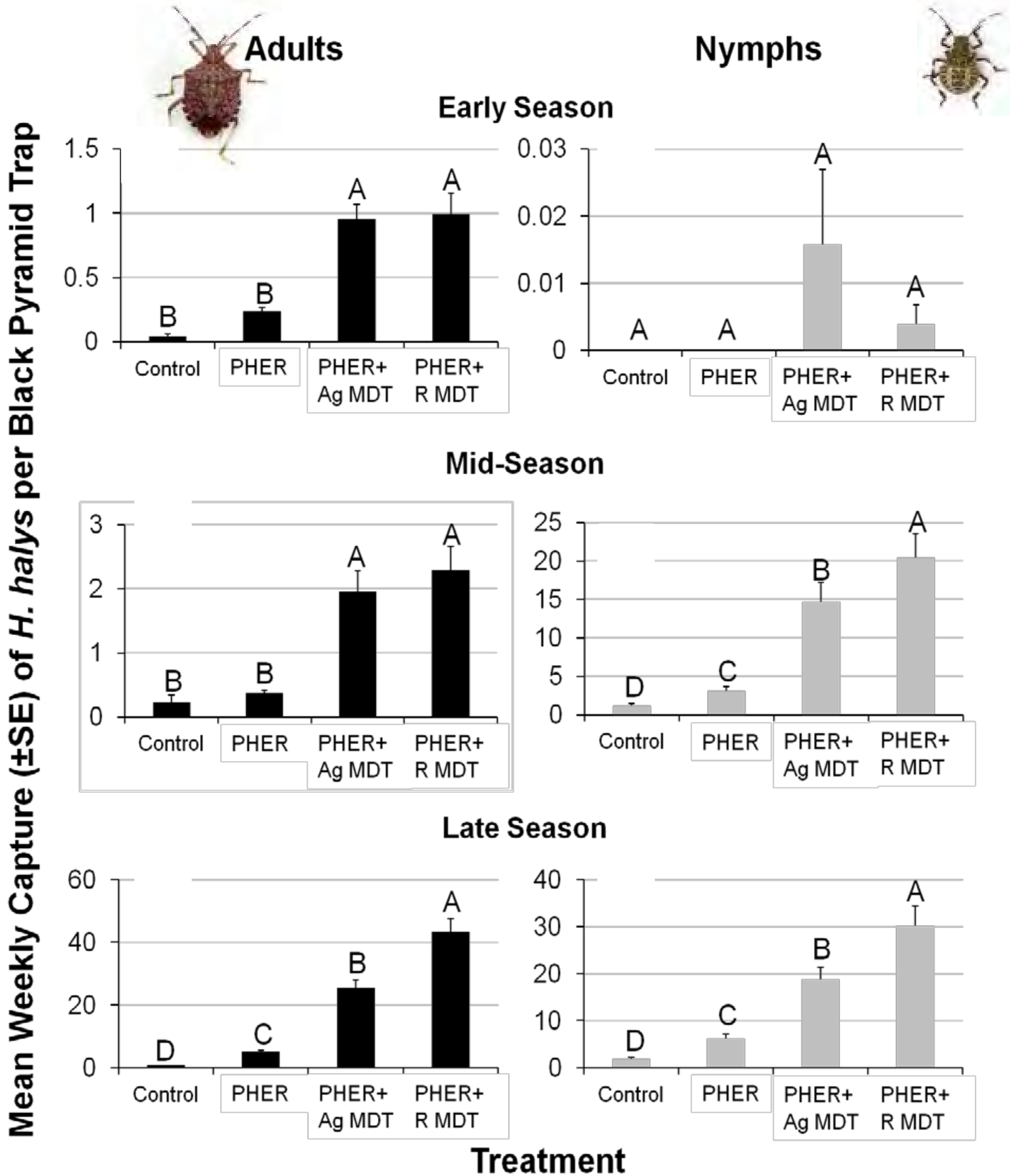
Synergism

Weber et al. 2014

General Protocol

- Black pyramid traps
- Three odor treatments
  - 1) #10 (10 mg)
  - 2) #10 (10 mg) + Rescue MDT (119 mg)
  - 3) #10 (10 mg) + AgBio MDT (66 mg)
  - 4) Unbaited control
- Traps are deployed between wild host habitat and agricultural production areas.
- Traps were deployed in mid-April and left in place season-long.

2013 Summary Results



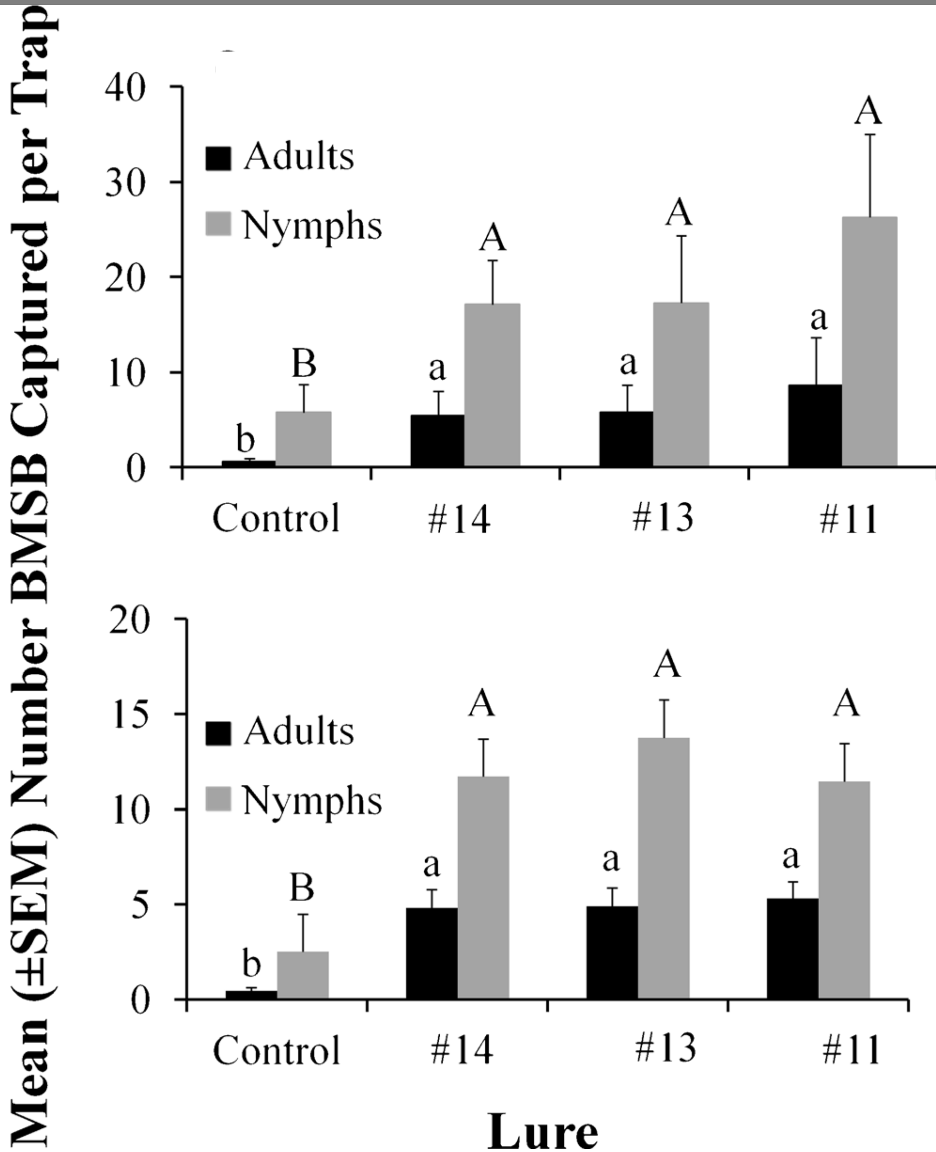
Leskey et al. 2015a

Do Pheromone Lures Need to Be Highly Purified?

- BMSB pheromone comprised of 3.5:1 mixture of (3S,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol and (3R,6S,7R, 10S)-10,11-epoxy-1-bisabolen-3-ol.

