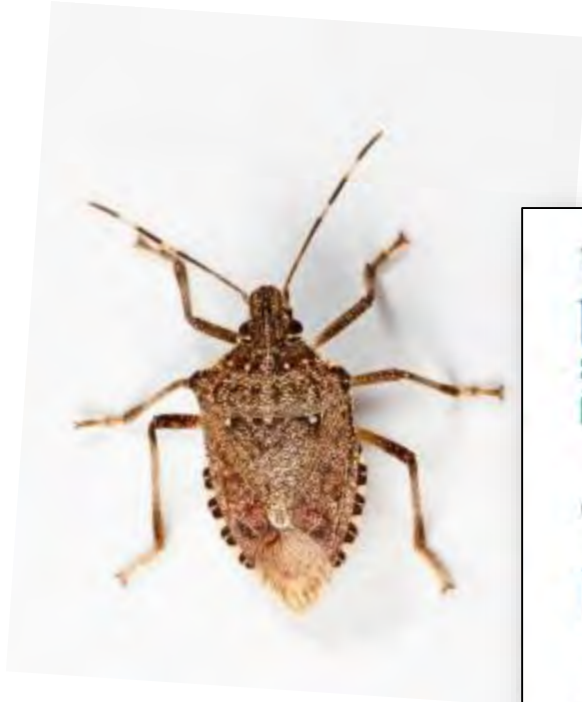


Subobjective 2.2.2. Development of BMSB-specific fungal Entomopathogens (St. Leger - U of Maryland & Lee – USDA Corvallis)



Funding

 **United States Department of Agriculture** **National Institute of Food and Agriculture** Specialty Crop Research Initiative Grant #2011-01413-30937

Collaborating Institutions

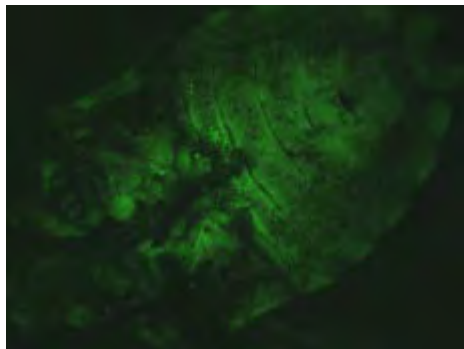
  

 **Cornell University**  **OSU Oregon State University**  **UNIVERSITY OF MARYLAND**

 **Virginia Tech**  **NC STATE UNIVERSITY**

Background

- *Metarhizium* species, a.k.a. green muscardine fungi, are recognized for their biological control potential against arthropods
- *Metarhizium* infects and kills BMSB at high humidities
- Poor performance of fungi at low humidities
 - Low humidities reduce germination
 - Increase susceptibility to volatiles
- BMSB relies heavily on volatiles for defense
 - BUT haemolymph defenses are weak as shown by injecting spores

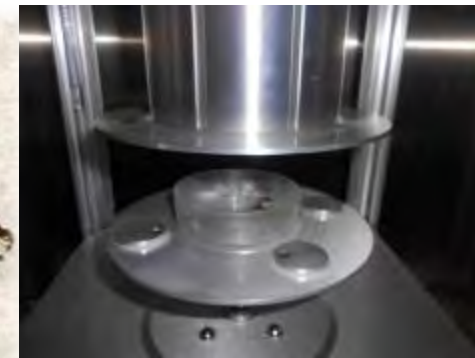
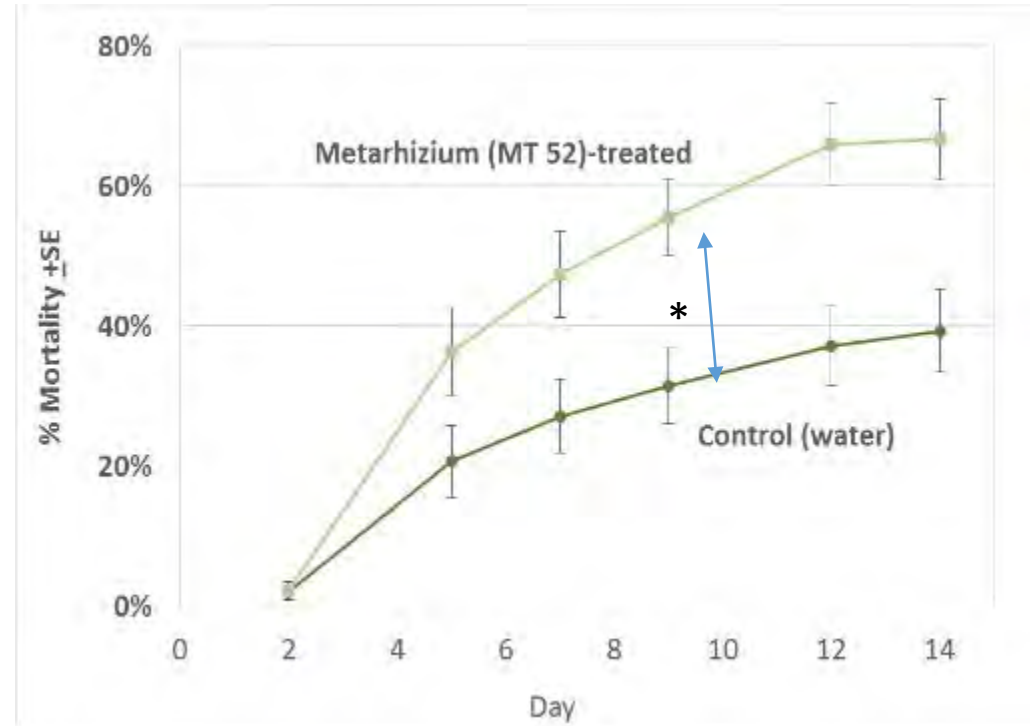


A “glow in the dark”
BMSB produced by
injecting 50 spores
of Ma-GFP



Metarhizium spray of nymphs (OR, Jana Lee)

- 66% of nymphs sprayed with Met 52 EC died
- Many nymphs (control or Met) still molted to adulthood
- While Met can kill nymphs, this rate under optimal lab conditions is not promising enough to meet grower needs

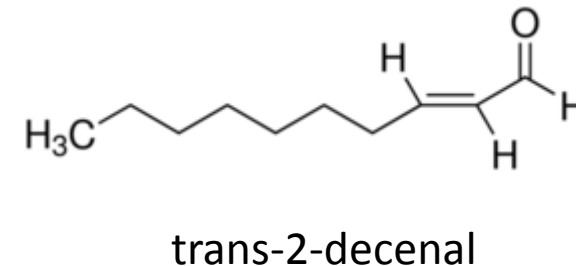
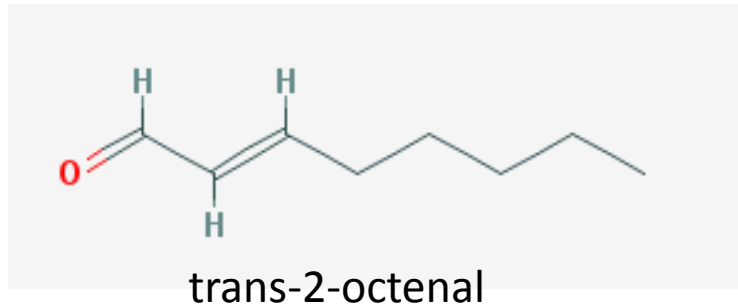


The Objective: (UMD, St. Leger)

Genetically manipulate fungal strains (*Metarhizium*) to overcome this defense, leading to sustainable BMSB management tactics

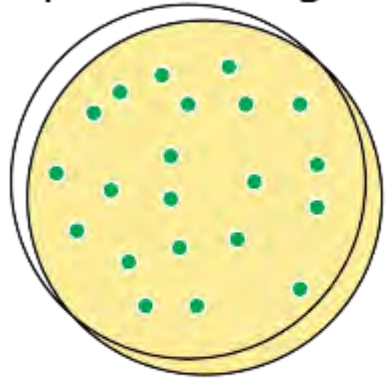
The Challenge: Low virulence of fungi against BMSB

- Brown marmorated stink bug defensive compounds may be the cause of poor fungal performance
- Two chemicals present in stink bug defensive secretions (trans-2-octenal and trans-2-decenal) found to strongly inhibit growth of entomopathogenic fungi





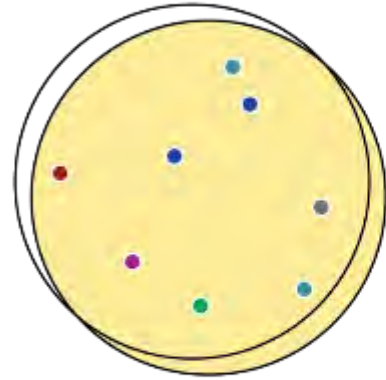
Stink Bug
Specific Fungus



UV-C Exposure



UV-Generated
Mutant Fungi

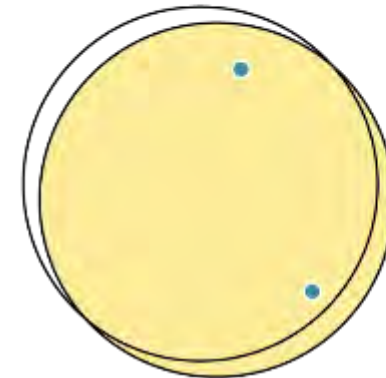


UV Mutagenesis Methodology

BMSB Volatile
Exposure



Volatile Resistant
Mutant Fungi



Bioassays With
Volatile Resistant
Mutants



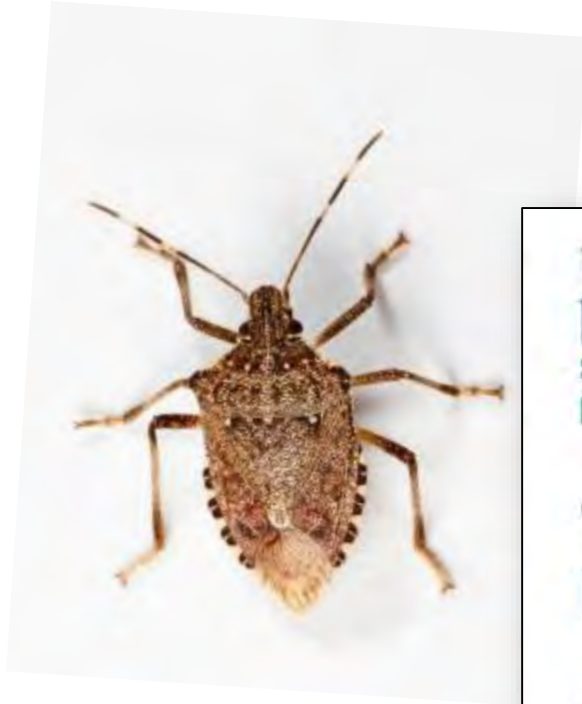


Logical Next Steps – BMSB-specific fungal Entomopathogens

- Resistant mutants have fluffy white growth – this could be because BMSB volatiles resemble chemicals that fungi use for auto-inhibition of spores
 - select for mutants that do not auto inhibit
 - transgenic approaches to alter regulation of sporulation genes
- Conduct mass spec analysis of volatile production by infected BMSB
- Determine role of microbial community on cuticle
- Employ field cages to test laboratory results that single insects are more susceptible than clustered insects
- Protoplast fusion of BMSB-infecting *Metarhizium* strains with *M. acridum* (desert strain that infects at low humidities).
- Use GE to convert volatile and/or RH resistant strain to hypervirulence



Subobjective 2.2.5. Asian Natural Enemies



Funding

 **United States Department of Agriculture** **National Institute of Food and Agriculture** **Specialty Crop Research Initiative Grant #2011-01413-30937**

Collaborating Institutions

 **UNIVERSITY OF DELAWARE**  **PENNSYLVANIA STATE UNIVERSITY**

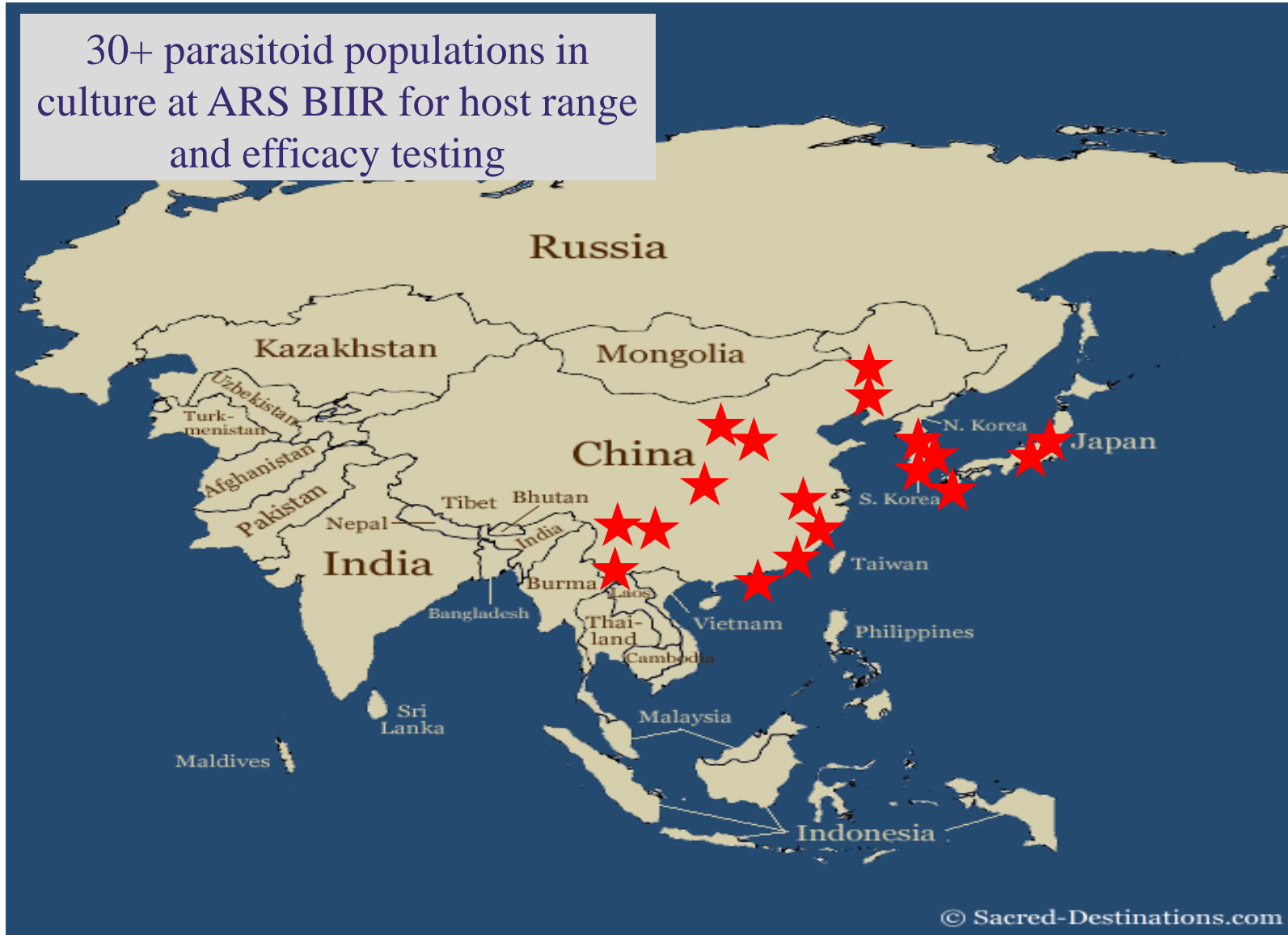
 **THE STATE UNIVERSITY OF NEW JERSEY RUTGERS**  **Northeastern IPM Center**  **WASHINGTON STATE UNIVERSITY**

