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

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

The State University of New Jersey Rutgers

Northeastern IPM Center

Washington State University

Cornell University

University of Maryland

Virginia Tech

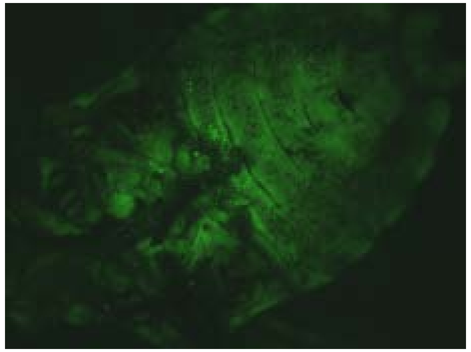
OSU Oregon State University

NC State University

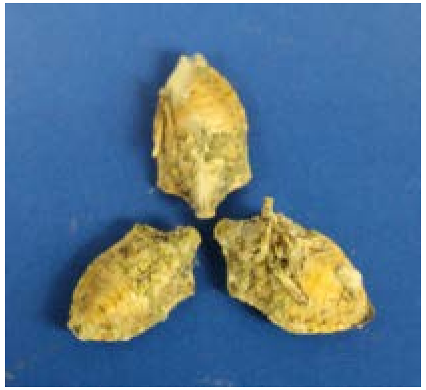
StopBMSB.org

Background

- *Metarizhium* 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

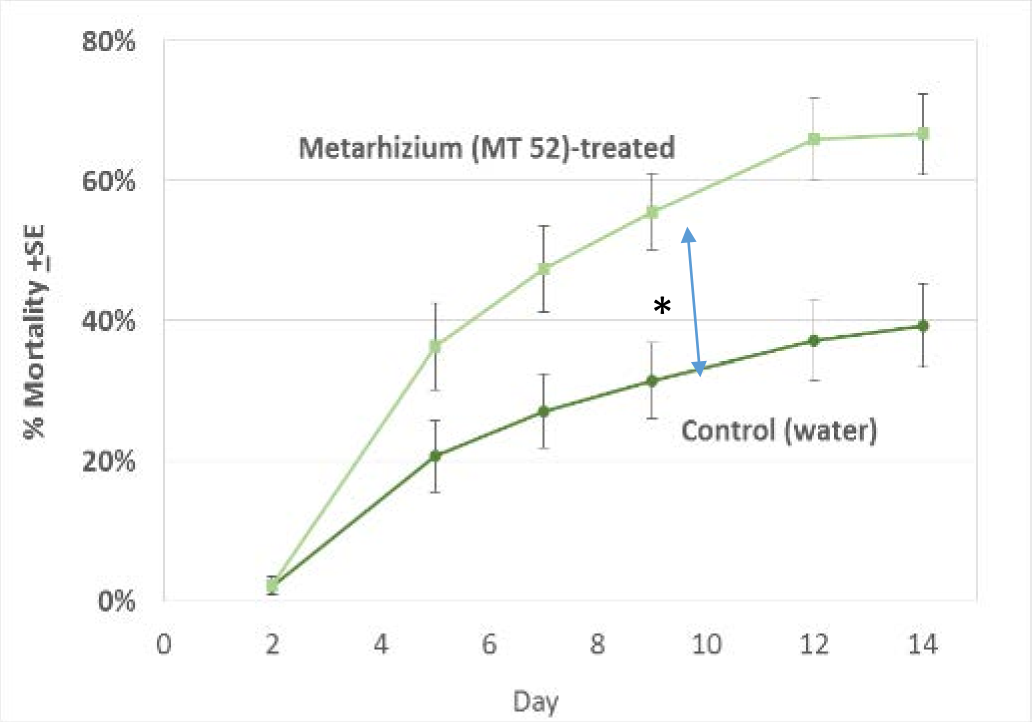


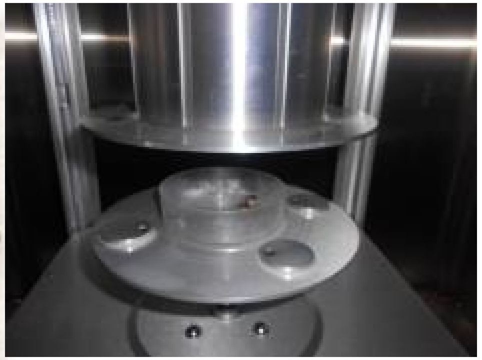
A “glow in the dark” BMSB produced by injecting 50 spores of Ma-GFP