- Two stereoisomers of a natural sesquiterpene with a bisabolane skeleton, potentially existing in 16 stereoisomeric forms.

No Significant Difference in BMSB Responses to Varying Levels of Purity

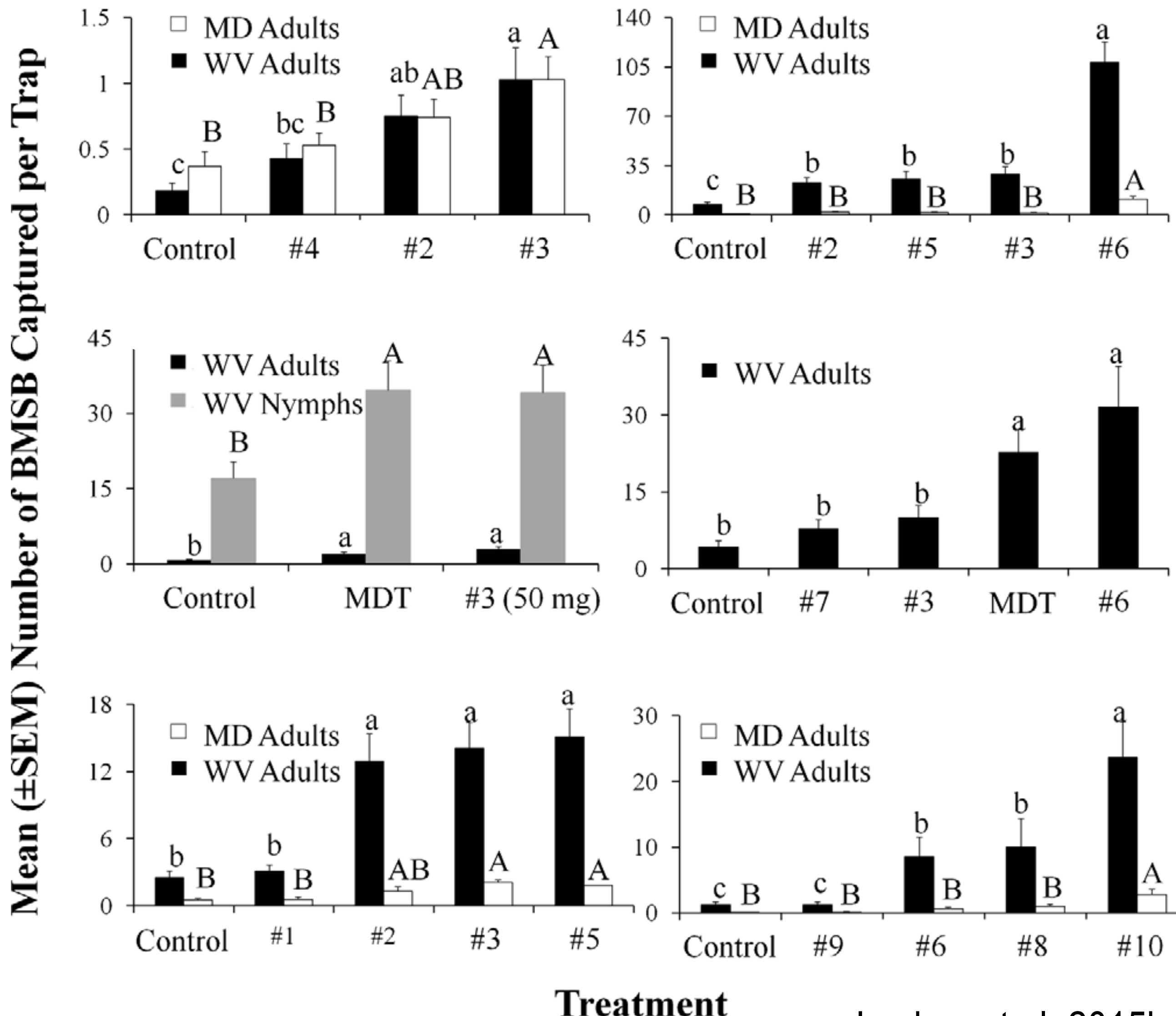


- #11 – off-ratio mixture of two components.
- #13 – all 16 stereoisomers including two components (purified once).
- #14 – all 16 stereoisomers including two components (no purification)

Leskey et al. 2015b

BMSB Attracted to Non-BMSB Stereoisomers

- #1,2,3,4,5 and 7 are non-BMSB stereoisomers.
- Traps baited with #2, 3, and 5 produced captures greater than control.
- Less attractive compared with BMSB-stereoisomers

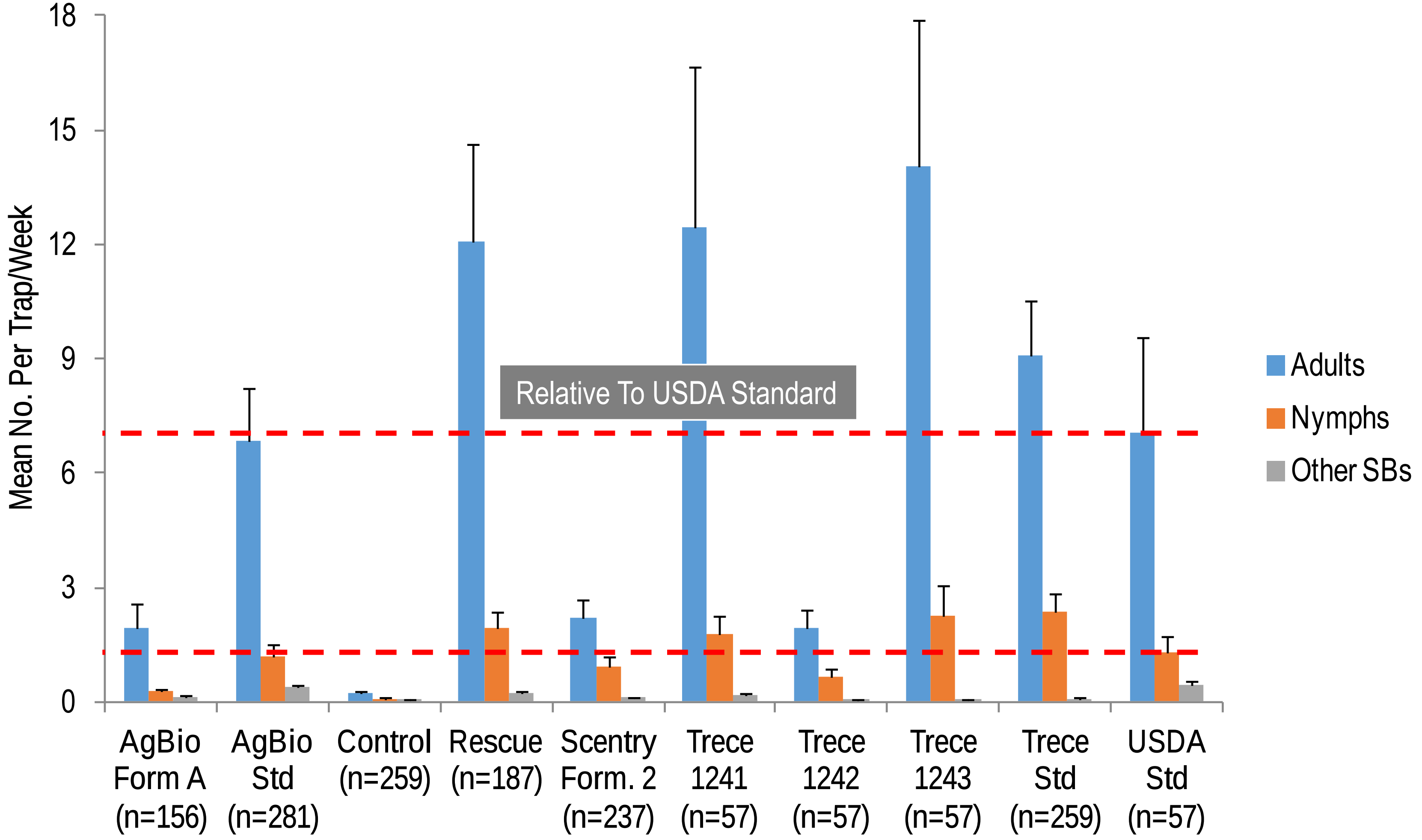


Leskey et al. 2015b

2.1.1.2. Optimization of Pheromone and Kairomone Dispensers

- Collaborations with commercial companies throughout the project.
- Provided commercial collaborators with samples of BMSB pheromone for formulation and testing.
- Coordinated lure trials in 2014 and 2015 with current commercial formulations.
- Most lures perform as well as experimental standard.