 **Cornell University**  **OSU Oregon State University**  **UNIVERSITY OF MARYLAND**

 **Virginia Tech**  **NC STATE UNIVERSITY**

Foreign exploration for Asian natural enemies of BMSB (2007-2015)

30+ parasitoid populations in culture at ARS BIIR for host range and efficacy testing

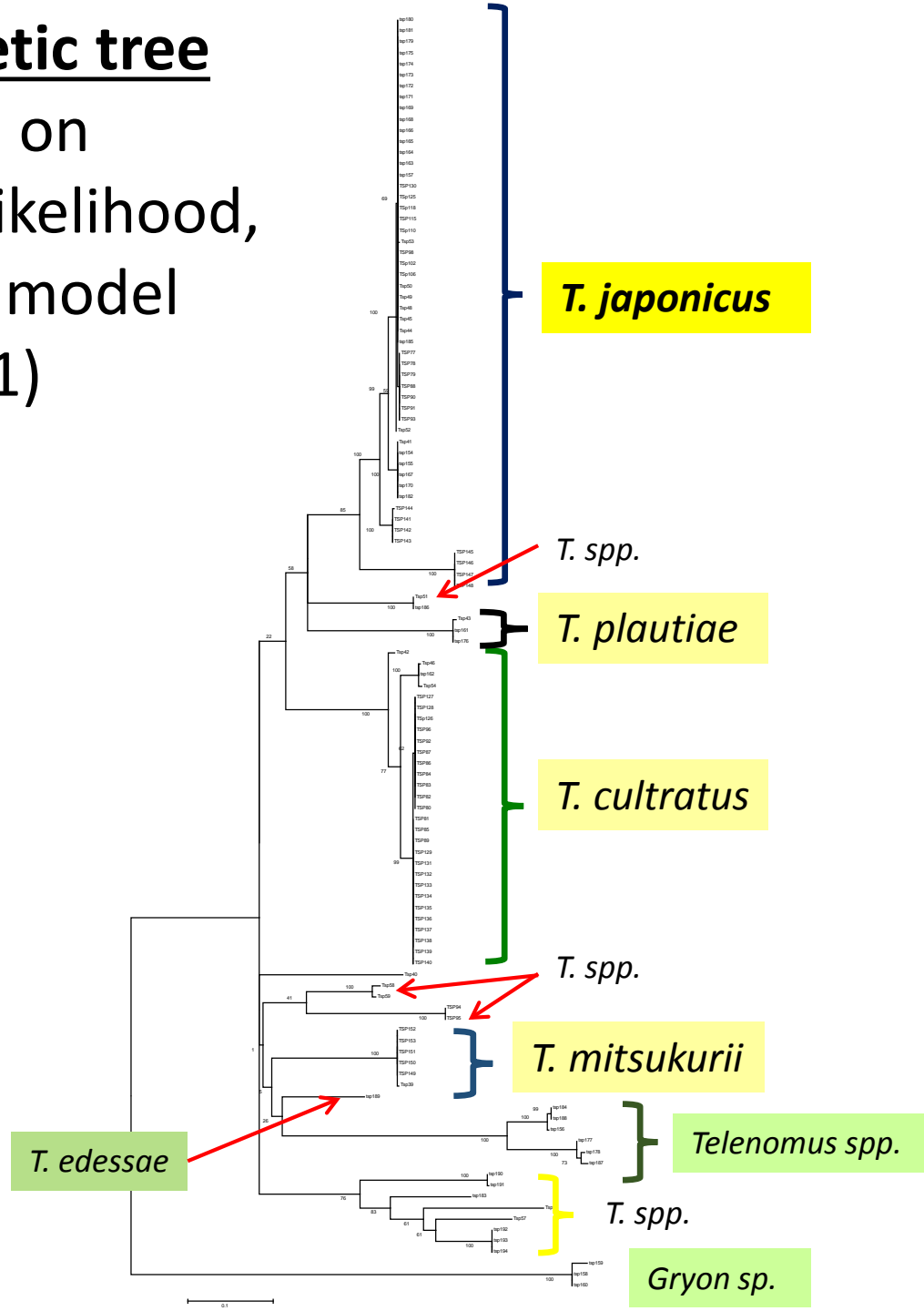




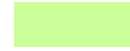
Phylogenetic tree

based on

Maximum Likelihood,
GTR+G+I model
(CO1)



Scelionidae recovered from Asian
&
Pentatomid eggs



Scelionidae recovered from North
American Pentatomid eggs

Contributors:

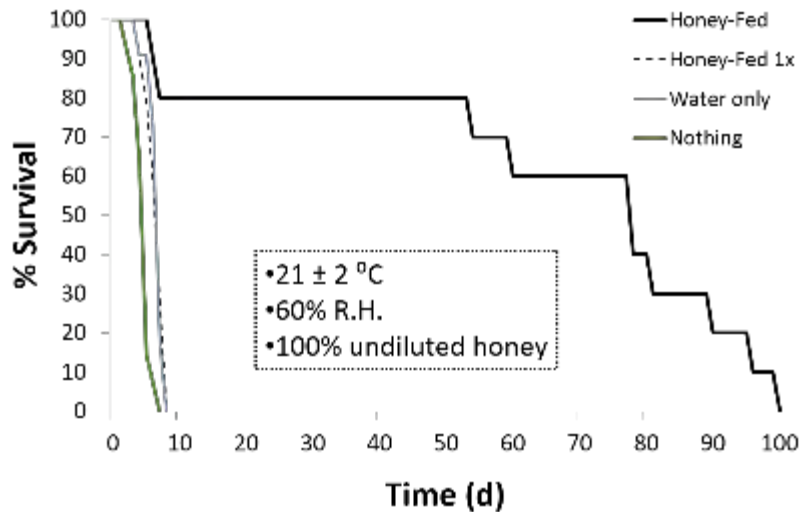
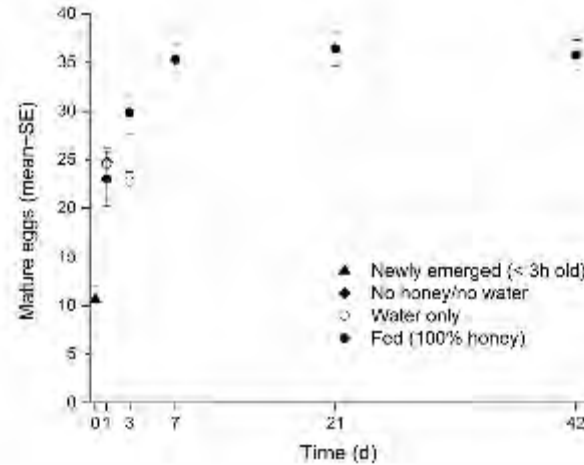
- M.-C. Bon – ARS/EBCL
- E. Talamas – ARS/SEL
- M. Buffington – ARS/SEL
- C. Dieckhoff – ARS/BIIR
- K. Hoelmer – ARS/BIIR



Brian T. Cutting

Trissolcus japonicus (Hymenoptera: Scelionidae)

(first described as *T. halyomorphae*)



•21 ± 2 °C
 •60% R.H.
 •100% undiluted honey

- solitary egg parasitoid
- 2 - 3 weeks/generation
- multiple generations/season
- female-biased sex ratio
- 65 to 90% parasitism on BMSB reported in Asia



Trissolcus japonicus is oligophagous - it attacks several Asian pentatomid species



Halyomorpha halys



Glaucias subpunctatus



Plautia crossota



Dolycoris baccarum



Erthesina fullo





Host Range Evaluations– A team effort to fast-track the evaluation process

Funding for Host Range Evaluations:

Farm Bill funding (APHIS PPQ)

NIFA SCRI multi-institution BMSB grant

Collaborators:

USDA-ARS (Newark, DE & EBCL, France)

University of Delaware (D. Tallamy)

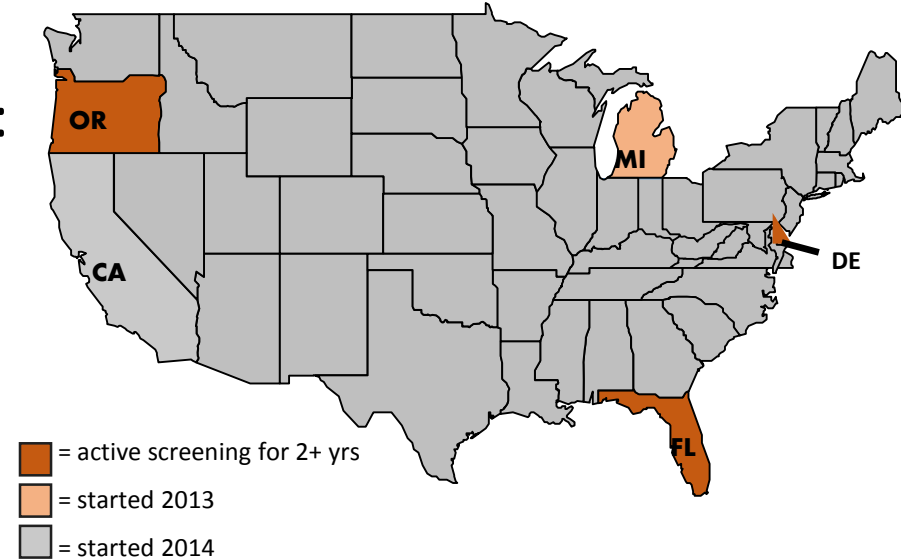
Florida Dept. Agriculture & Consumer Services, Division of Plant Industry

MSU – Michigan State University – Department of Entomology (E. Delfosse)

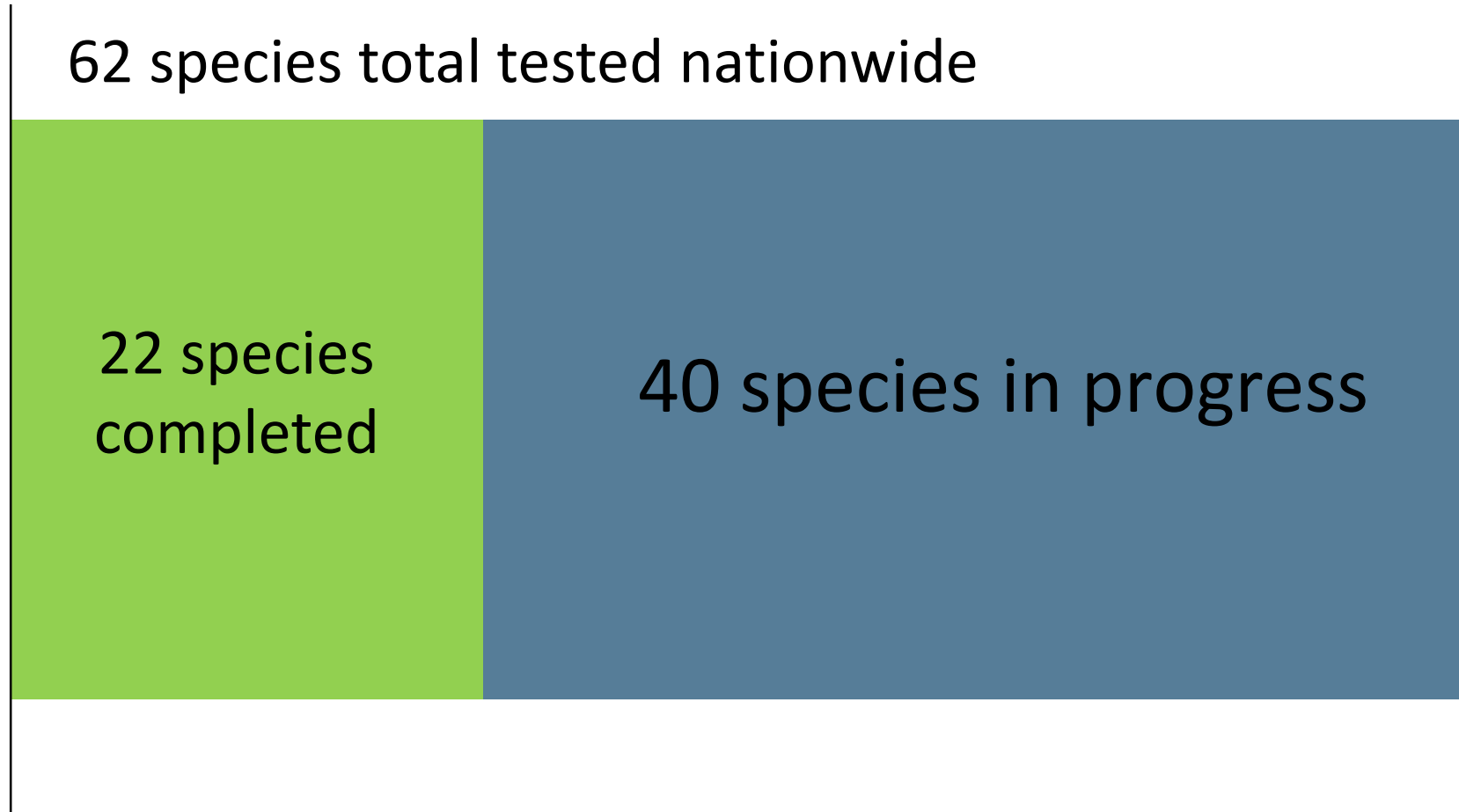
Oregon Department of Agriculture

Oregon State University – Department of Horticulture (V. Walton, P. Shearer)

University of California, Riverside & CDFA (M. Hoddle, C. Pickett)



Host Range Evaluations: Progress



Host range testing procedures



No Choice Test

Exposure to non-target species egg mass only – for 24h:



Followed by a BMSB target egg mass as control for another 24h:



If parasitism
on non-target
is recorded



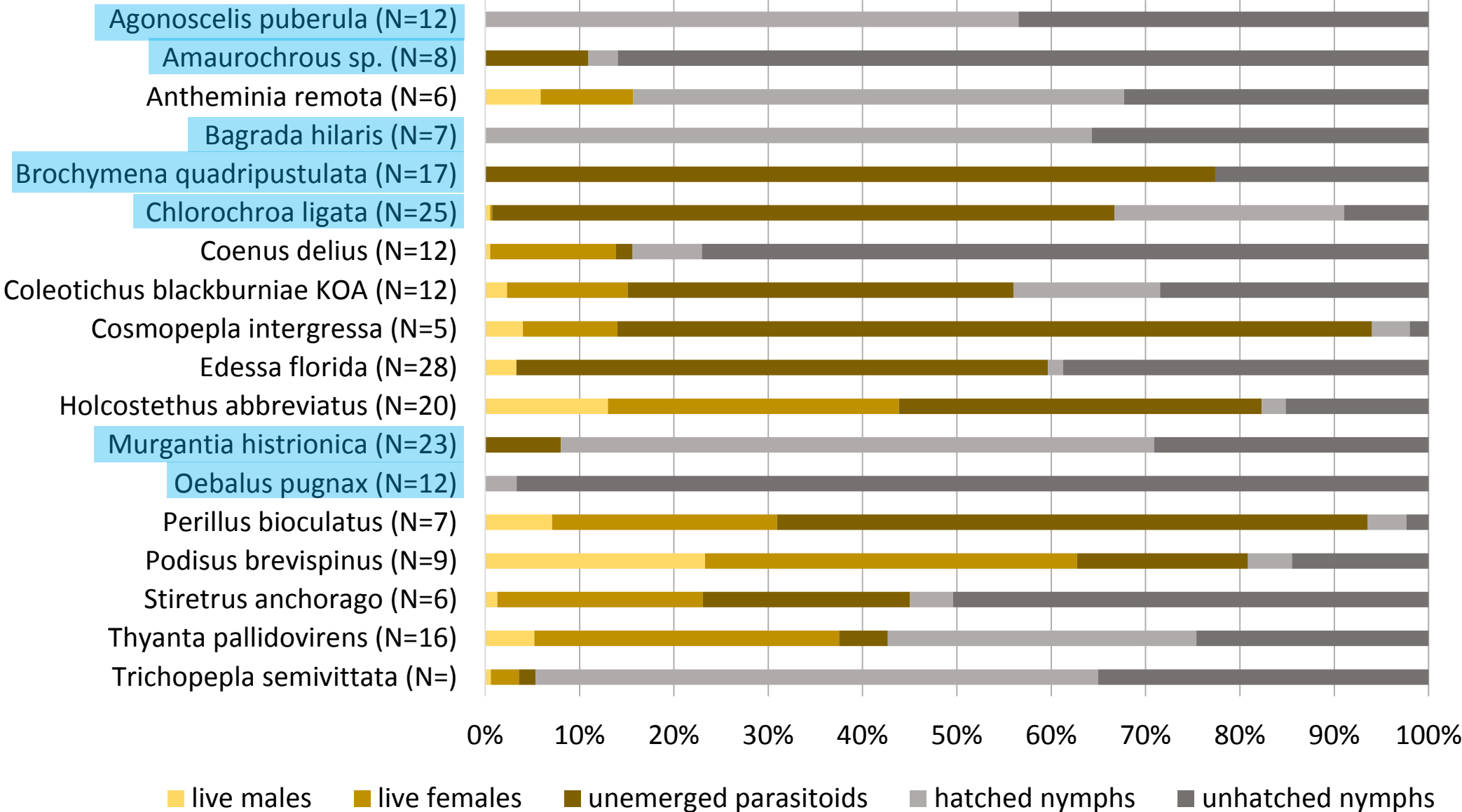
Choice Test

Egg masses of non-target species and BMSB presented together for 24h:



No-Choice Test Outcome - Part 1

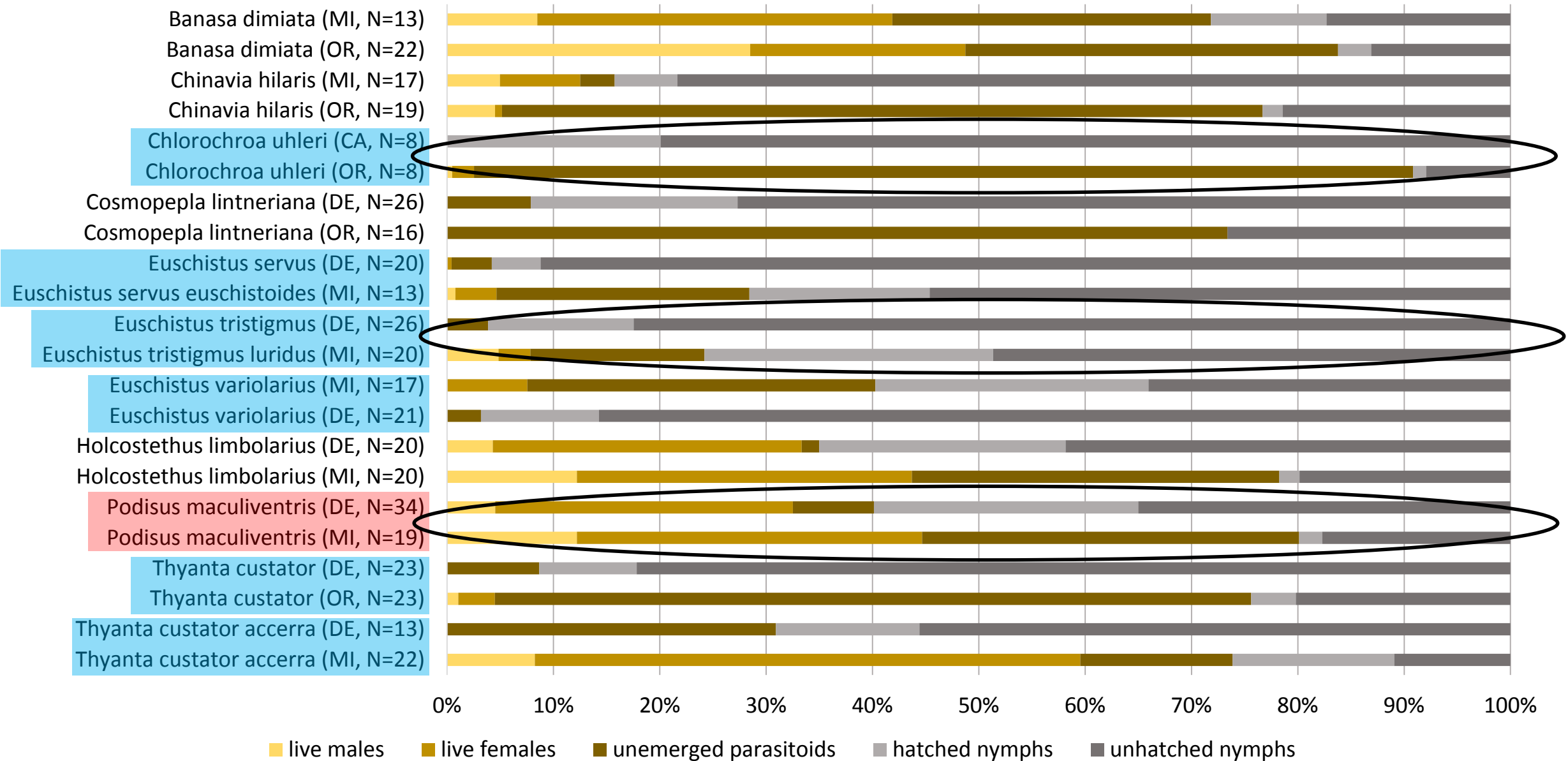
(as of 2015)





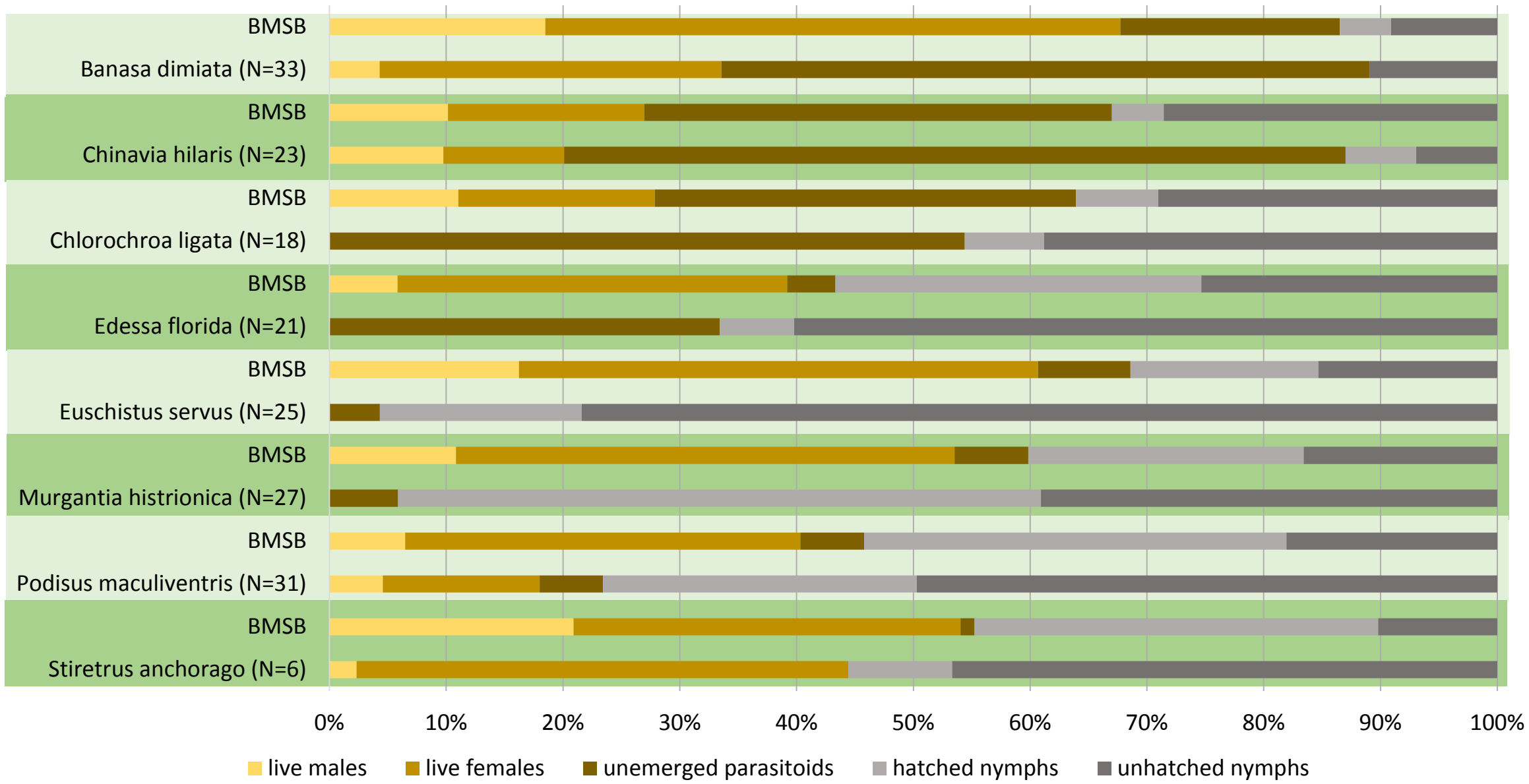
No-Choice Test Outcome - Part 2

(as of 2015)



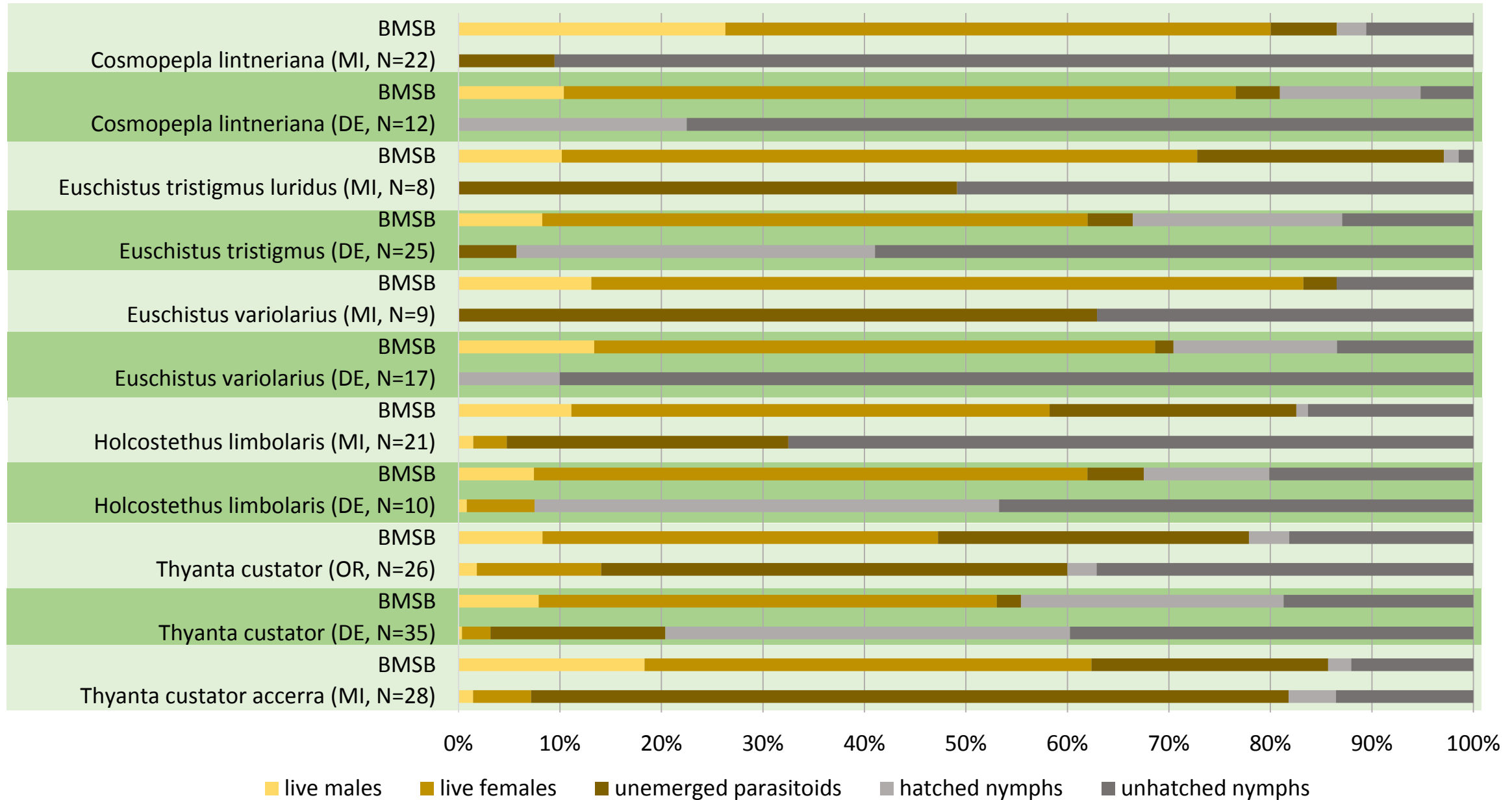
Choice Test Outcome - Part 1

(as of 2015)



Choice Test Outcome - Part 2

(as of 2015)



Logical next steps

A Closer Look at Host Choice Behavior in *T. japonicus*

Influence of arena size and complexity



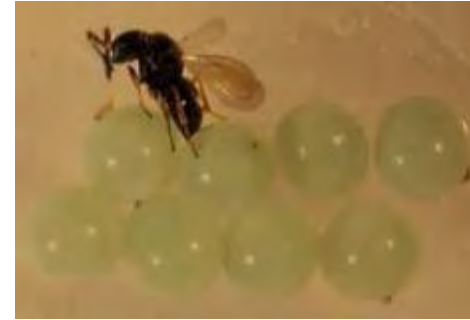
➤ **Size (Finished)**

- 10 dram
- 100 dram
- 500 dram
- 1000 dram
- 2000 dram

➤ **Complexity (Started)**

- Choice tests on plants

Role of parasitoid physiology & experience



- Parental experience
- Parental physiology
- Effect of host choice on offspring physiology & behavior

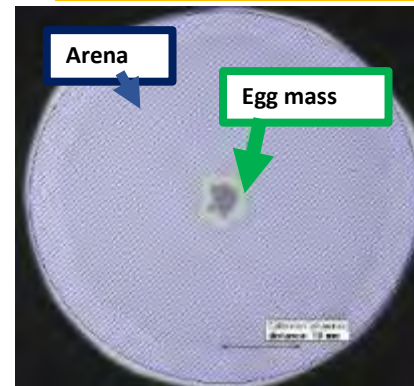
Influence of time of exposure



➤ **Finished**

- 1 h
- 4 h
- 6 h
- 24 h

Behavioral observations



➤ **Ongoing**

- Searching behavior
- Oviposition behavior
- Host choice
- ...