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**





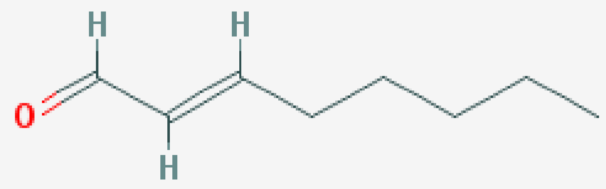
Skillman, Lee -USDA Corvallis

<u>The Objective</u>: (UMD, St. Leger)

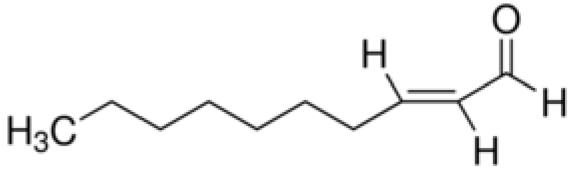
Genetically manipulate fungal strains (*Metarhizium*) to overcome this defense, leading to sustainable BMSB management tactics

<u>The Challenge</u>: 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

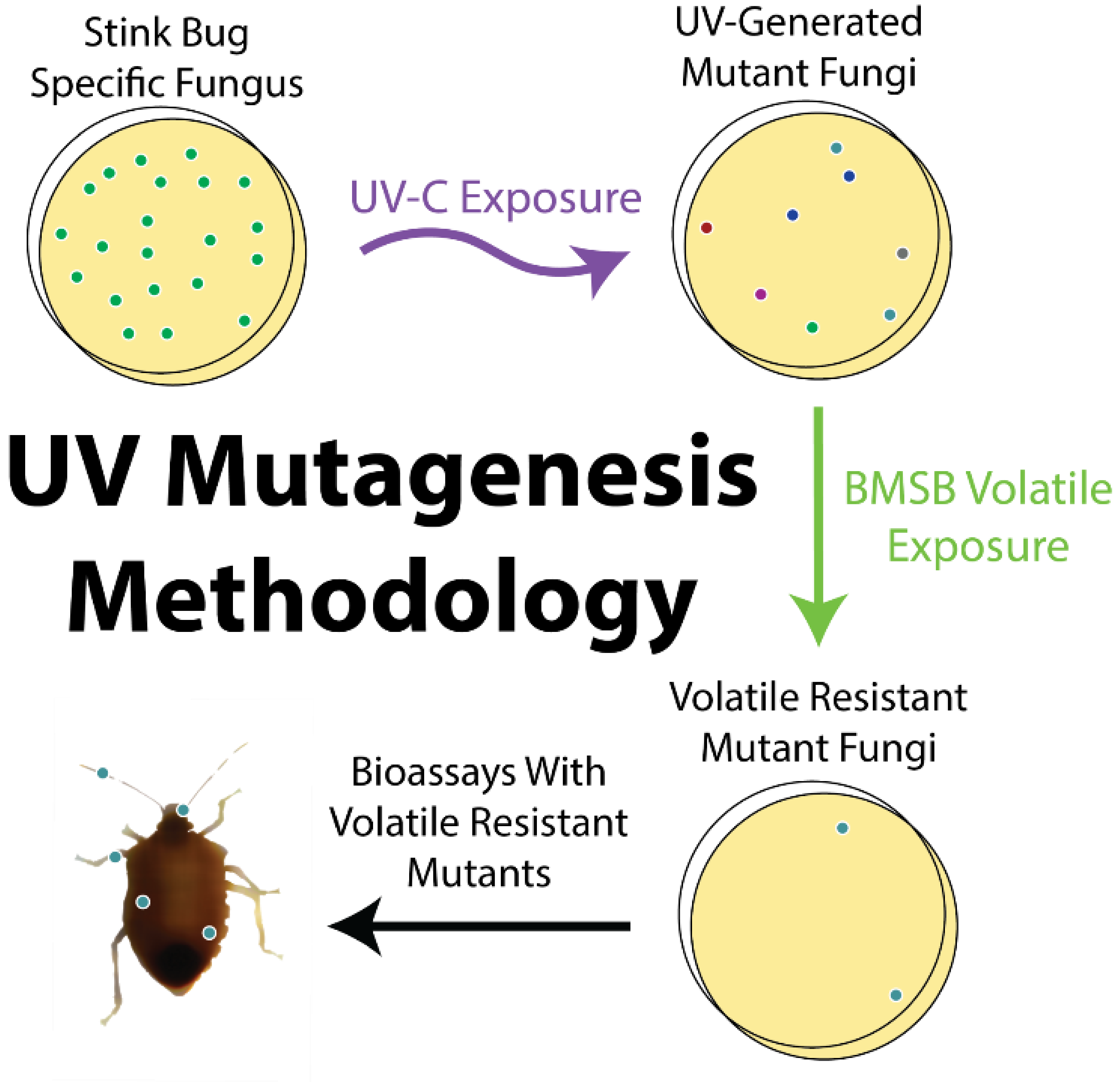


trans-2-octenal



trans-2-decenal

http://pubchem.ncbi.nlm.nih.gov/compound/trans-2-Octenal
http://www.sigmaaldrich.com/catalog/product/aldrich/w236608?lang=en&region=US


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

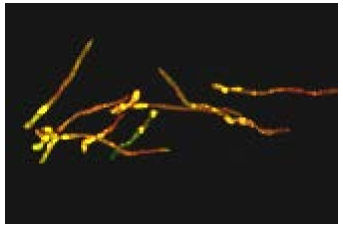
- Resistant mutants have fluffy white growth – this could be because BMSB volatiles resemble chemicals that fungi use for <u>auto-inhibition of spores</u>
  - ➢ select for mutants that do not auto inhibit
  - ➢ transgenic approaches to alter regulation of sporulation genes

➢ Conduct mass spec analysis of <u>volatile production by infected BMSB</u>

➢ Determine <u>role of microbial community on cuticle</u>

➢ Employ <u>field cages</u> to test laboratory results that single insects are more susceptible than clustered insects

➢ Protoplast <u>fusion of BMSB-infecting *Metarhizium* strains with *M. acridum*</u> *(desert strain that infects at low humidities)* .