2015 Results From Season-Long Trial



Next Steps

- Standardized dose/release rate for monitoring lures. Need enough captures to be biologically relevant, but not excessive such that trap maintenance becomes a burden.
- Standardized dose/release rate for exclusion/detection lures. Reliable detection under low density situations.
- Biological information generated by baited traps translated into thresholds and recommendations.
- Traps and lures are optimized to establish industry standards for monitoring and management.
- New synergist.

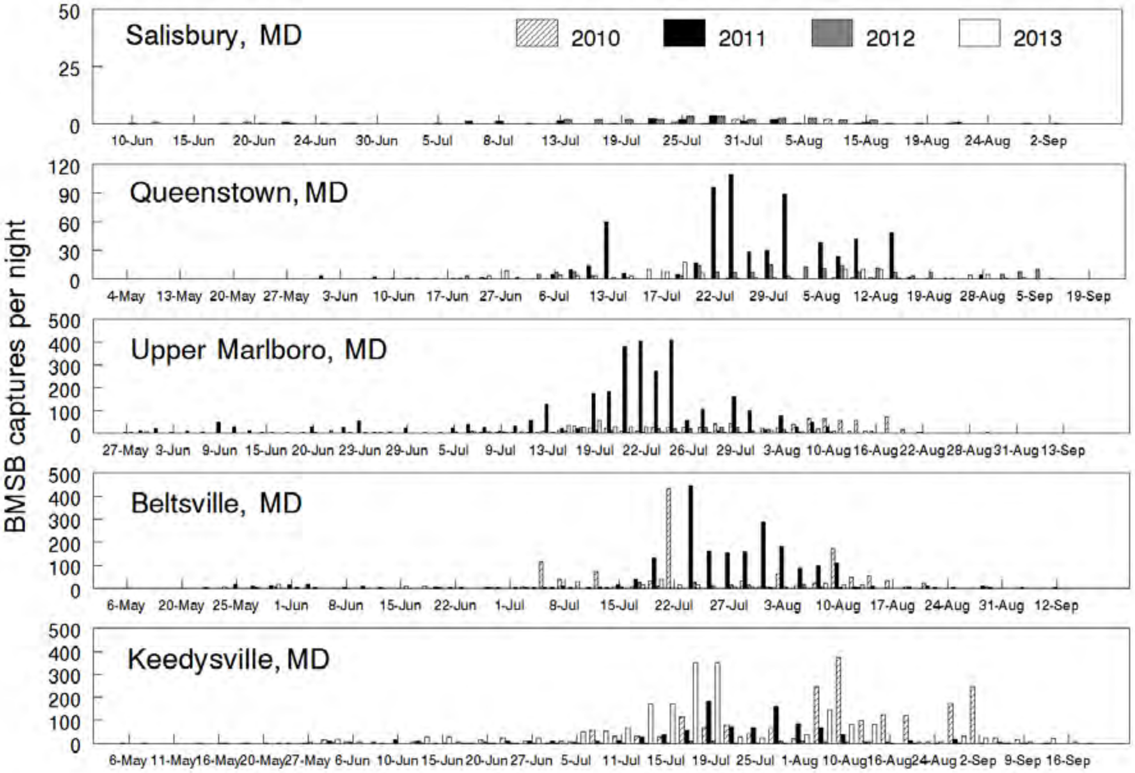
2.1.1.3. Refining Utility of Light-Based Traps

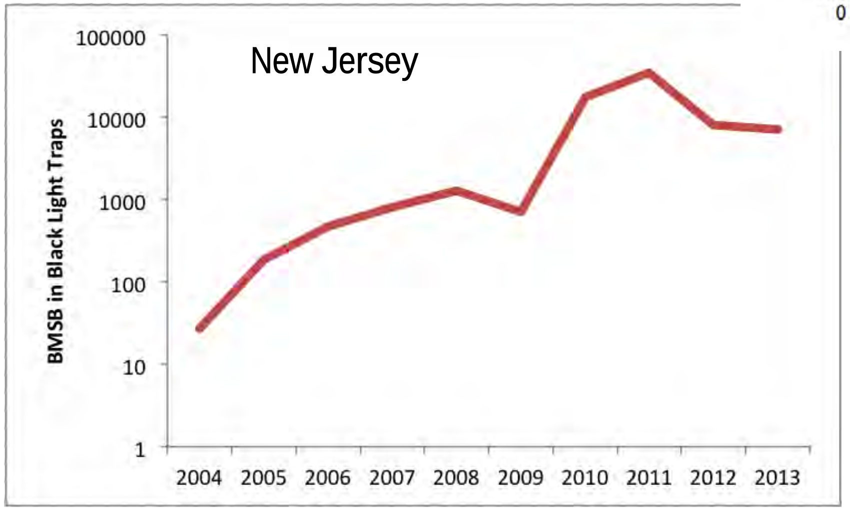
Published Manuscripts

Wallner, A.M., Hamilton, G.C., Nielsen, A.L., Hahn, N., Green, E., and Rodriguez-Saona, C.R. 2014. Landscape factors facilitating the invasive dynamics and distribution of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae), after arrival in the United States. PLoS ONE 9(5): e95691. doi:10.1371/journal.pone.0095691.

Leskey, T.C., D-H. Lee, D.M. Glenn and W.R. Morrison. 2015. Behavioral responses of the invasive *Halyomorpha halys* (Stål) to light-based stimuli in the laboratory and field. Journal of Insect Behavior. (in press).