Olfactometer Studies

(FL, MI) - ongoing



www.ars-fla.com



Ecological Host Range of *T. japonicus* in Asia

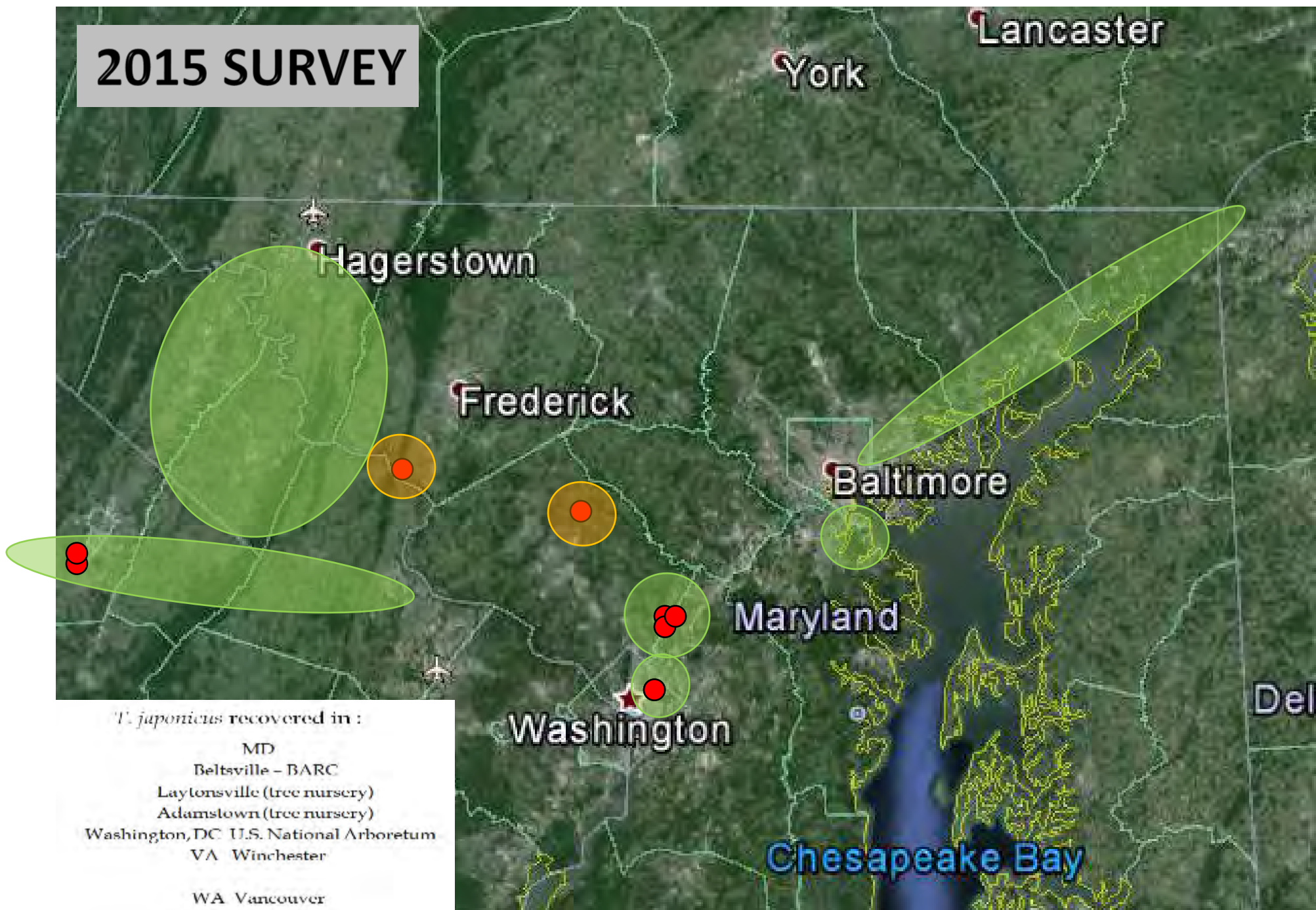


Recovery of *Trissolcus japonicus* in Maryland in 2014



a potential game changer?

2015 SURVEY

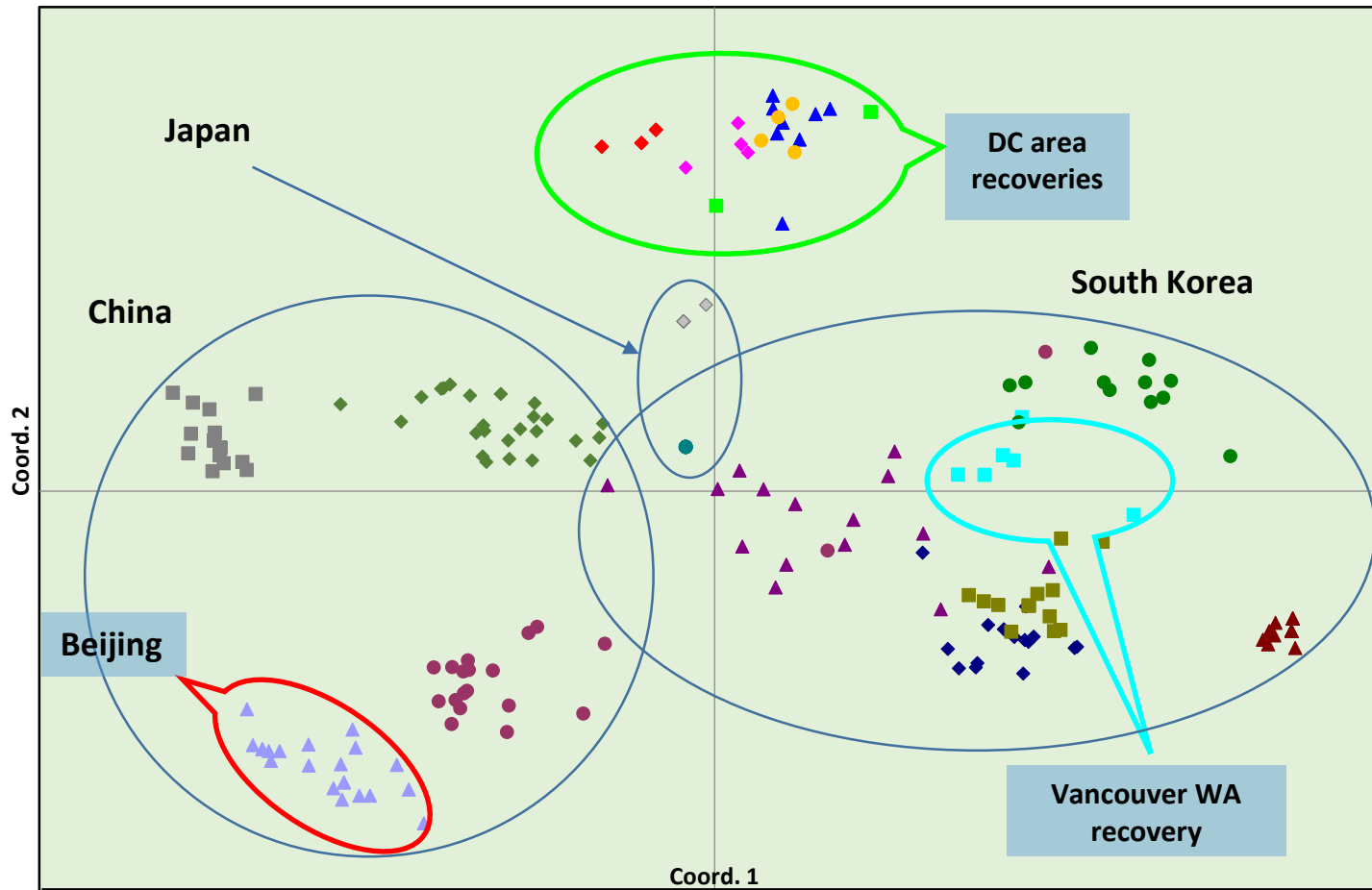


T. japonicus recovered in :

- MD
 - Beltsville - BARC
 - Laytonsville (tree nursery)
 - Adamstown (tree nursery)
- Washington, DC U.S. National Arboretum
- VA Winchester
- WA Vancouver

Origin of the adventive *T. japonicus* populations

Principal coordinates analysis of genetic diversity among 23 microsatellite markers in *T. japonicus*



Genetic distance between populations

Axis 1 & axis 2 explain, 22% and 20% respectively, of the distribution

Take home message:

- 1) These populations are adventive – they were not released nor did they escape quarantine!
- 2) DC area populations genetically similar to populations sampled in Japan and S. Korea
- 3) WA population genetically similar to populations sampled in S. Korea

Logical next steps – Asian Natural Enemies

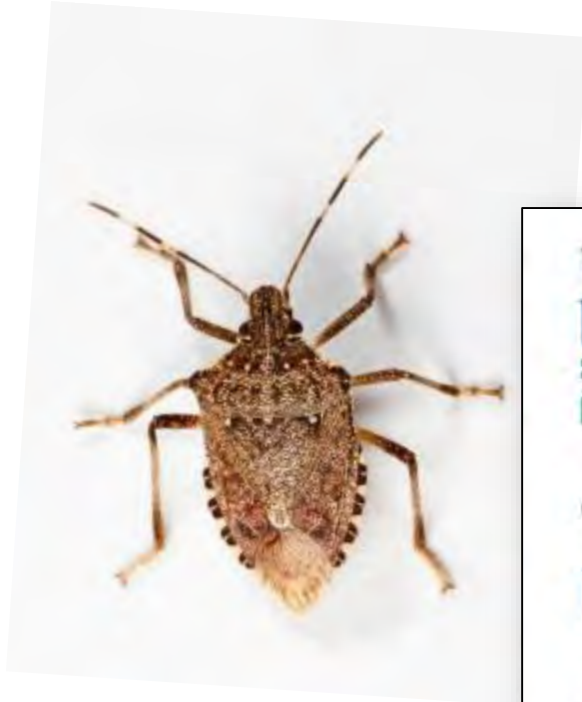
Quarantine Host Range Evaluations:

- Continue laboratory host range research (pending evidence of establishment and dispersal of adventive populations) towards a Petition to Release (APHIS requires a Petition to Release for each state)

Adventive *Trissolcus japonicus*:

- Expand surveys initiated in 2015 to determine the extent of establishment, incl. an increased focus on wooded habitats and a widened survey area to see how quickly populations spread
- Analyze recovered parasitoid microsatellite DNA to determine heterogeneity of the adventive populations
- Increase monitoring of parasitism of BMSB & non-target pentatomid egg masses in the field

Subobjective 2.2.6. Native Natural Enemies



Funding

 **United States Department of Agriculture** **National Institute of Food and Agriculture** Specialty Crop Research Initiative
Grant #2011-01413-30937

Collaborating Institutions

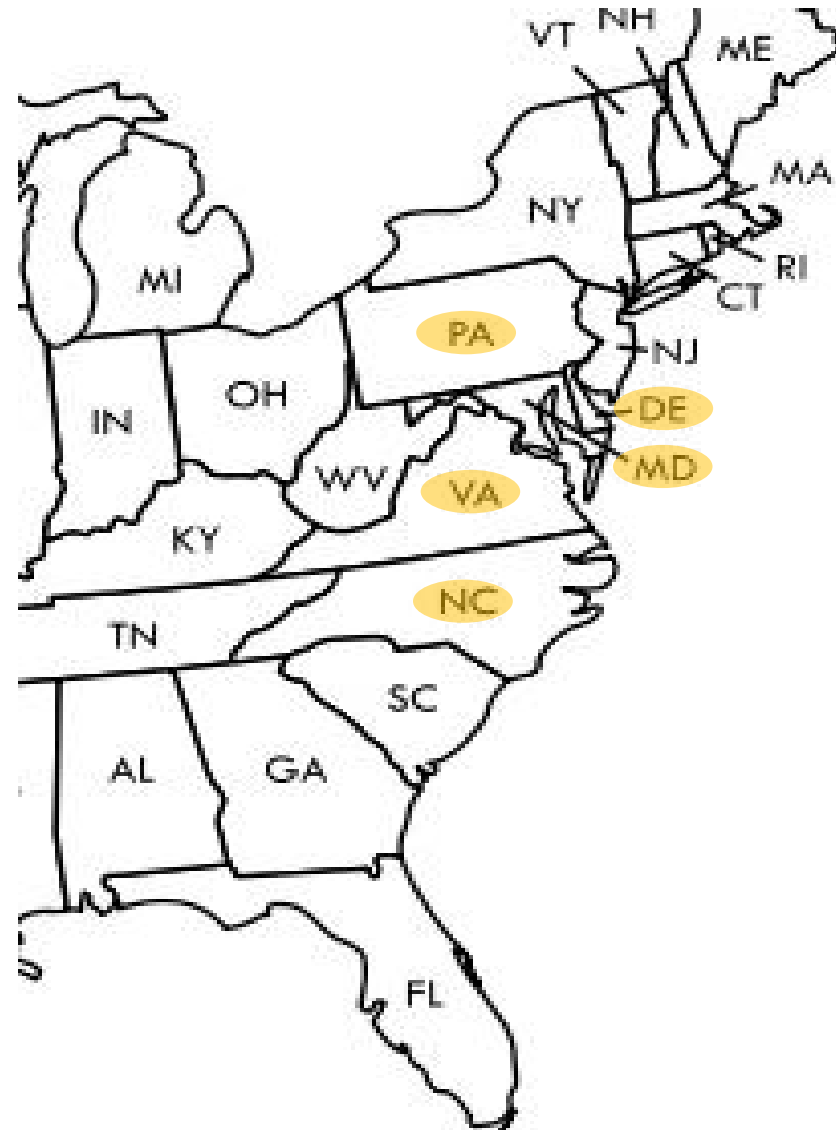
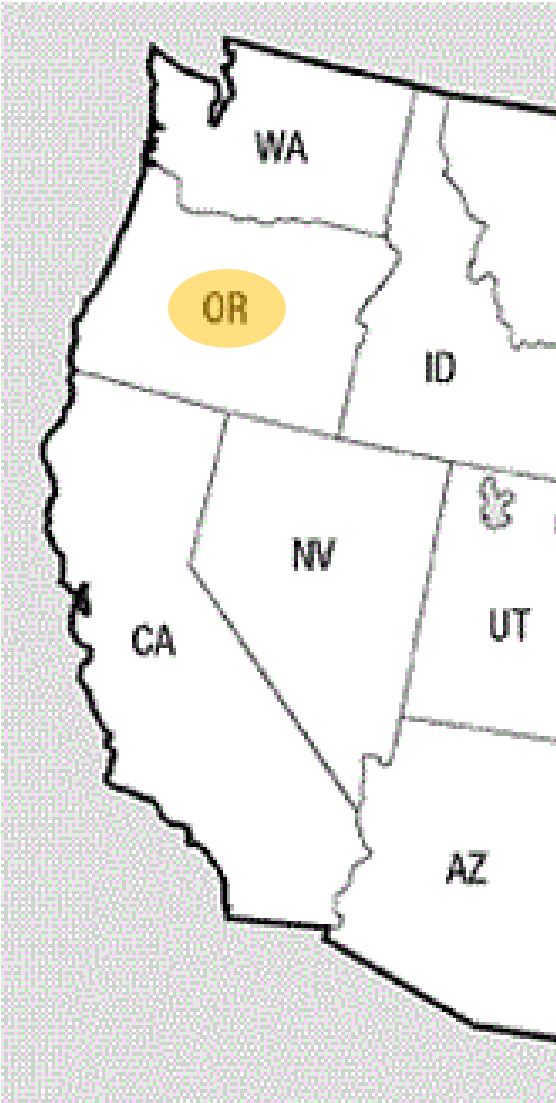
 **UNIVERSITY OF DELAWARE**  **OSU**  **PENNSYLVANIA STATE UNIVERSITY**