➢ Use GE to <u>convert volatile and/or RH resistant strain to hypervirulence</u>

Subobjective 2.2.5. Asian Natural Enemies

Funding

USDA United States Department of Agriculture

National Institute of Food and Agriculture

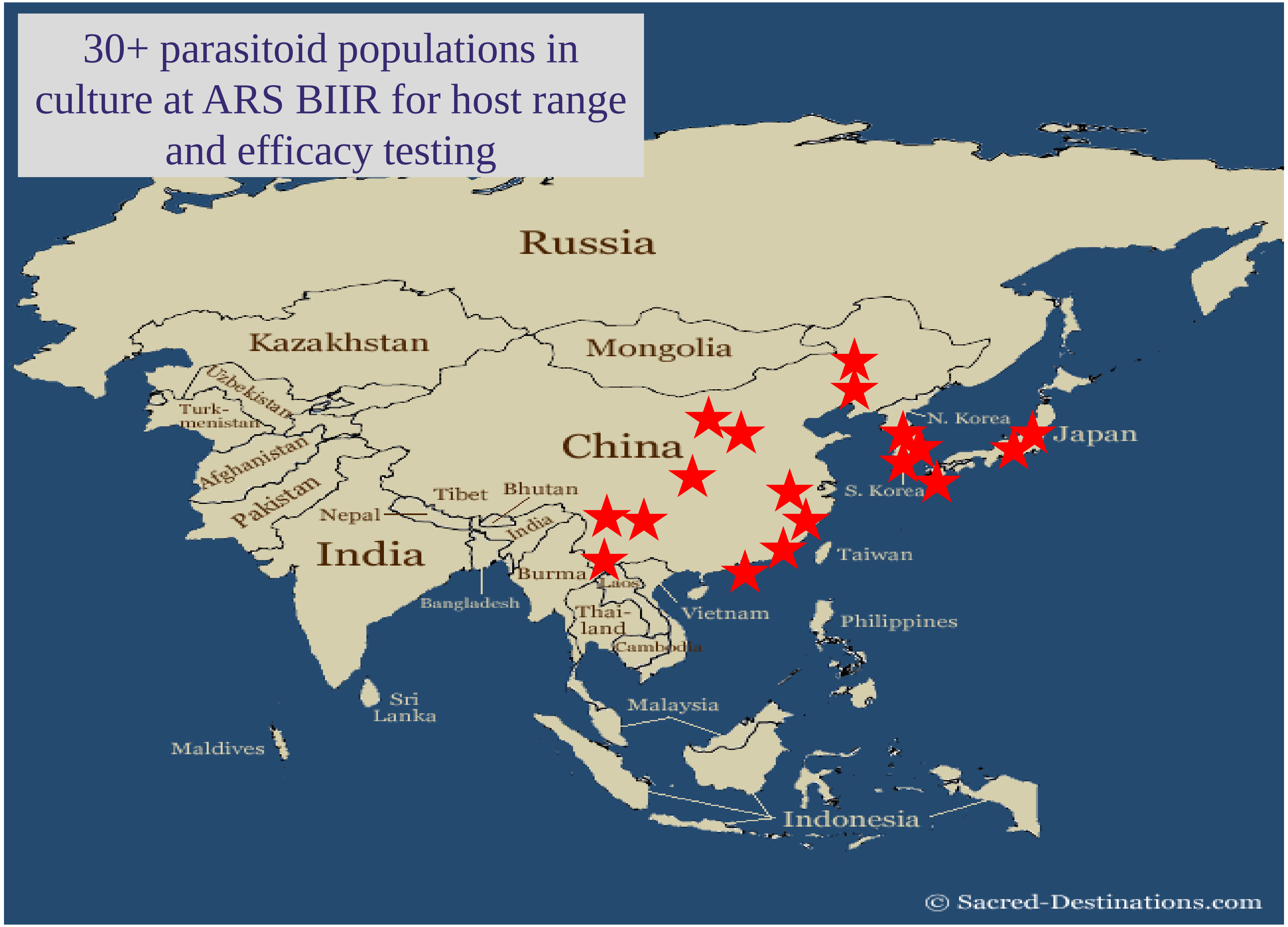
Specialty Crop Research Initiative
Grant #2011-01413-30937

Collaborating Institutions

University of Delaware
ARS
Penn State
The State University of New Jersey Rutgers
Northeastern IPM Center
Washington State University
Cornell University
University of Maryland
Virginia Tech
OSU Oregon State University
NC State University