Landscape-Level Monitoring With Blacklight Traps

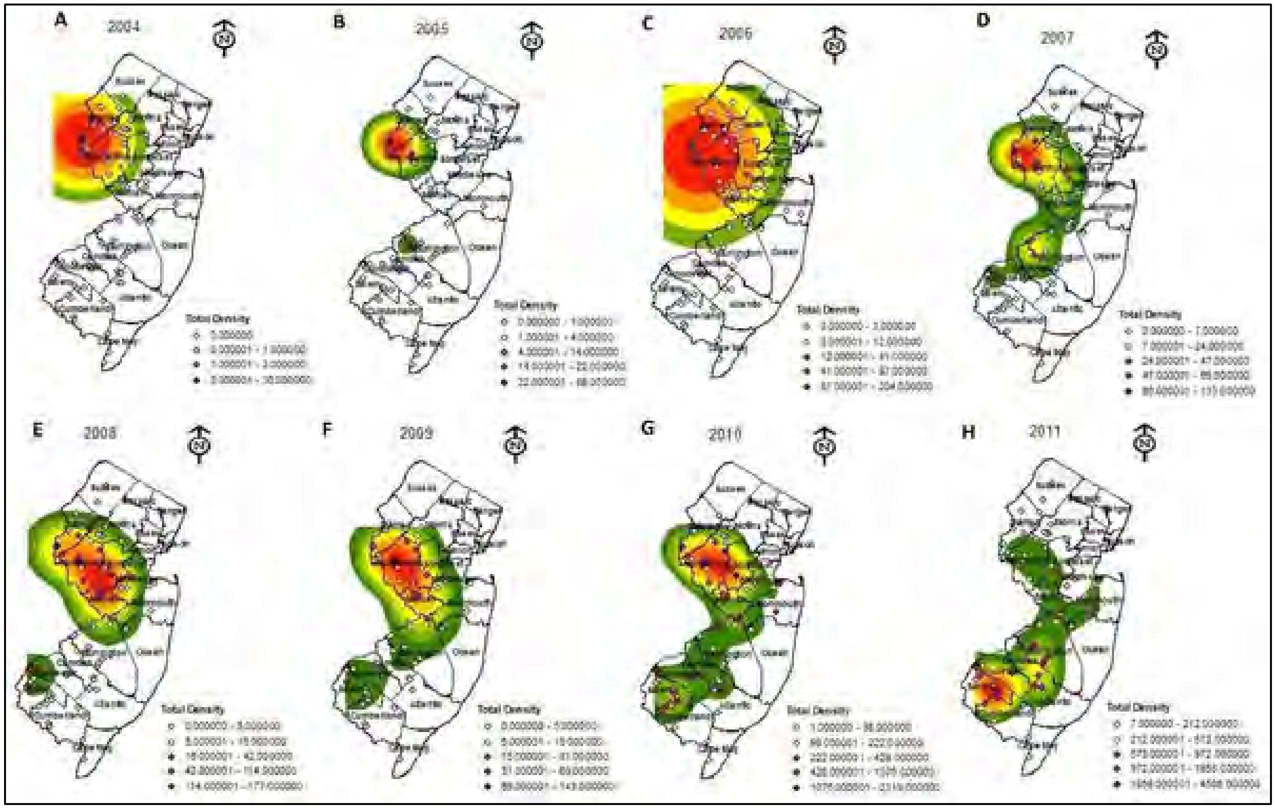




Seasonal and annual trends in populations

Blacklight Traps Used To Predict Spread and Risk Factors

Figure 2. Kernel Density Estimation (KDE) graphs of the density of *Halymorpha halys* captured from black light traps placed throughout New Jersey from (A) 2004, (B) 2005, (C) 2006, (D) 2007, (E) 2008, (F) 2009, (G) 2010, (H) 2011.



Wallner AM, Hamilton GC, Nielsen AL, Hahn N, Green EJ, et al. (2014) Landscape Factors Facilitating the Invasive Dynamics and Distribution of the Brown Marmorated Stink Bug, Halyomorpha halys (Hemiptera: Pentatomidae), after Arrival in the United States. PLoS ONE 9(5): e95691. doi:10.1371/journal.pone.0095691
http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0095691

Identification of attractive visual stimuli including color and light

Can we augment ordinary pyramid traps with light sources and capture BMSBs reliably?

Night View

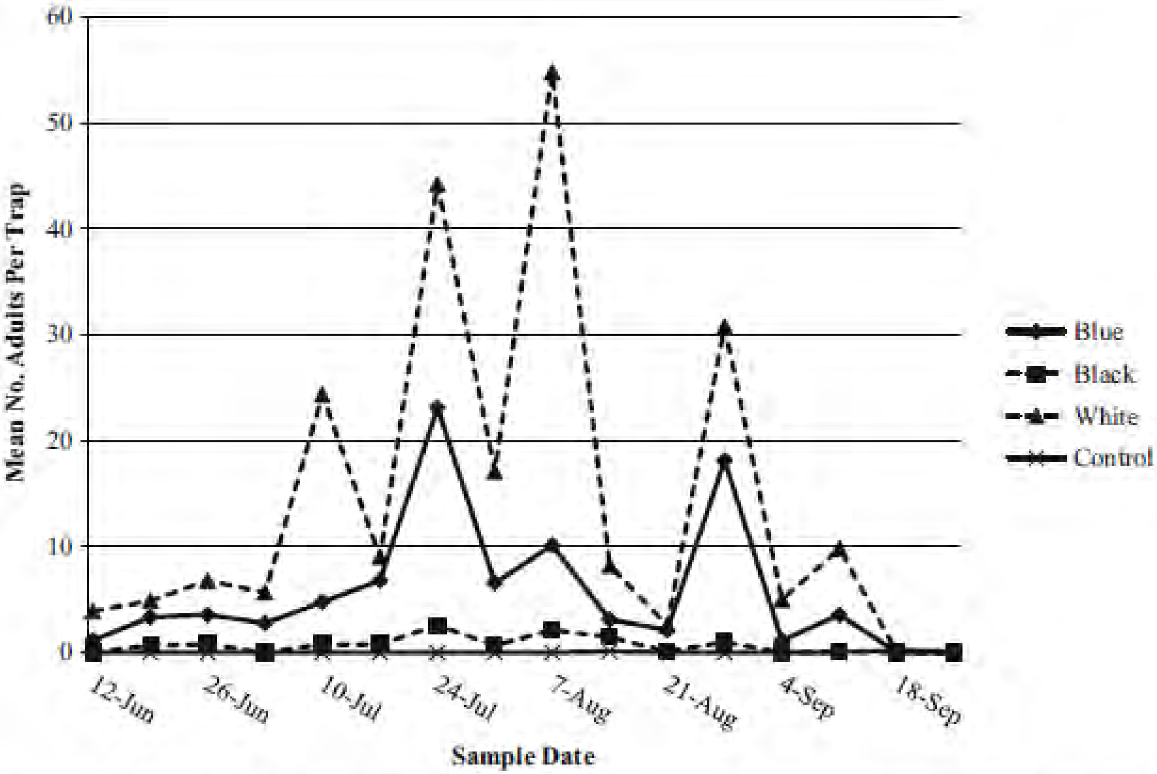


Fig. 8 Mean number adult *Halyomorpha halys* captured per trap across three commercial orchards in traps baited with light-based stimuli from 12 June through 30 September, 2012 ($n = 9$)

White light sources were most attractive in the field, but blue light sources were most specific for BMSB

2.1.1.4. Active Space of Traps, Efficient Trap Designs, and Deployment Strategies

Published Manuscripts

Acebes-Doria, A.L., T.C. Leskey and J.C. Bergh. 2015. Development and comparison of trunk traps to monitor movement of *Halyomorpha halys* (Hemiptera: Pentatomidae) nymphs on host trees. Entomologia Experimentalis et Applicata. (accepted).

Morrison, III W.R., J.P. Cullum, and T.C. Leskey. 2015. Evaluation of trap design and deployment strategy for capturing *Haylomorpha halys* (Hemiptera: Pentatomidae). Journal of Economic Entomology. DOI: http://dx.doi.org/10.1093/jee/tov159

Joseph, S., C. Bergh, S.E. Wright and T.C. Leskey. 2013. Factors affecting captures of brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae) in baited pyramid traps. Journal of Entomological Science. 48: 43-51.

Leskey T.C., S.E. Wright., B.D. Short. and A. Khrimian. 2012. Development of behaviorally based monitoring tools for the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae) in commercial tree fruit orchards. Journal of Entomological Science. 47: 76-85.

Can we make trapping simpler for growers?

- <u>Visual Stimulus</u>
  - Large black pyramid (trunk-mimicking stimulus)
- <u>Olfactory Stimulus</u>
  - PHER + MDT
- <u>Capture Mechanism</u>
  - Tapered pyramid attached to inverted funnel jar with DDVP strip
- <u>Deployment Strategy</u>
  - Traps placed in peripheral row or border area

Can we utilize other trap styles?

Experimental Standard Wooden Pyramid

Coroplast Pyramid

Small Pyramid (Ground)