 **RUTGERS**  **Northeastern IPM Center**  **WASHINGTON STATE UNIVERSITY**

 **Cornell University**  **OSU Oregon State University**  **UNIVERSITY OF MARYLAND**

 **Virginia Tech**  **NC STATE UNIVERSITY**

Survey area and Collaborators



Delaware – USDA-ARS:

K. Hoelmer, K. Tatman & C. Dieckhoff

Maryland – University of Maryland:

P. Shrewsbury & A. Jones

North Carolina – NC State University:

J. Walgenbach & E. Ogburn

Oregon – USDA-ARS Corvallis:

Jana Lee

Pennsylvania – Penn State University:

D. Biddinger & N. Joshi

Virginia – Virginia Tech:

T. Kuhar, D. Pfeiffer & T. Trope

Native Biocontrol of BMSB In PA

D. Biddinger & N. Joshi

Penn State University Entomology



by Alex Surcică



by Alex Surcică



BMSB Adult Parasitism by *Trichopoda pennipes* (Diptera: Tachinidae)

Date	No BMSB Collected & Examined	No. Locations	No. Parasitized	% Parasitized (range by location)	No. Eggs Found	Av. # Eggs/Host (range of eggs/host)
2012	4,595	7	115	2.44 (0-10%)	157	1.37 (up to 9)
2013	3,087	8	57	1.81 (0-7%)	75	1.32 (up to 5)

Date	% Males	% Females	% of Eggs on Dorsum	% Eggs on Venter	% Eggs on Head	% Eggs on Thorax	% Eggs on Abdomen	% Eggs on Legs
2012	59.62	40.38	39.49	61.15	1.91	56.69	39.49	0.64
2013	49.12	50.88	36.00	64.00	1.33	65.33	33.33	0.00

Objective: Survey and Identify native natural enemies of BMSB



Sentinel BMSB egg mass placement (lab-reared) - DE, NC



Naturally laid (wild) BMSB egg masses – DE, MD, NC, VA

Habitats surveyed

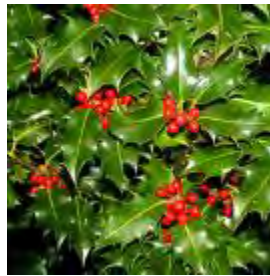
Egg masses on plant hosts in non-managed habitats (wild and sentinel) –
DE, NC & VA



Paulownia



Tree of heaven
Ailanthus sp.



Other species

Naturally laid egg masses in 3 MD nurseries
- MD only
Tree genera – Maple (*Acer*), Cherry (*Prunus*),
and Elm (*Ulmus*)