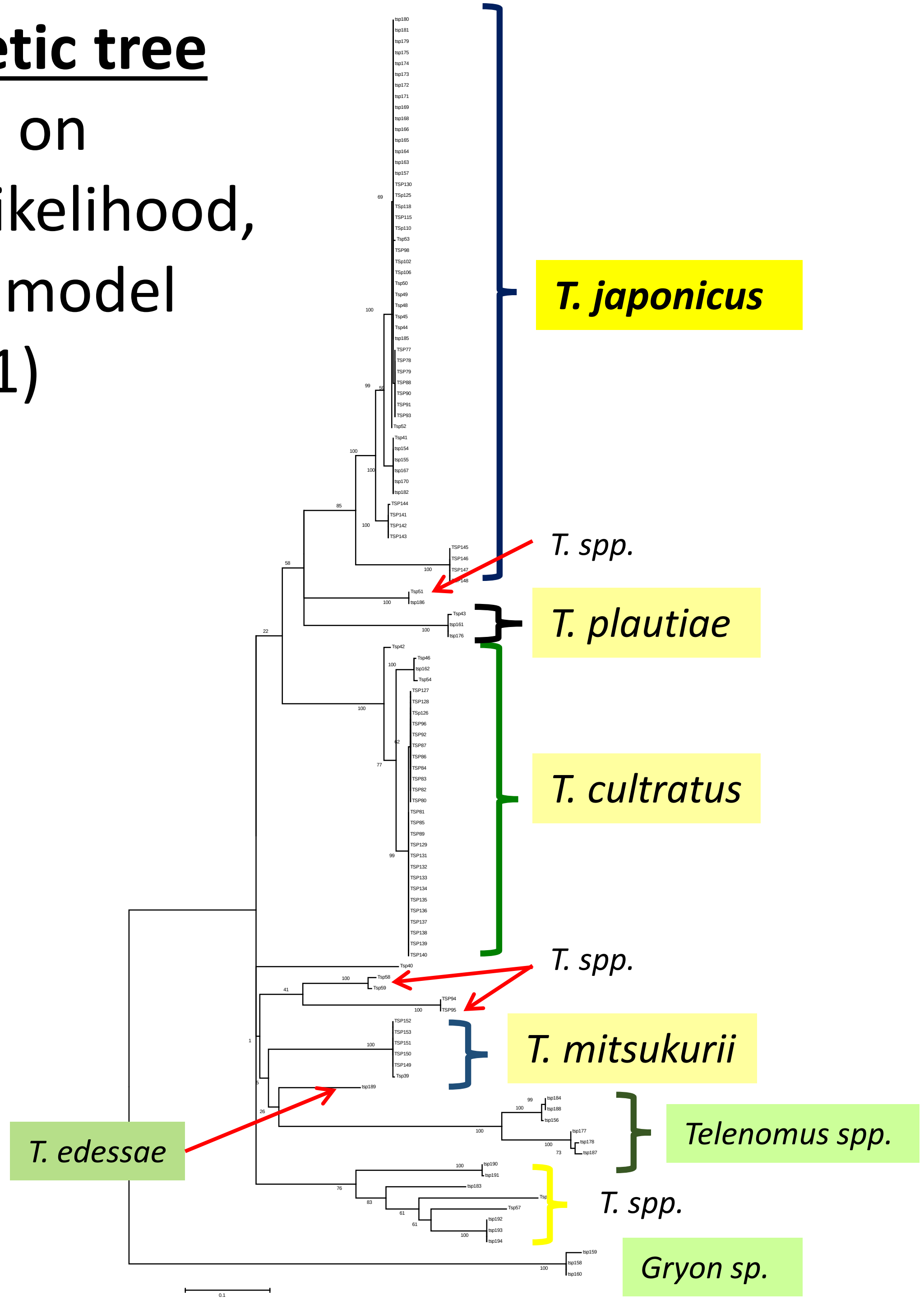
StopBMSB.org

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



Phylogenetic tree

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



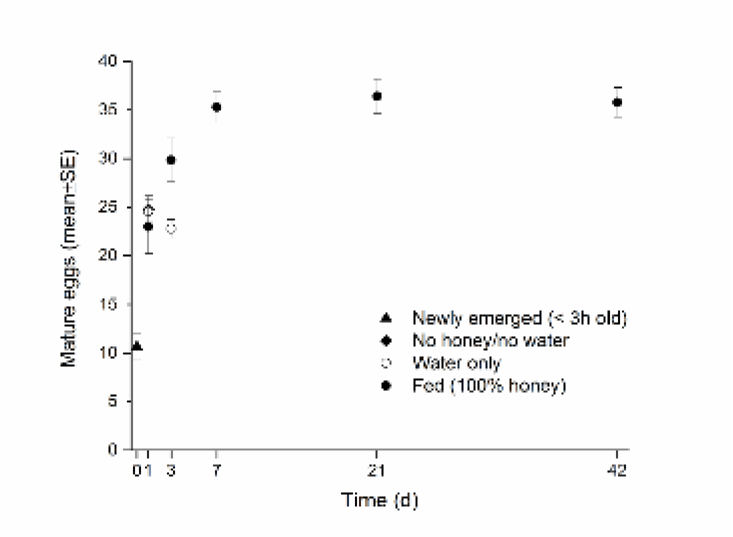
&
Scelionidae recovered from Asian Pentatomid eggs