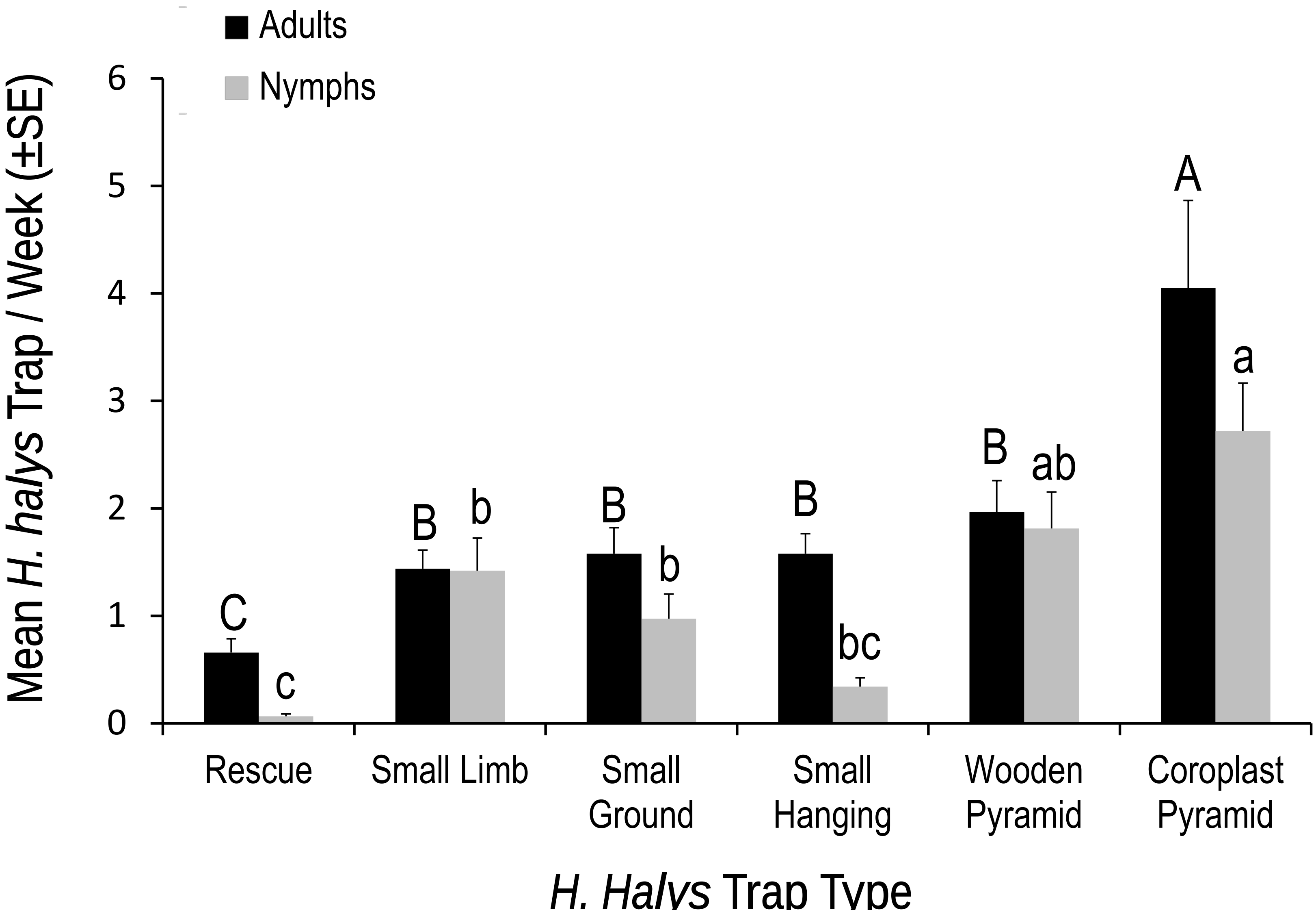
Small Pyramid (Limb)

Small Pyramid (Hanging)

Rescue (Hanging/ Foilage)

- Are captures similar among other trap types and deployment strategies compared with our experimental standard?
- Baited with BMSB Pheromone + MDT synergist. Two years of data from commercial orchards.

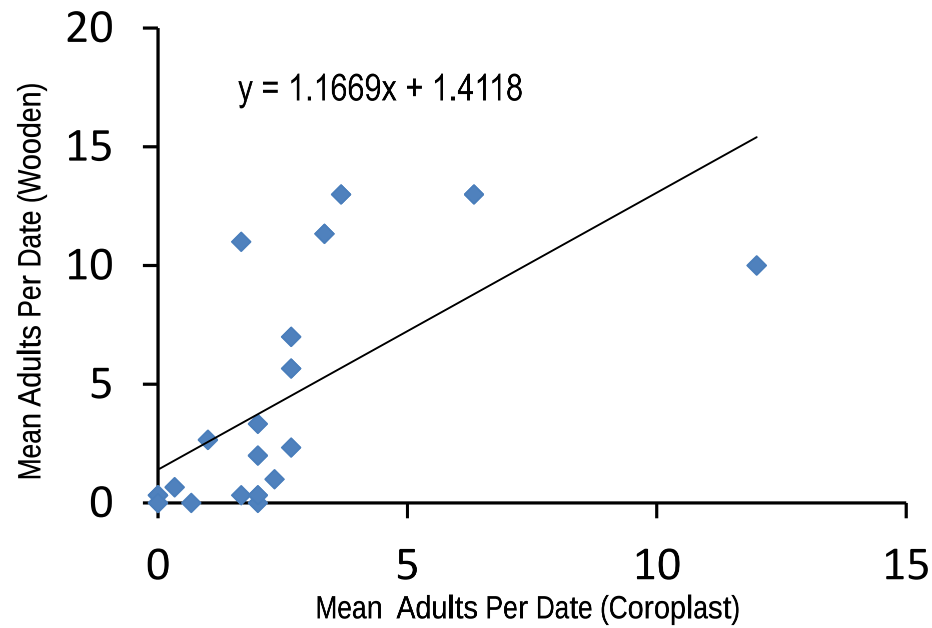
Season-Long Trap Captures / Sensitivity



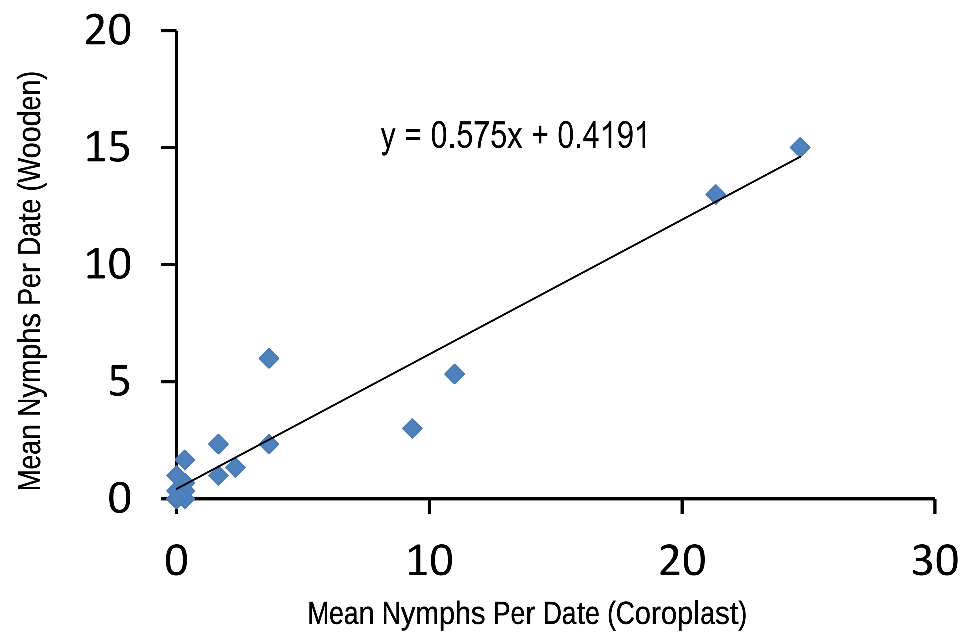
(Morrison et al. 2015)

Coroplast vs. Standard Wooden Pyramids

Spearman Rank Correlation
ρ=0.735
P < 0.0001

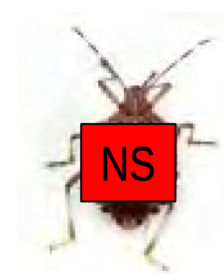


Spearman Rank Correlation
ρ=0.900
P < 0.0001



(Morrison et al. 2015)

Coroplast vs. All Others

Coroplast Pyramid		Small Pyramid (Ground)	Small Pyramid (Hanging)	Small Pyramid (Limb)	Rescue (Hanging/ Foilage)
					
		 SIG.	 SIG.	 SIG.	 NS
	(Morrison et al. 2015)	 SIG.	 SIG.	 SIG.	 SIG.