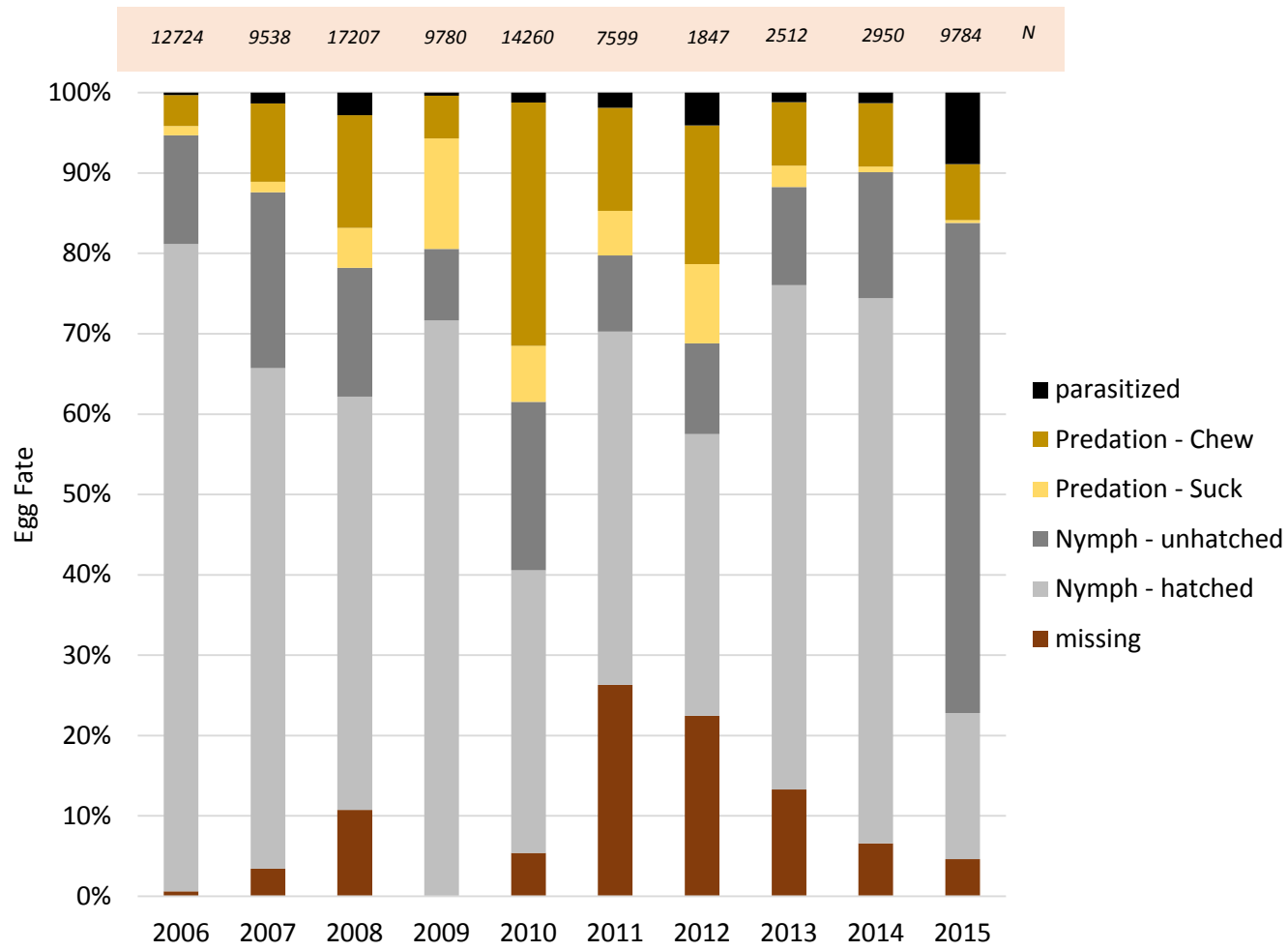


Photo by Steve Black, Raemelton Farm

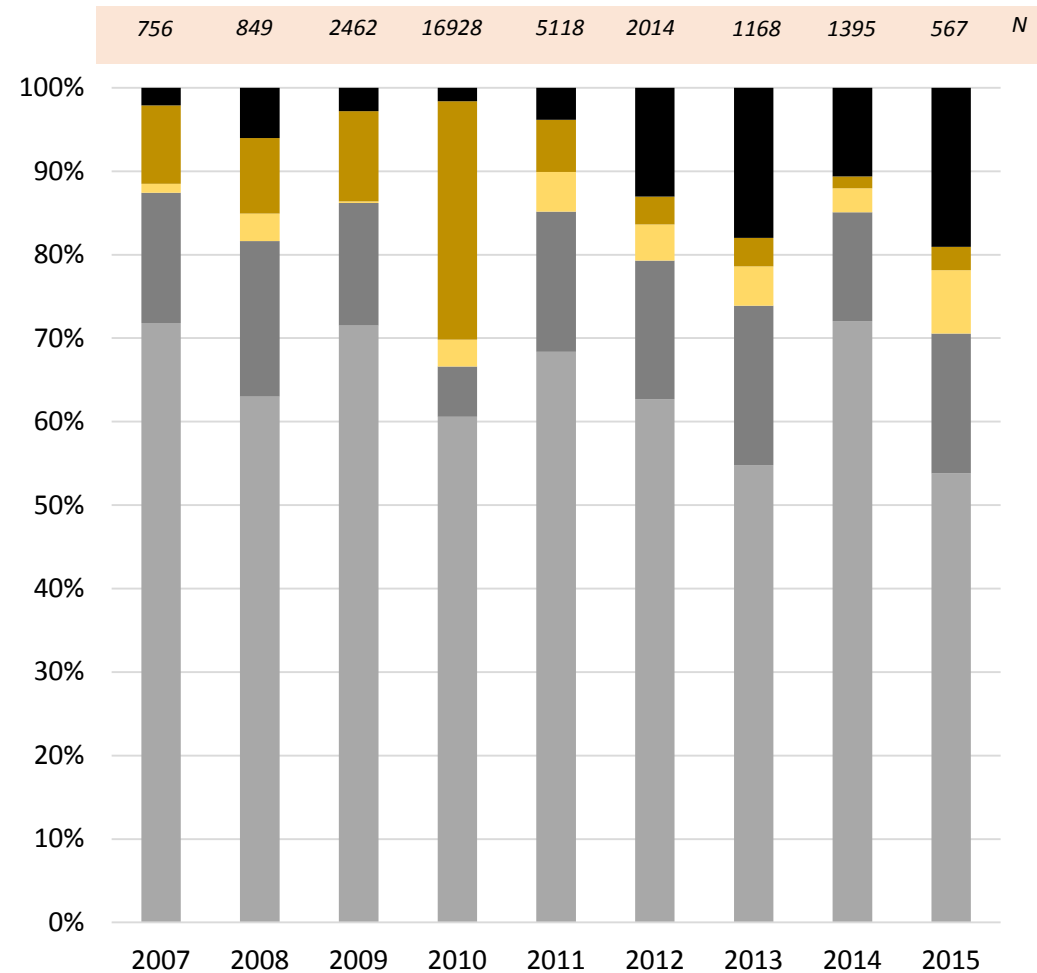


Photo by Ruppert Nurseries

Fate of sentinel and naturally laid BMSB eggs



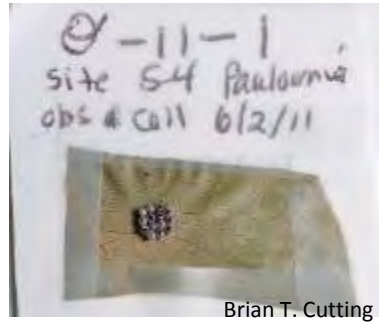
Sentinel BMSB eggs



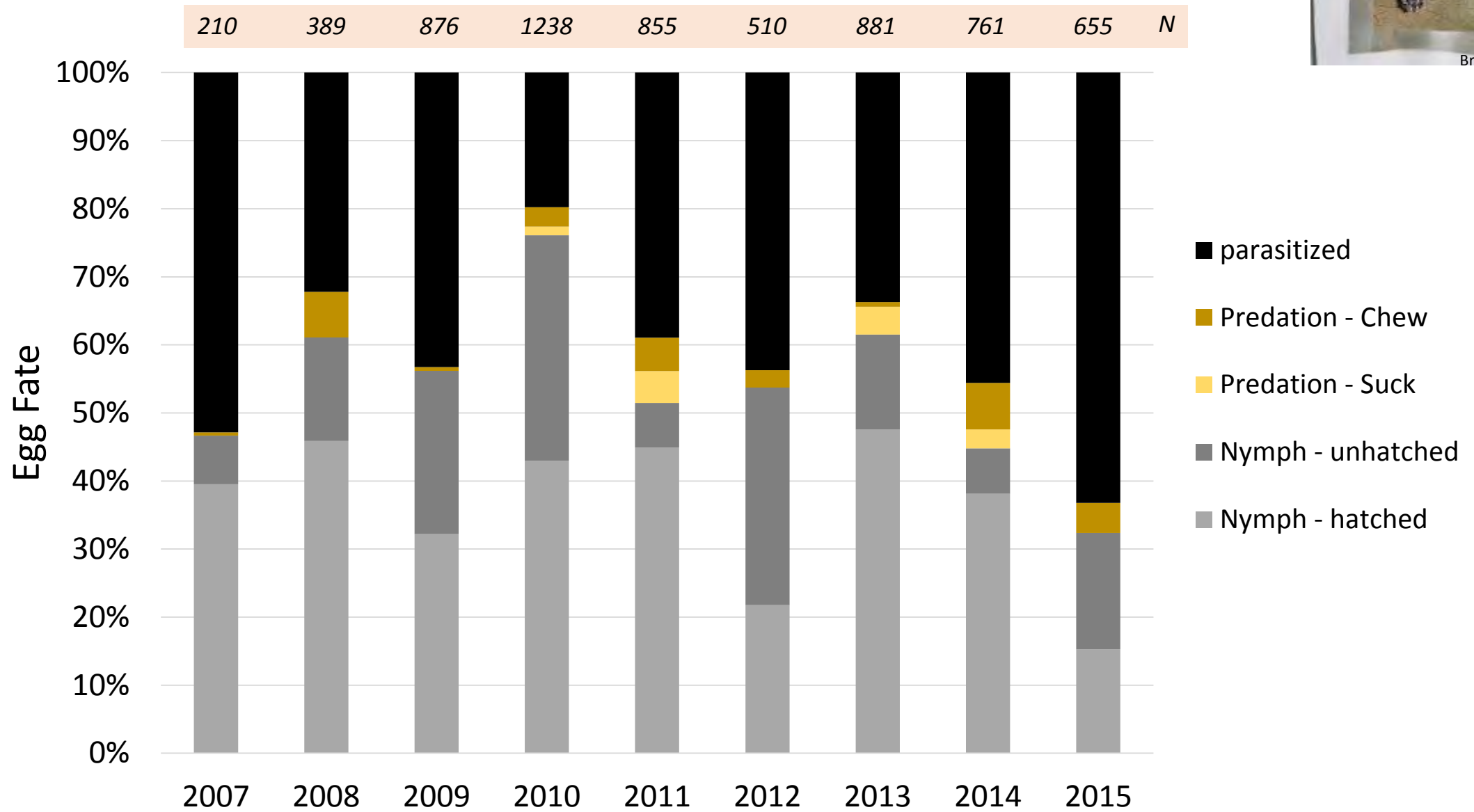
Naturally laid BMSB eggs



Fate of naturally laid non-BMSB eggs



Brian T. Cutting



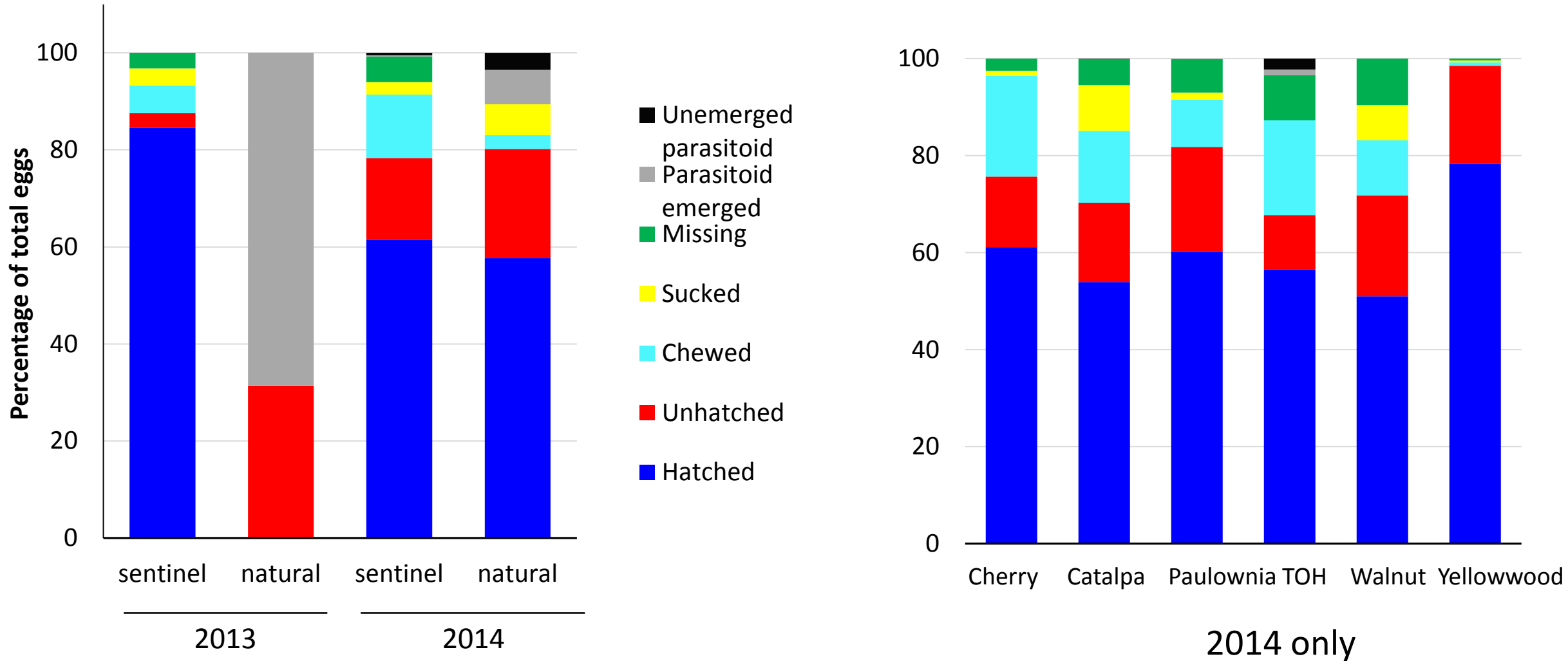
Parasitism of sentinel and naturally laid eggs in NC sites, 2013-2014



Parasitism rates of sentinel and naturally laid eggs recovered in 2013 and 2014 ranged from 0.26% to 27.54%

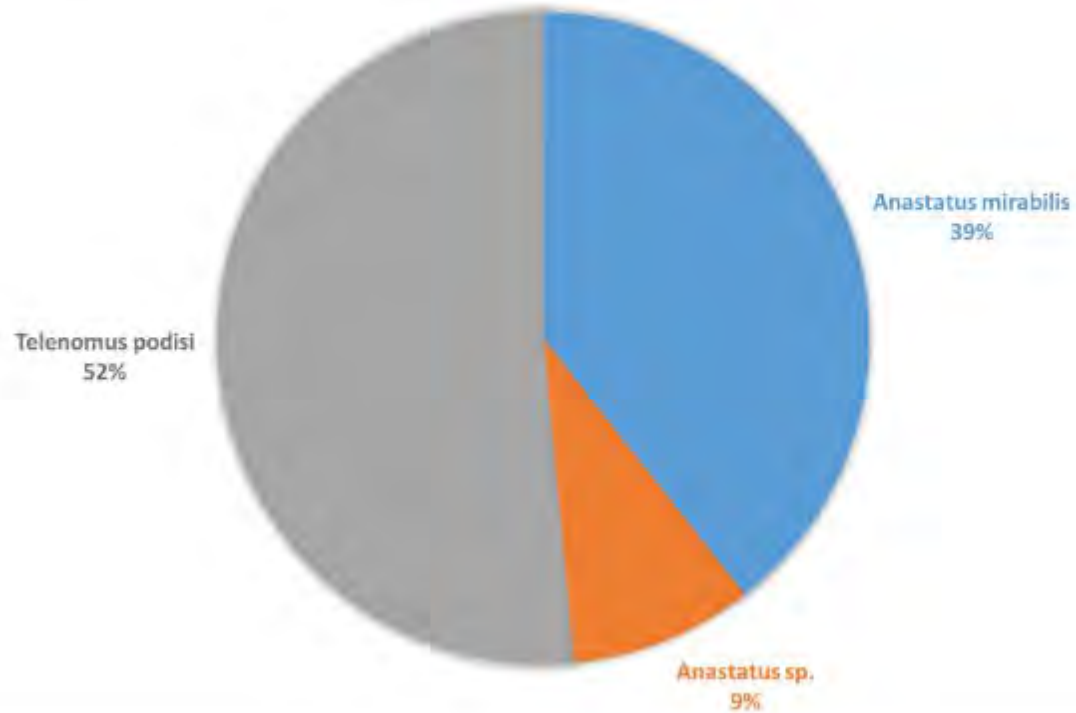
- In 2013, **150** naturally laid eggs were found with **57** (27.54%) successfully emerged parasitoids.
 - Also in that season, **749** sentinel eggs were placed in the wild for 5 days, **none** of which were parasitized.
- In 2014, **772** naturally laid eggs were found with **55** (6.65%) successfully emerged parasitoids.
 - And **7545** sentinel eggs were placed in the wild for 5 days and **20** (0.26%) were parasitized, and 17 that were dissected for identification.

Fate of Sentinel and Naturally Laid BMSB Eggs on Trees in Non-Managed NC Habitats – 2013-2014

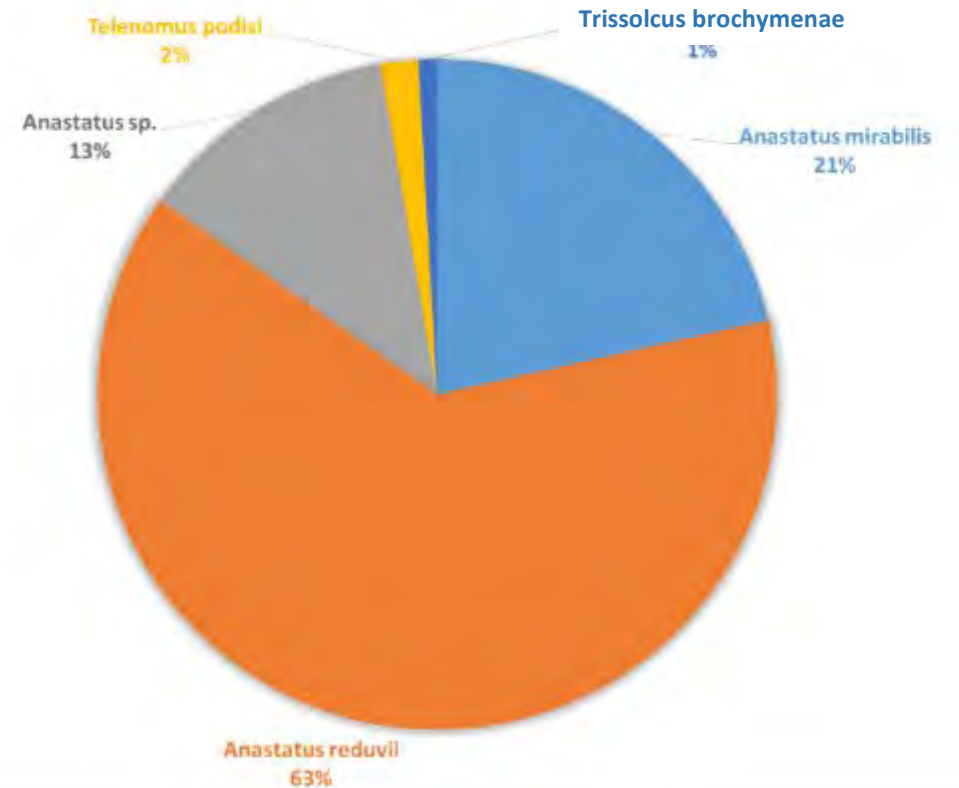


Species composition of parasitized sentinel and naturally laid BMSB eggs in NC sites

% Parasitoids on sentinel BMSB eggs in 2014



% Parasitoids on naturally laid BMSB eggs in 2013-2014



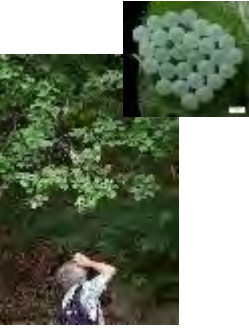
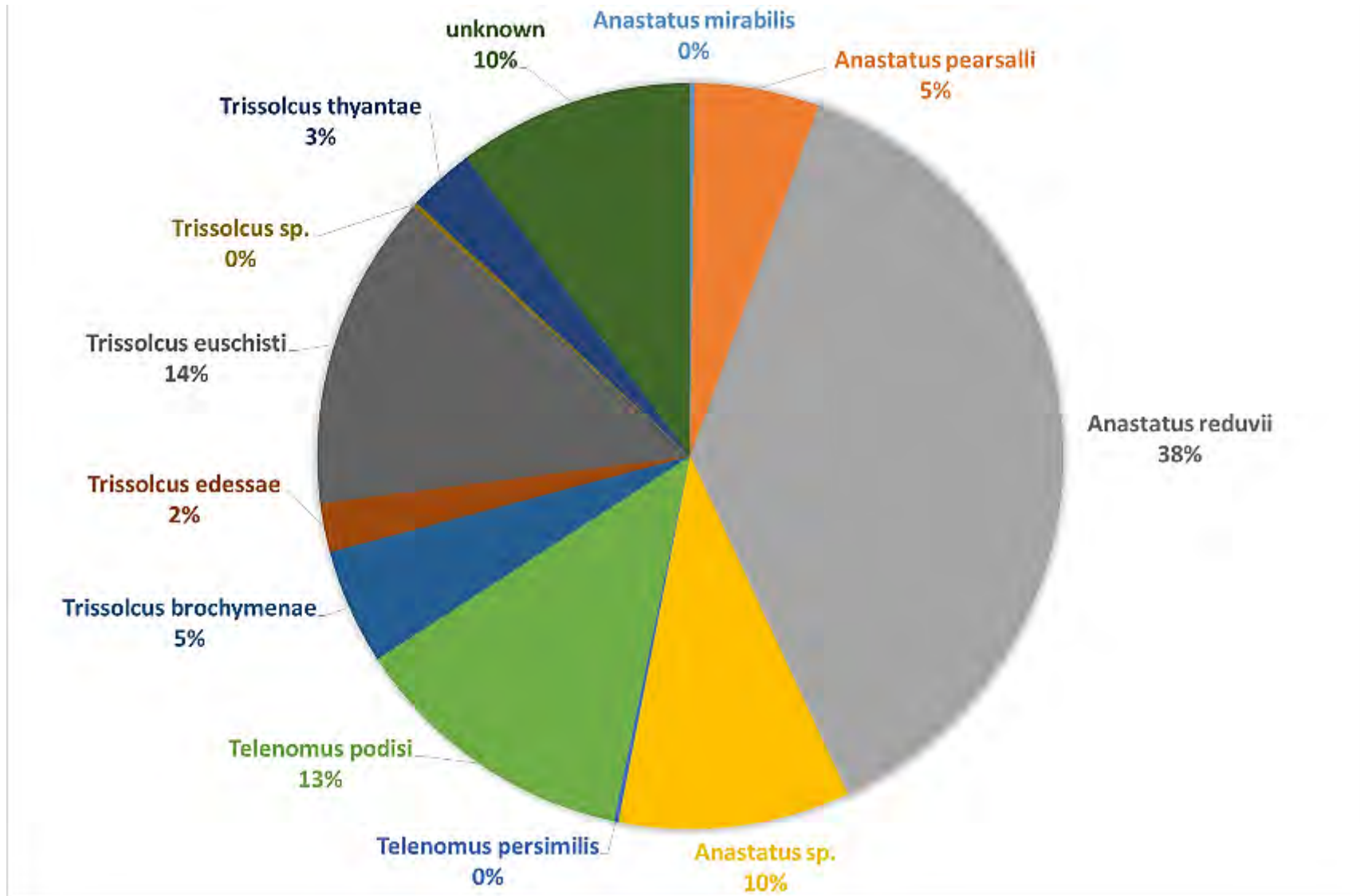
Parasitism of naturally laid eggs in VA sites, 2011 - 2015

Parasitism rates of naturally laid eggs recovered between 2011 and 2015 ranged from 1.97% to 27.57%



- In 2011, 2896 BMSB eggs were found with 106 (3.53%) successfully emerged parasitoids.
- In 2012, 4687 eggs were found with 94 (1.97%) successfully emerged parasitoids.
- In 2013, 2120 eggs were found with 166 (7.26%) parasitized and successful emergence of 87 parasitoids.
- In 2014, 2590 eggs were found with 81 (3.03%) parasitized and successful emergence of 46 parasitoids and 35 that were undeveloped and could not be identified.
- And in 2015, 394 eggs were found with 150 (27.57%) parasitized, successful emergence of 120 parasitoids and 30 that were either dissected for identification or undeveloped.