Scelionidae recovered from North American Pentatomid eggs

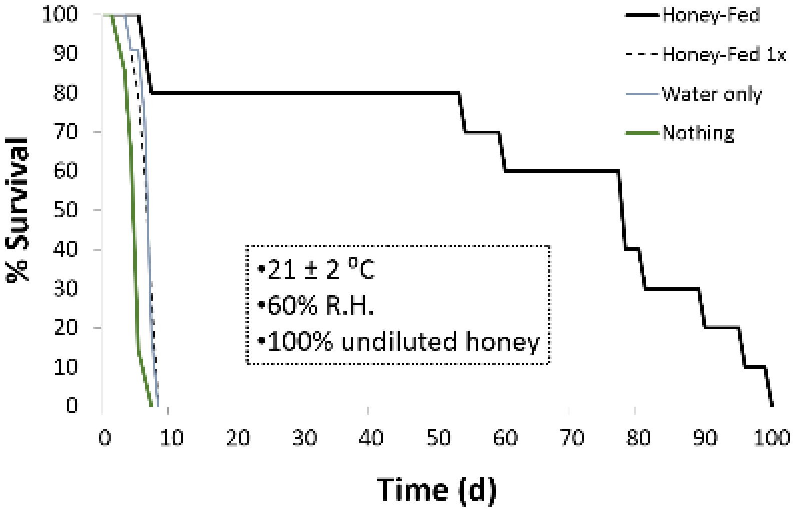
Contributors:
M.-C. Bon – ARS/EBCL
E. Talamas – ARS/SEL
M. Buffington – ARS/SEL
C. Dieckhoff – ARS/BIIR
K. Hoelmer – ARS/BIIR

Trissolcus japonicus (Hymenoptera: Scelionidae)

(first described as *T. halyomorphae*)



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



Yang et al. (2009) Annals of the Entomological Society of America . 102: 39-47

Trissolcus japonicus is oligophagous - it attacks several Asian pentatomid species