New Trap Comparisons

Delta Trap

Yellow Sticky Card

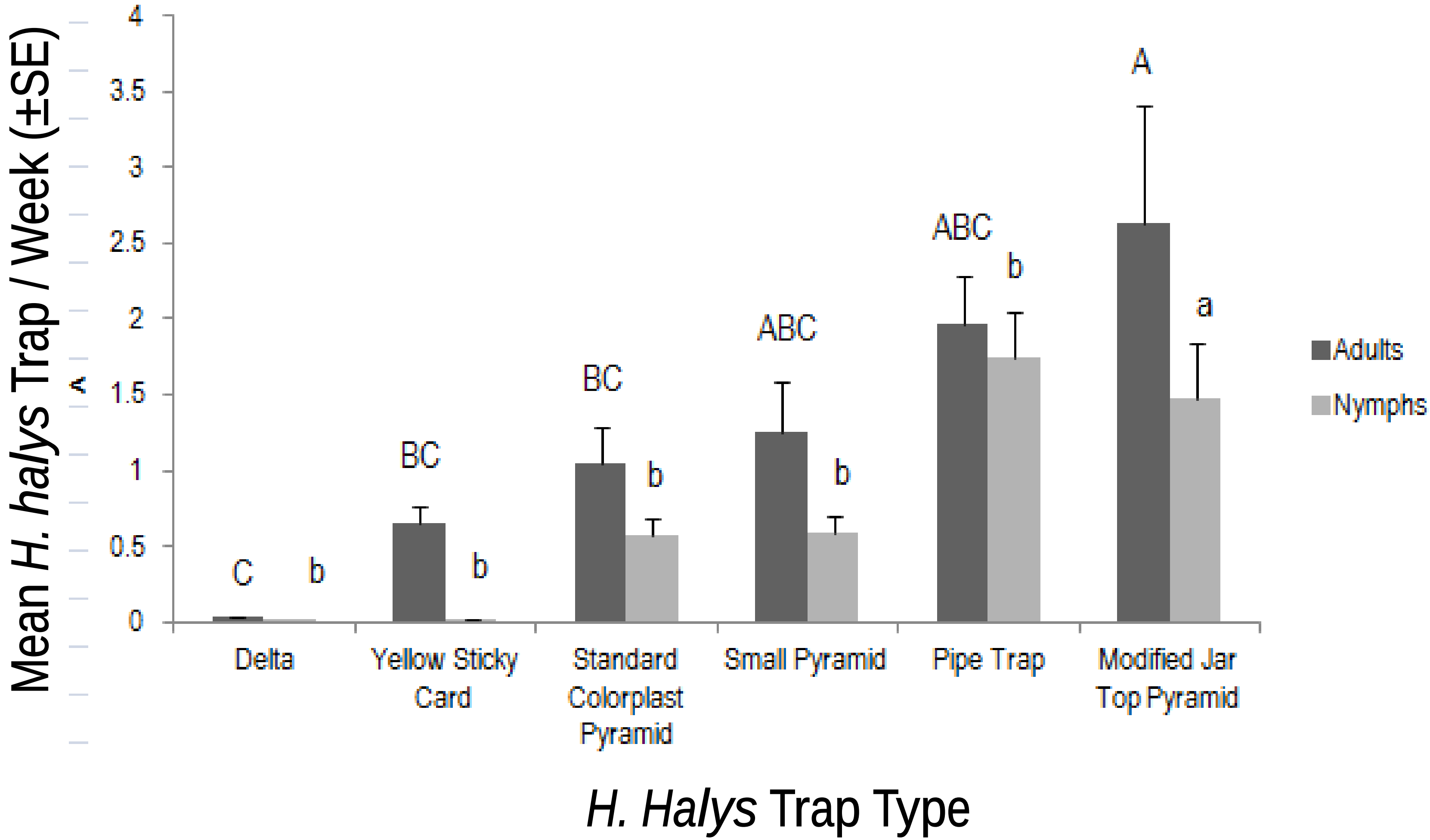
Standard Coroplast Pyramid

Small Black Pyramid

Pipe Trap

Modified Jar Top Pyramid

Season-Long Trap Captures / Sensitivity



Standard Pyramid vs. All Others

Delta Trap

Yellow Sticky Card

Standard Coroplast Pyramid

Small Black Pyramid

Pipe Trap

Modified Jar Top Pyramid

PSU Trap comparison for monitoring BMSB - 2015

Traps lure combinations:

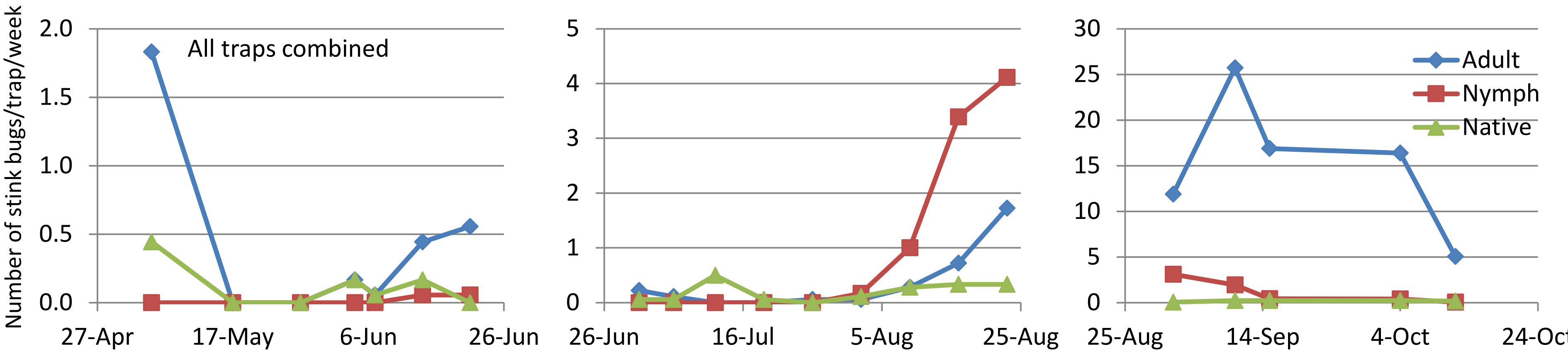
- Dead – Inn Pyramid trap (Ag-Bio) x Ag-Bio BMSB X-tra lure
- Clear sticky trap (AlphaScent) x Rescue lure
- Rescue Stink Bug Trap (Sterling Int.) x Rescue lure

Project description:

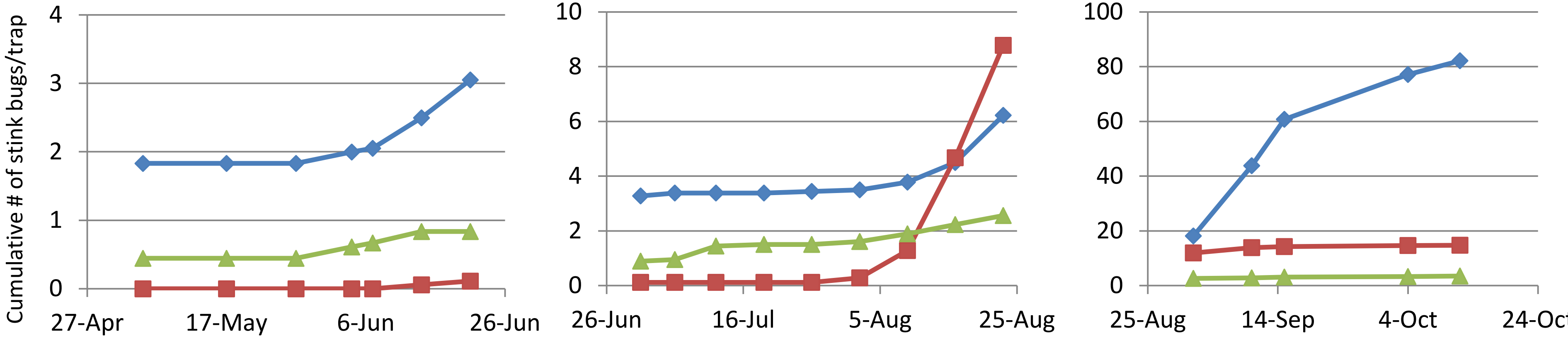
- Two commercial fruit orchards
- Three replicates per orchard
- Two locations (inside/outside) for each trap/lure combination per replicate