Species composition of parasitized naturally laid BMSB eggs in Virginia from 2011-2015





Biological Control of naturally laid BMSB egg masses in MD Nurseries



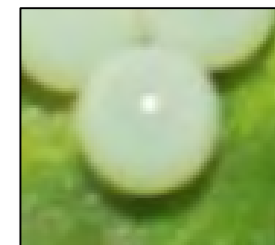
- **Sampled naturally laid eggs at 3 MD nurseries**
 - **late May through September in 2012 and 2013**
- **Tree genera – Maple (*Acer*), Cherry (*Prunus*), and Elm (*Ulmus*)**
- **Eggs returned to lab and monitored**



Photo by Steve Black, Raemelon Farm

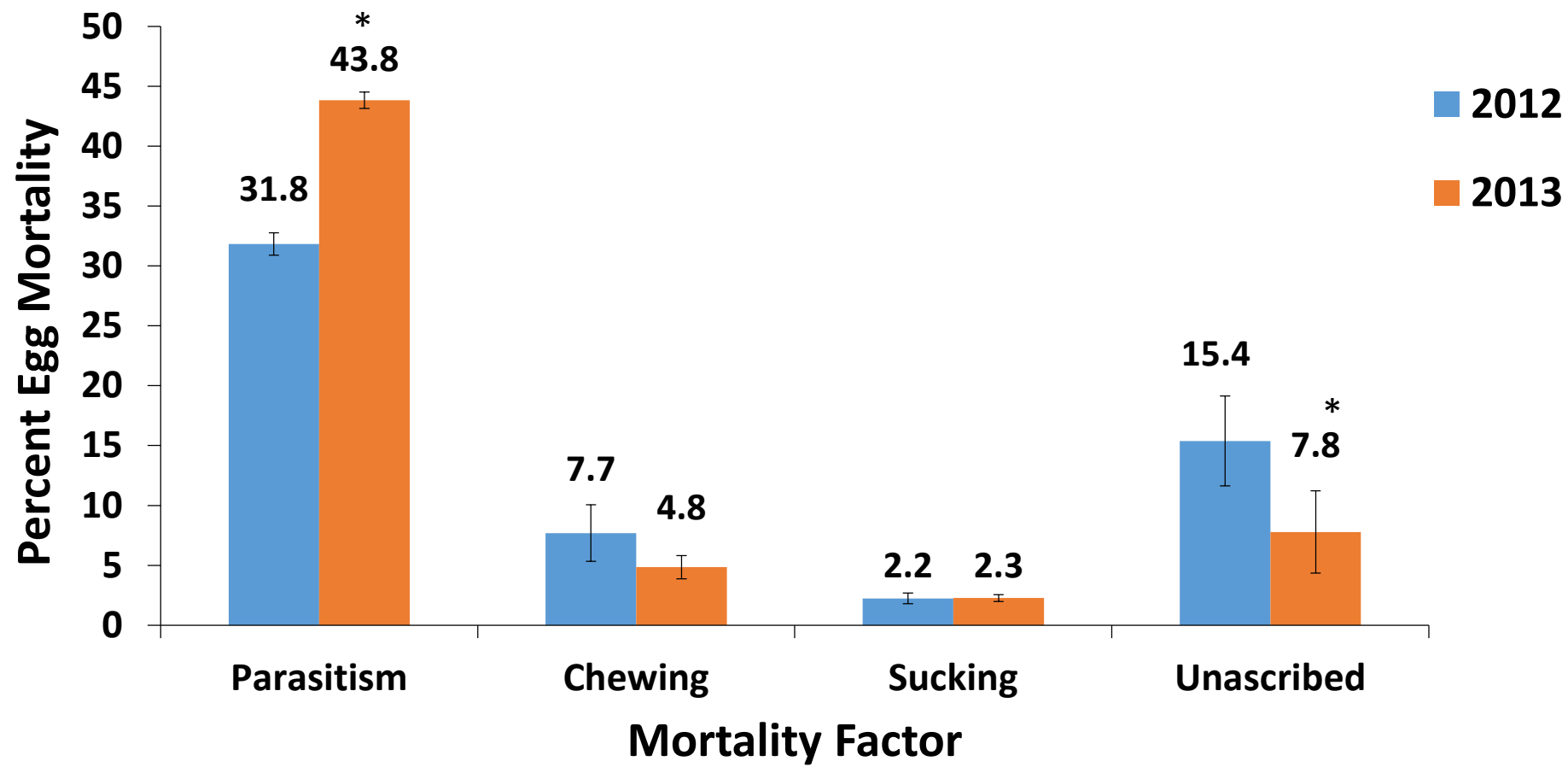


Photo by Ruppert Nurseries



BMSB Egg Mortality Factors

Total Mortality ~ 58%



Parasitoid species composition in Maryland, 2012-2013

2012

*-Trissolcus
brochymenae*
1.1%

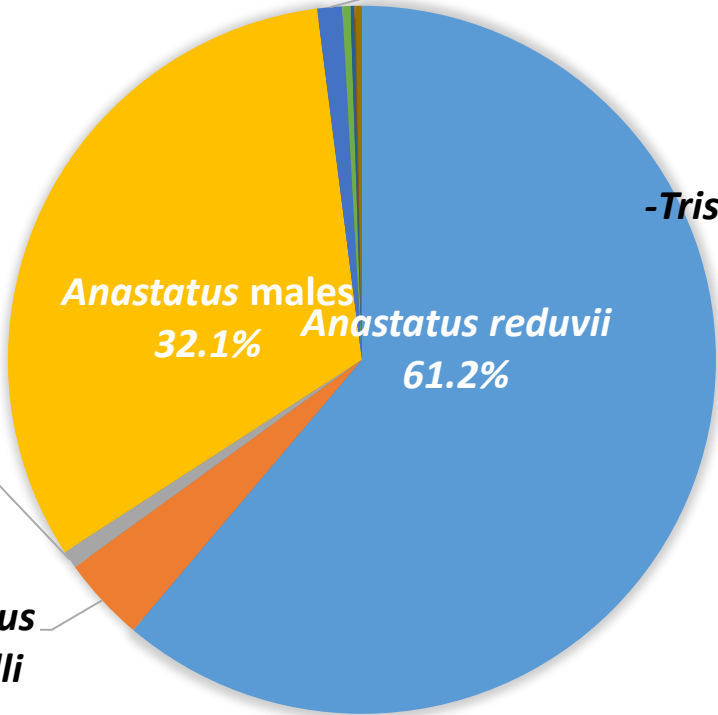
*-Trissolcus
euschisti*
0.4%

-Trissolcus males
0.2%

*-Telenomus
podisi*
0.04%

-Telenomus
males
0.04%

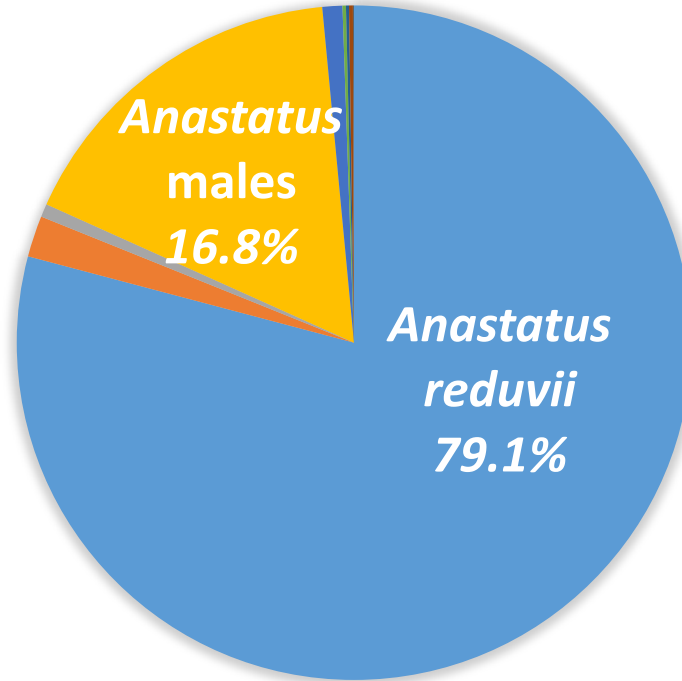
-Ooencyrtus sp.
0.3%



5,638 parasitoids
24,124 eggs

1 : 2.05 M:F ratio

2013



9,719 parasitoids
32,073 eggs

1 : 4.86 M:F ratio

Summary

- Parasitism of sentinel and naturally laid eggs low throughout across years and locations (2006/2007 through 2015)
- Survey of parasitism of non-BMSB naturally laid eggs suggests that native egg parasitoid species are present and active in the same habitats as BMSB
 - Failure to adapt?
 - Failure to recognize BMSB as a host?
 - BMSB defense mechanism to parasitism by native egg parasitoids?
- Species composition of sentinel and naturally laid eggs:
 - dominated by *Anastatus spp.* (Eupelmidae)
 - highly variable between years
 - proportion of undeveloped eggs highly variable between year, partly due to state of egg mass at deployment (frozen vs. fresh)



Summary – Maryland survey

- Egg mortality from all sources was approximately 58%
- Mortality increased throughout the season
- Parasitism is the greatest cause of egg mortality (range 7-80% parasitism)
- Female-biased sex ratio
- *Anastatus redivii* was the most abundant parasitoid
 - **BUT**: Generalist across orders



Logical next steps

- Continue sentinel surveys
 - Examine any adaptation by native natural enemies to BMSB
- Interaction between *T. japonicus* and local natural enemy communities and their impact on BMSB dynamics
- Long-term monitoring for successful native parasitoid populations (various locations)
 - laboratory selection
 - for augmentative biocontrol
- Examine what habitat factors influence various natural enemies
 - identification and conservation of those habitats/crops
 - Compare natural enemy complexes and impacts between different habitats

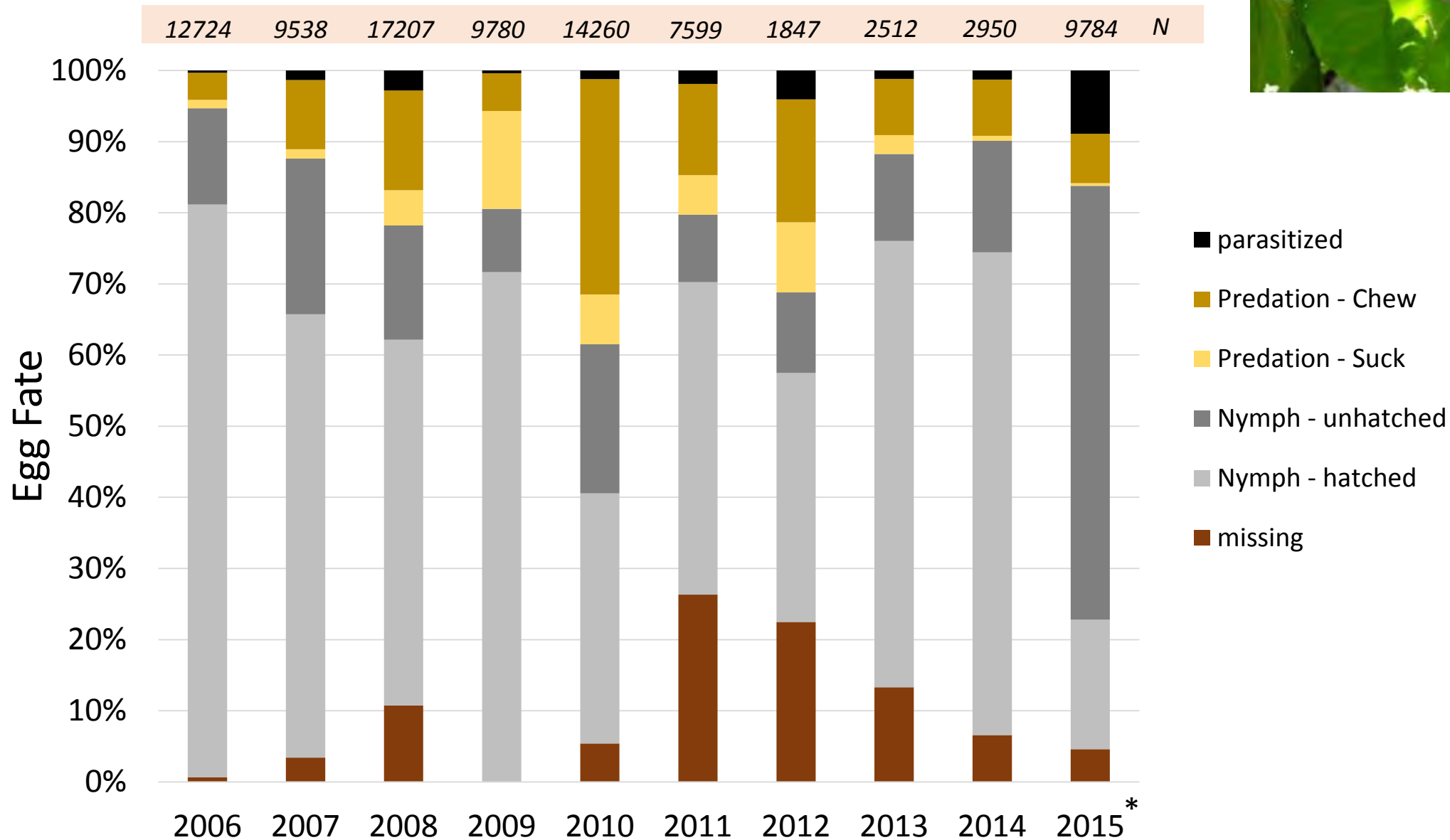
Acknowledgements

- **Funding source: USDA-NIFA
SCRI Award # 2011-51181-
30937**

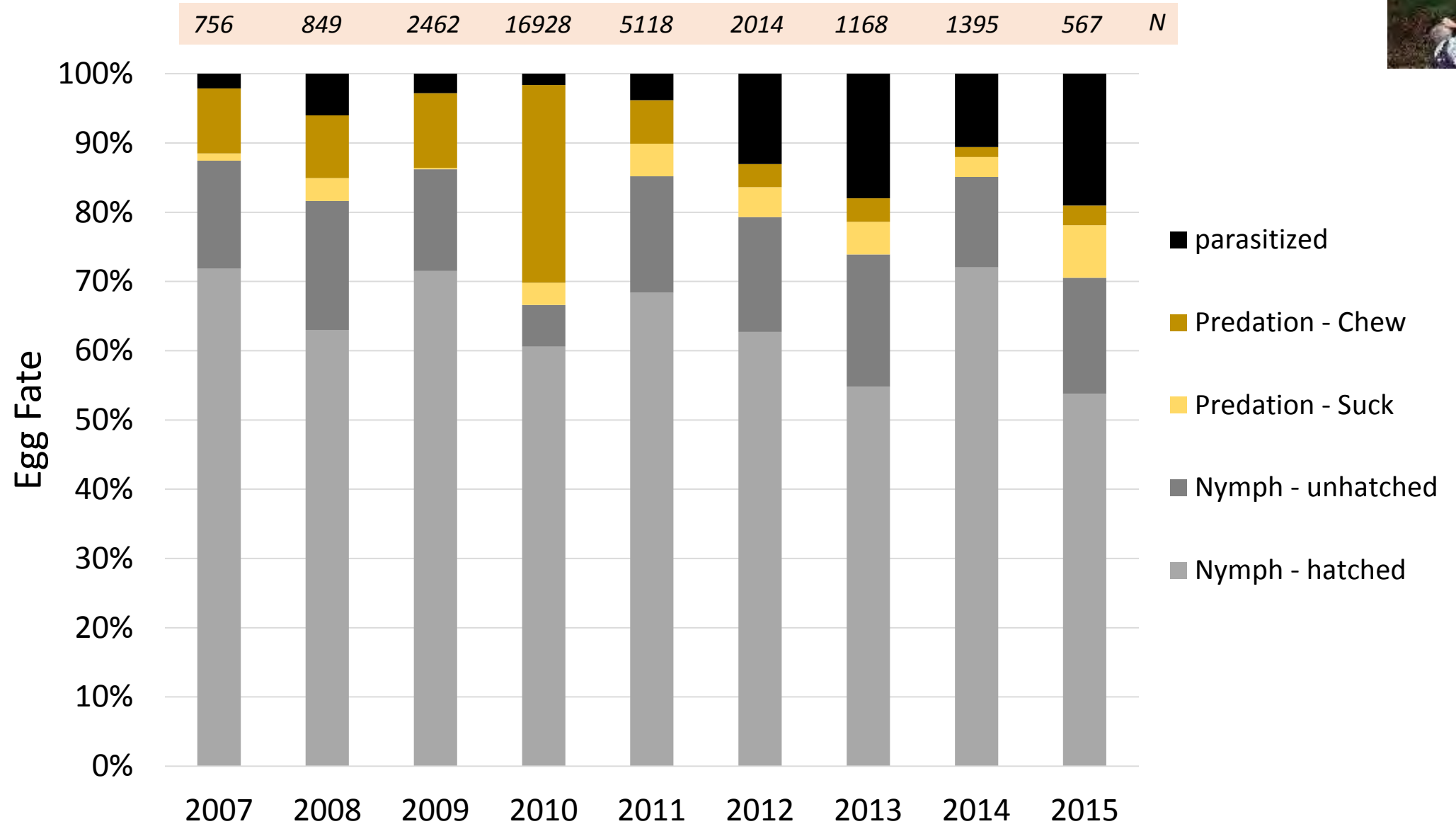
- **Brian Cutting, UD**
- **Amanda Stout, UD**
- **Hoelmer lab undergraduate students**
- **Elijah Talamas, USDA-SEL**
- **Matthew Buffington, USDA-SEL**
- **VA Tech & NCSU undergraduate students
assisting in the lab and field**
- **Cerruti Hooks, UMD**
- **Mike Raupp and lab, UMD**
- **Galen Dively and lab, UMD**
- **Shrewsbury lab, UMD**
- **Raemelon Farm, Adamstown, MD**
- **Ruppert Nursery, Laytonsville, MD**
- **Many stakeholder cooperators, students,
post-docs**