Observations period : May 01 - Oct 14, 2015

BMSB Trap Comparison PSU 2015 - Orchard No. 1



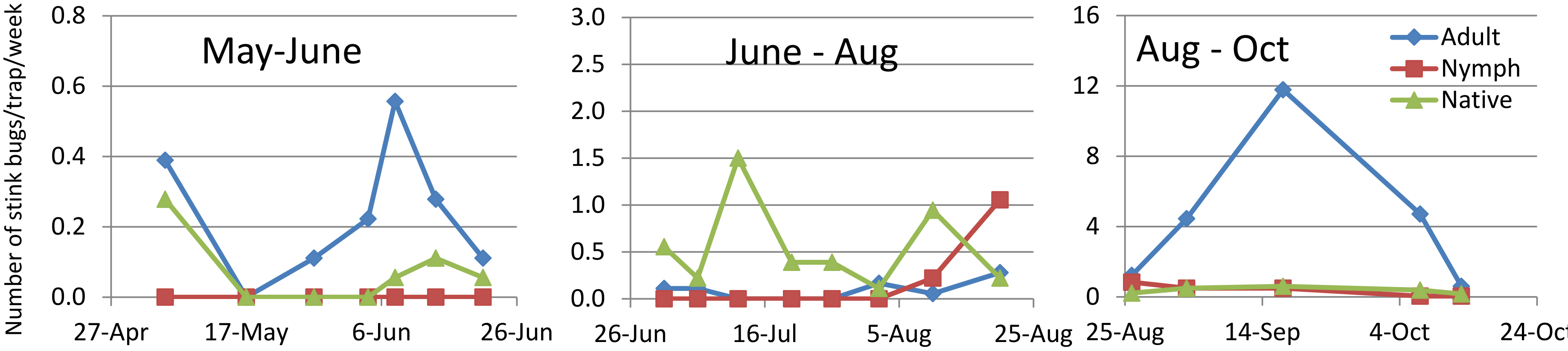
Seasonal activity



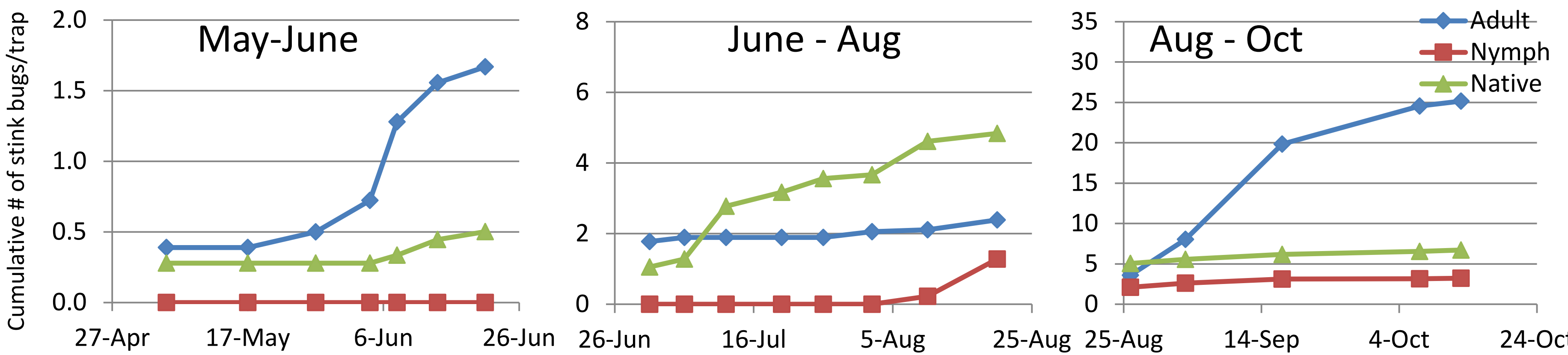
Cumulative captures per period

Trap data from all traps combined, n=18 traps per location

BMSB Trap Comparison PSU 2015 - Orchard No. 2



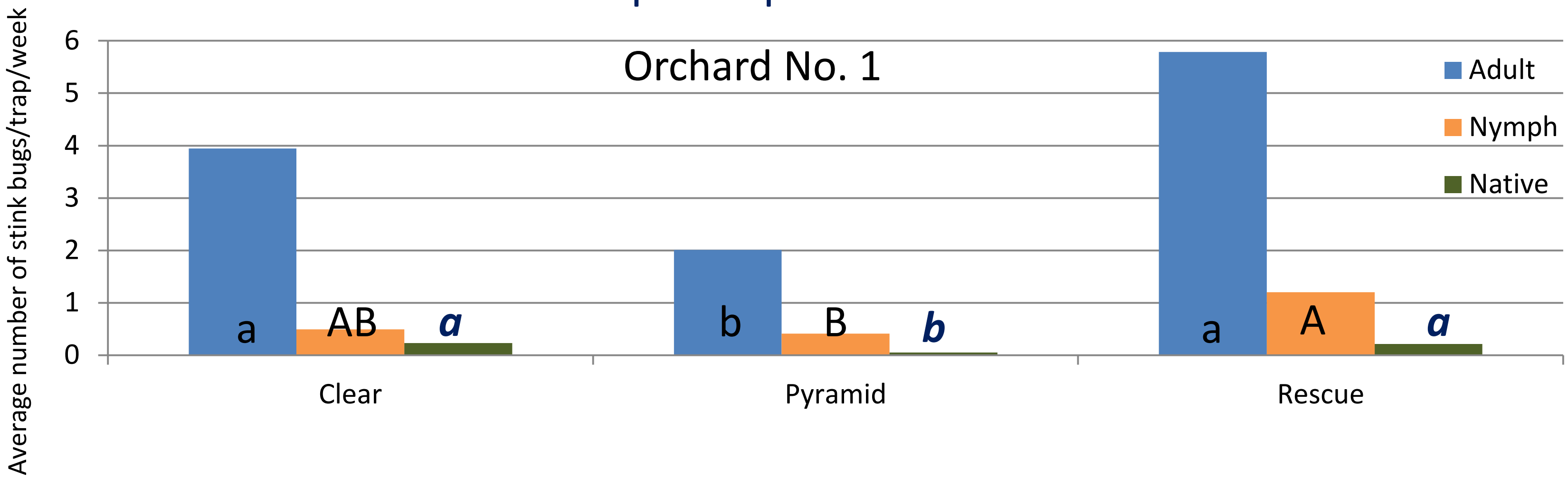
Seasonal activity

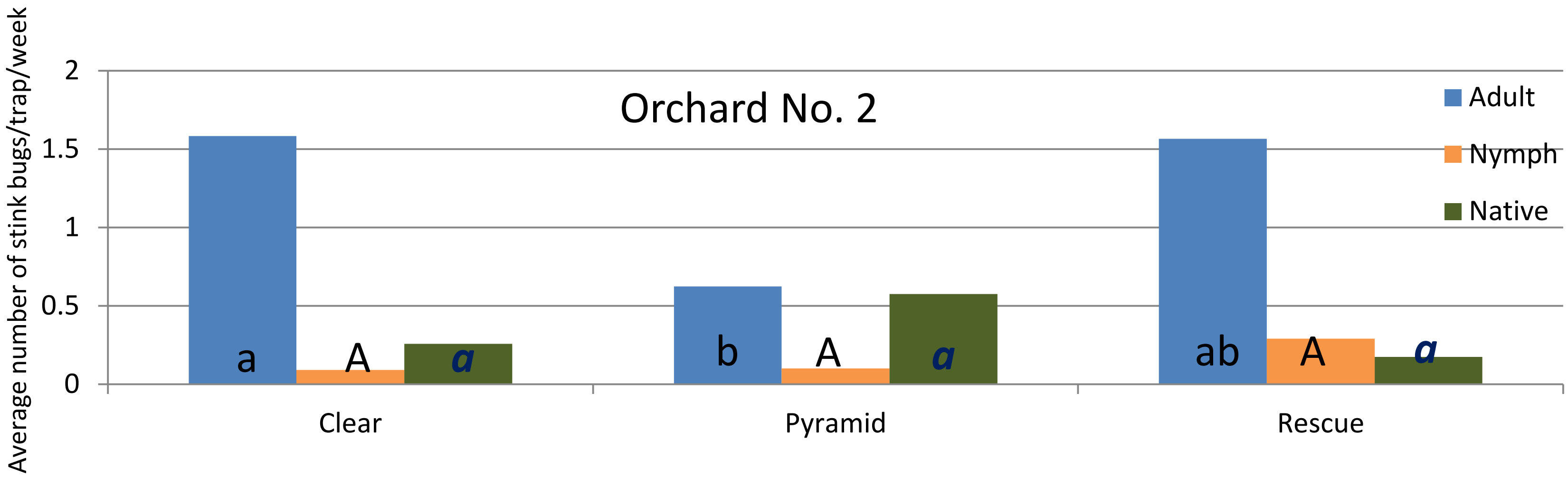


Cumulative captures per period

Trap data from all traps combined, n=18 traps per location

BMSB Trap Comparison – PSU 2015





Trap data from all traps combined, n=6 traps per location;
Bars within the same category (adults, nymphs and native) for the same location with the same letter are not different (ANOVA, sqrt transformation, LSD All pairwise, $p < 0.05$)

Monitoring Nymphal Movement

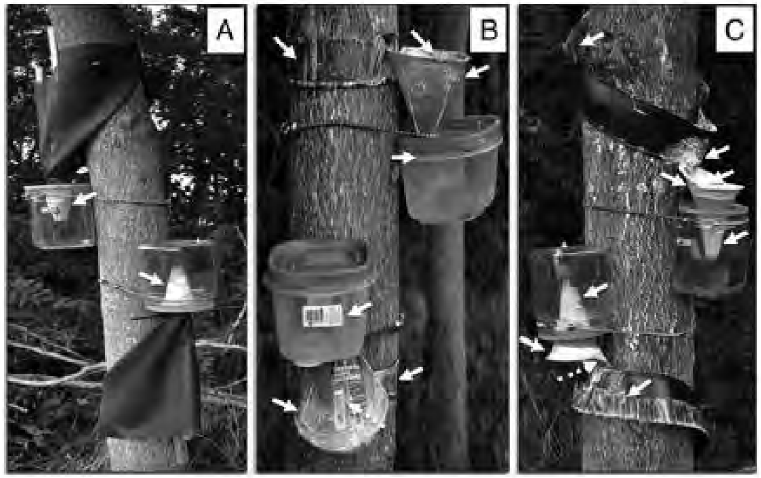


Figure 1 (A) Circle, (B) Hanula, and (C) M&M (Moeed & Meads) traps used to capture *Halyomorpha halys* nymphs walking on *Ailanthus altissima* logs and trees in laboratory and field studies, respectively, at Winchester (VA, USA). Solid arrows indicate surfaces coated with fluon and the broken arrow indicates the roughened area in the interior of the funnel.

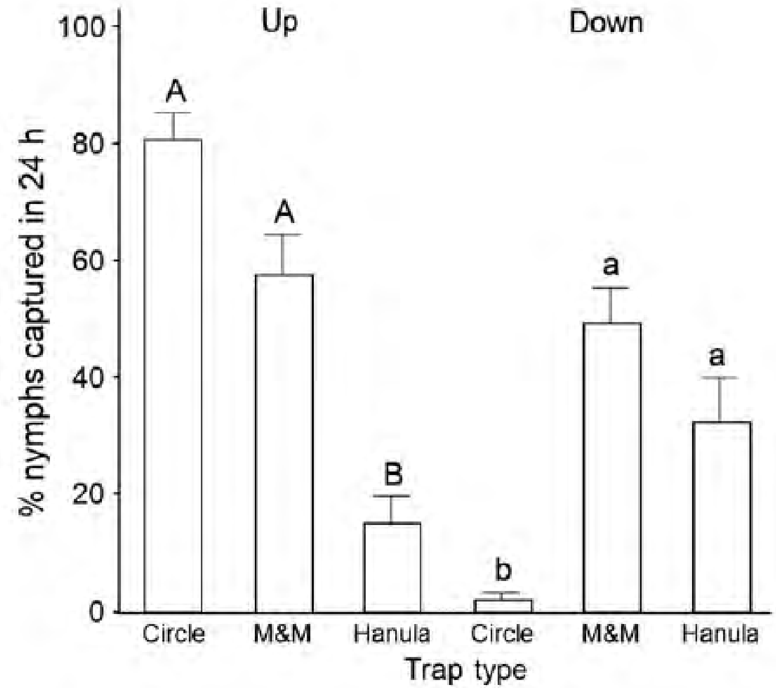


Figure 2 Mean (+ SE) percentage of second instar *Halyomorpha halys* captured in three types of trunk trap (see Figure 1) and two orientations (up, capturing upward-walking nymphs; down, capturing downward-walking nymphs) at 24 h after their release at the bottom or top of *Ailanthus altissima* logs in a growth chamber (n = 4 per replicate per trap design and orientation). The experiment was repeated 4×. Bars within an orientation capped with the same letters are not significantly different (Tukey's HSD test: P>0.05).

Have been used successfully to document nymphal dispersal onto and from host trees season-long. Implications for importance of diet-mixing.

(Acebes-Doria et al. 2015)

2.1.2. Other Monitoring Tools

Understanding the temporal patterns of abundance and dispersal of BMSB adults and nymphs among wild and cultivated hosts will enhance our understanding of the risk posed to specialty crops throughout the growing season.

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RESEARCH ARTICLE

Handheld Lasers Allow Efficient Detection of Fluorescent Marked Organisms in the Field

Kevin B. Rice, Shelby J. Fleischer, Consuelo M. De Moraes, Mark C. Mescher, John F. Tooker, Moshe Gish

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Abstract

Marking organisms with fluorescent dyes and powders is a common technique used in ecological field studies that monitor movement of organisms to examine life history traits, behaviors, and population dynamics. External fluorescent marking is relatively inexpensive and can be readily employed to quickly mark large numbers of individuals; however, the ability to detect marked organisms in the field at night has been hampered by the limited detection distances provided by portable fluorescent ultraviolet lamps. In recent years, significant advances in LED lamp and laser technology have led to development of powerful, low-cost ultraviolet light sources. In this study, we evaluate the potential of these new technologies to improve detection of fluorescent-marked organisms in the field and to create new possibilities for tracking marked organisms in visually challenging environments such as tree canopies and aquatic habitats. Using handheld lasers, we document a method that provides a fivefold increase in detection distance over previously available technologies. This method allows easy scouting of tree canopies (from the ground), as well as shallow aquatic systems. This novel detection method for fluorescent-marked organisms thus promises to significantly enhance the use of fluorescent marking as a non-destructive technique for tracking organisms in natural environments, facilitating field studies that aim to document otherwise inaccessible aspects of the movement, behavior, and population dynamics of study organisms, including species with significant economic impacts or relevance for ecology and human health.

(Rice et al. 2015)

Summer = Yellow Fall = Orange
Winter = White Spring = Pink

OBJECTIVE 2. DEVELOP AND REFINE MONITORING AND MANAGEMENT TOOLS FOR

OBJ.	Description	Participants
2.1	Develop monitoring tools for BMSB	
2.1.1	Trap-based monitoring	
2.1.1.1	Identification of pheromone and other attractants	Khriman, Leskey, Landolt, Lee, Wiman, Shearer, Rondon
2.1.1.2	Optimization of pheromone and kairomone dispensers for monitoring BMSB	Khrimian, Leskey, Bergh, Krawczyk, Saunders, Rodriguez-Saona, Hamilton, Polk, Wiman, Shearer
2.1.1.3	Refining utility of light-based traps for BMSB	Leskey, Hamilton, Krawczyk, Jacobs, Wiman, Shearer, Agnello, Jentsch
2.1.1.4.	Define behavioral characteristics of BMSB and active space of baited traps to develop efficient traps and deployment strategies	Leskey, Bergh, Krawczyk, Saunders, Shearer Wiman
2.1.2	Assess other types of monitoring tools	Leskey, Wright, Bergh, Krawczyk, Saunders, Wiman, Shearer

What We Didn't Accomplish Though We Have Preliminary Data

- Identification of other attractants (additional synergists and host plant volatiles).
- Optimized pheromone dispensers. Standardized dose/release rate for monitoring particular crops.
- Use of combination light and pheromone-based stimuli.
- Distance of response to baited traps.
- Optimized trap design and deployment strategy for specific specialty crops.
- Simpler trap designs.

Next Steps

- Continued collaboration with commercial companies to ensure reliable pheromone-based products and traps are available.
- Further validations of pheromone-based trapping in commercial orchards and other crops.
- Attract and kill strategies for spatially precise management and overall population reduction.

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