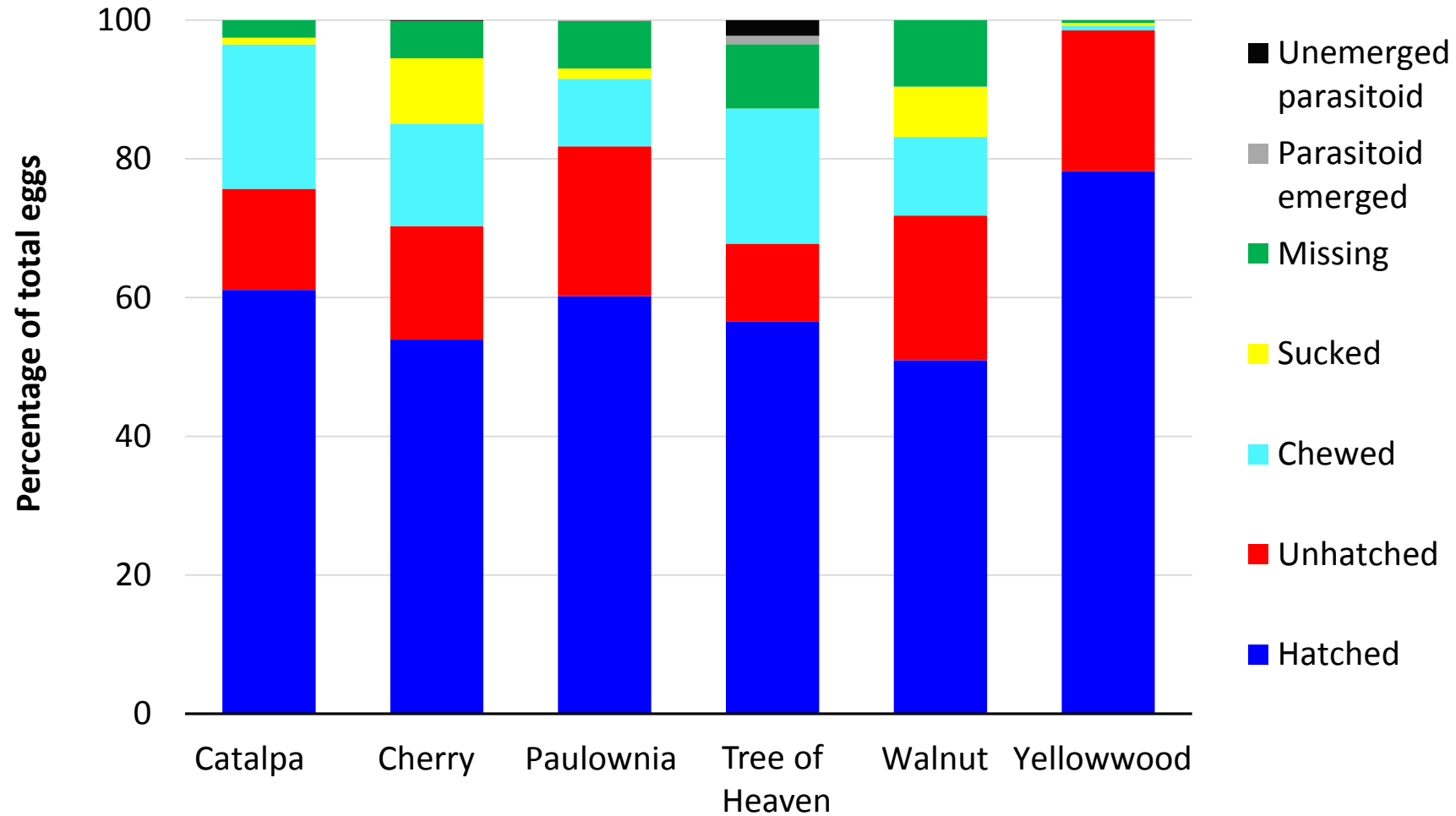
Fate of sentinel BMSB eggs



Fate of naturally laid BMSB eggs



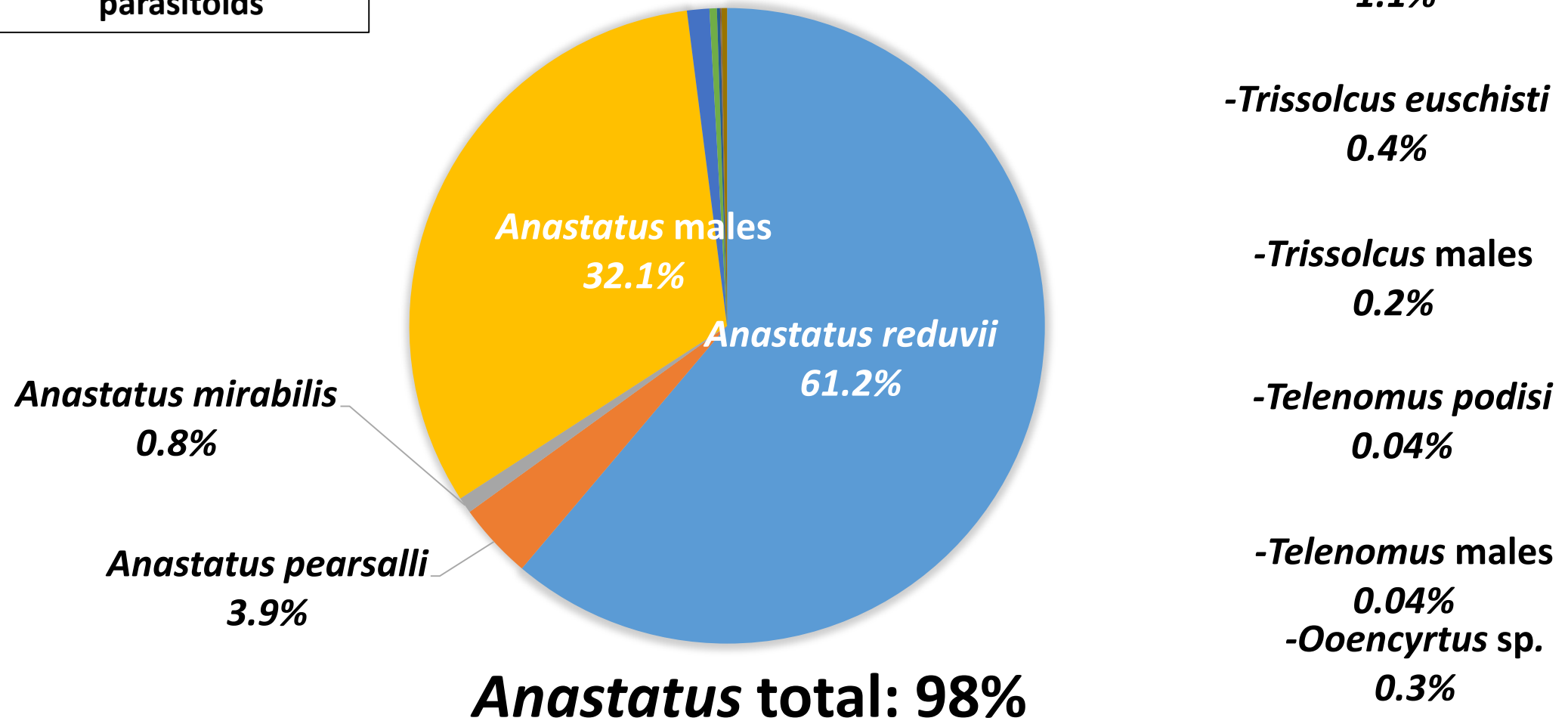
Fate of Naturally Laid Eggs on Trees in Non-Managed NC Habitats 2014



Parasitoid species composition in Maryland, 2012

2012

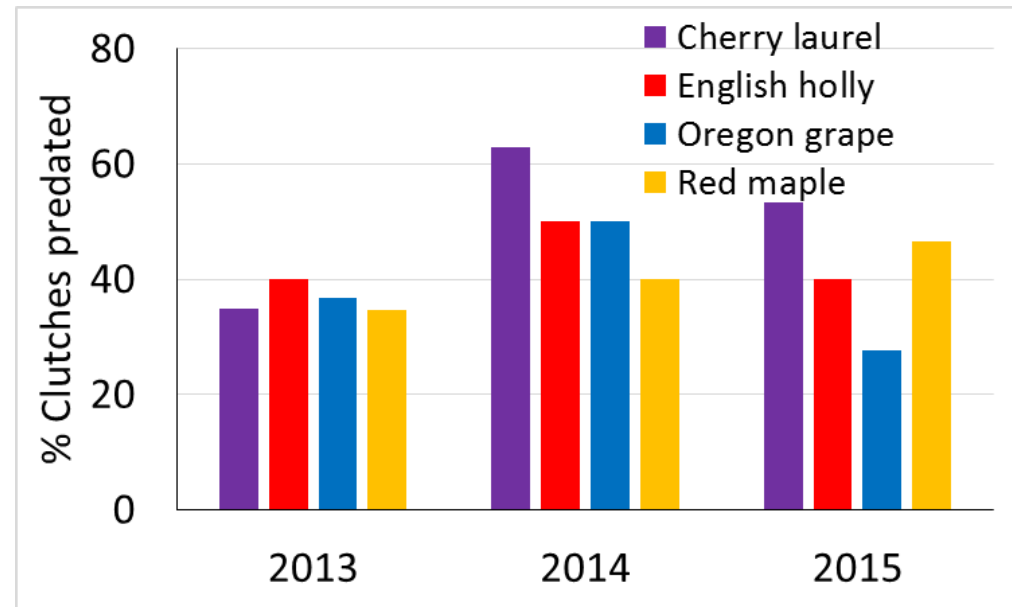
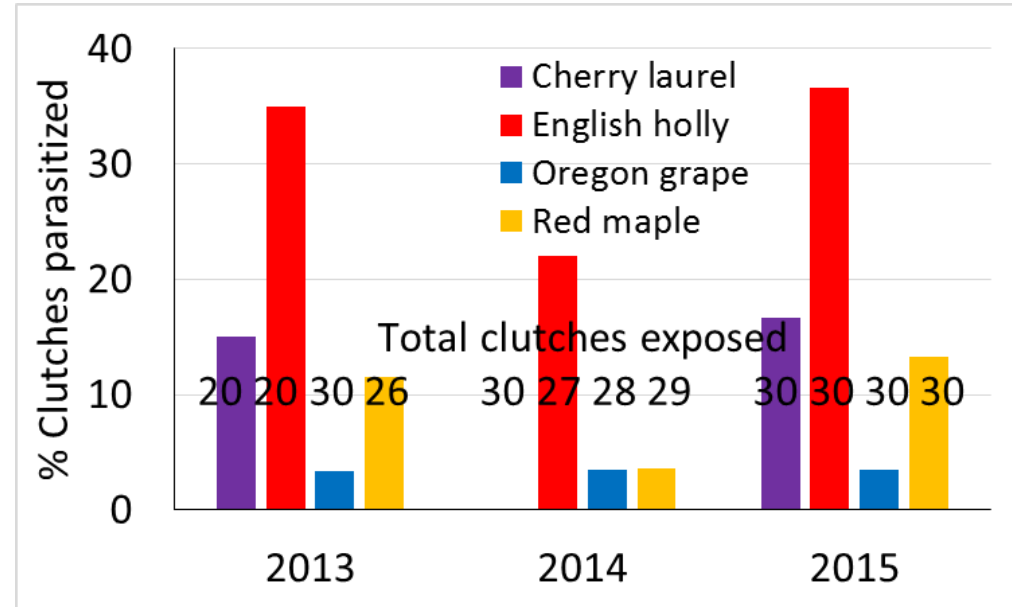
2012: 5,638 parasitoids



Parasitism and predation of eggs in landscape ornamentals (Jana Lee)



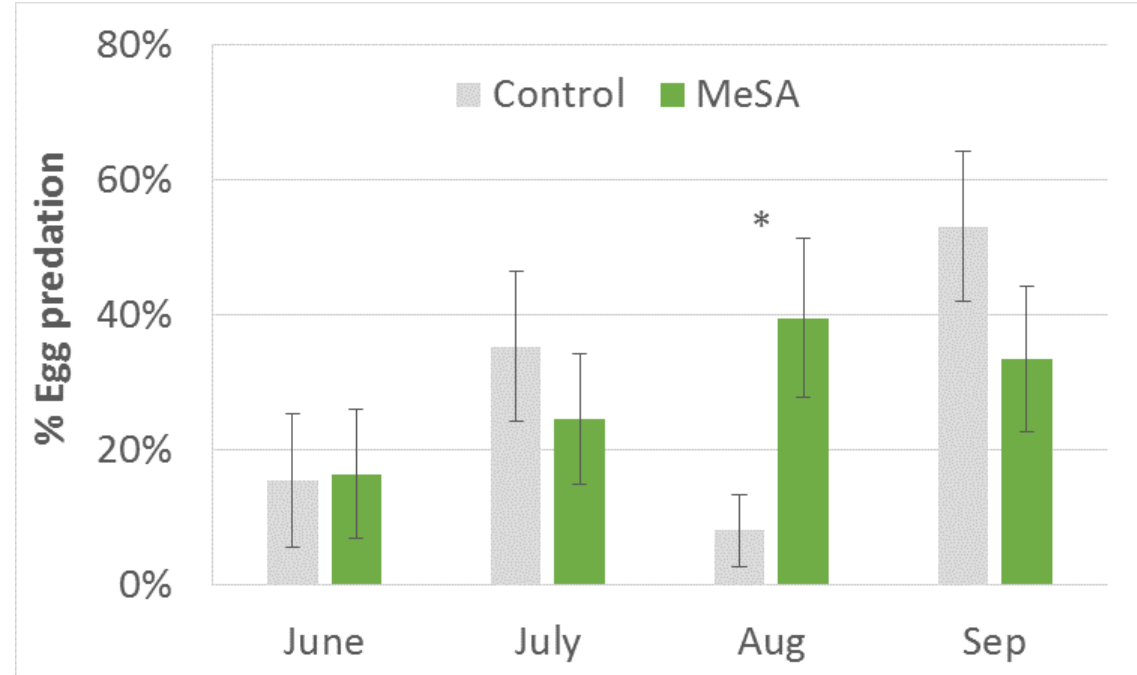
- 5 sites per host, with/without mesh cages at each site
- 1 wk exposure
- Monthly June – Aug/Sep
- **Frozen (!)** sentinel eggs





Does Methyl salicylate enhance egg biocontrol through predation? (Jana Lee)

- Methyl salicylate (MeSA) is a plant volatile that attracts natural enemies
- Sentinel egg masses next to MeSA had 4.8 X higher predation in Aug
- Green lacewing adults and lady beetles were caught more in MeSA sites
- Lacewing larva observed feeding on sentinel egg mass



Commercial lure