Halyomorpha halys

Glaucias subpunctatus

Plautia crossota

Dolycoris baccarum

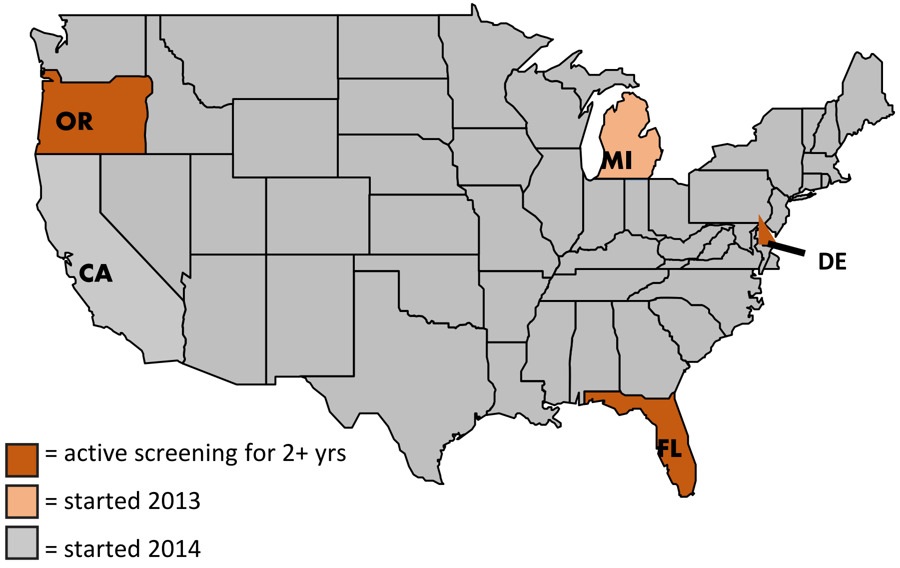
Erthesina fullo

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

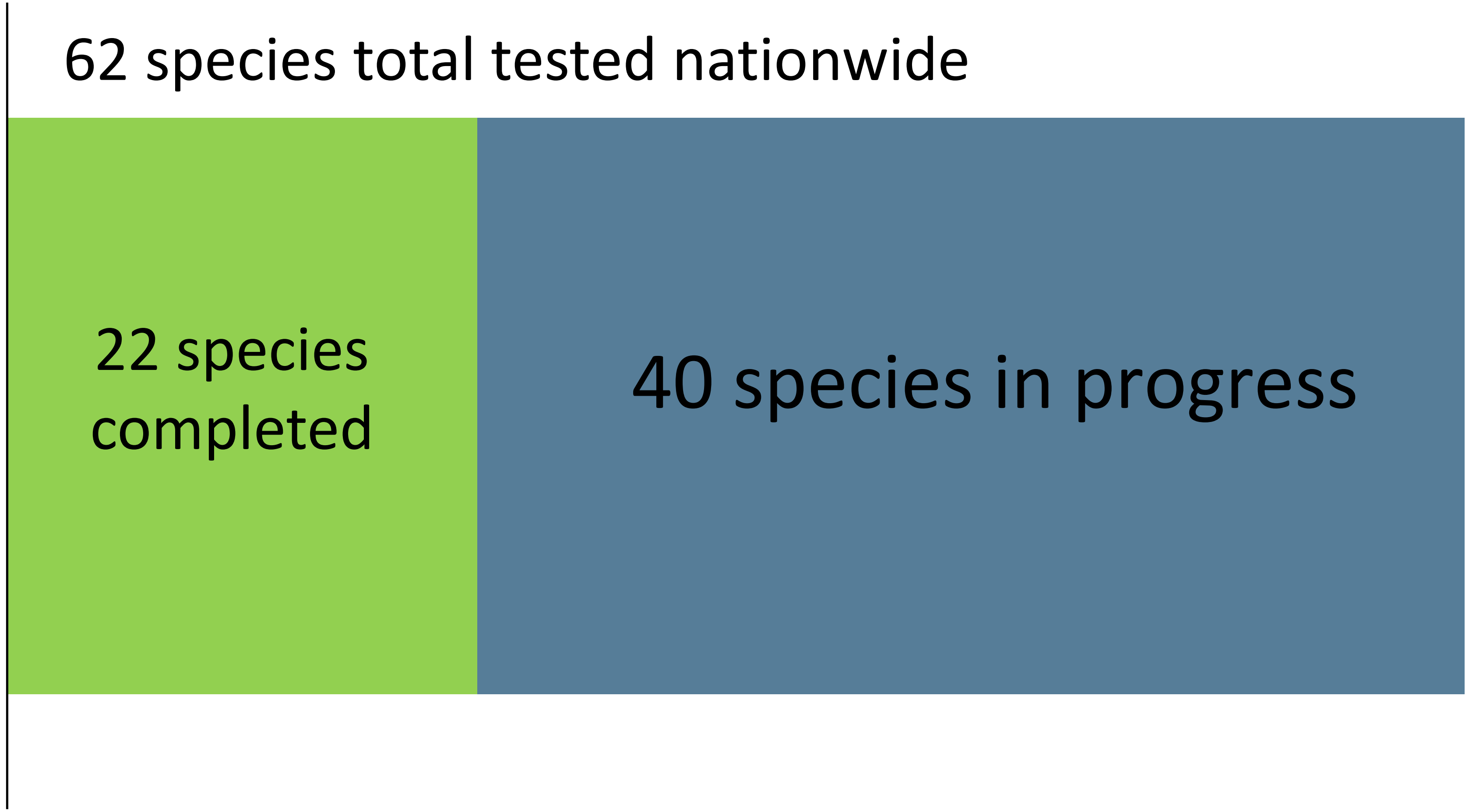
MSU – Michigan State University – Department of Entomology (E. Delfosse)

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:

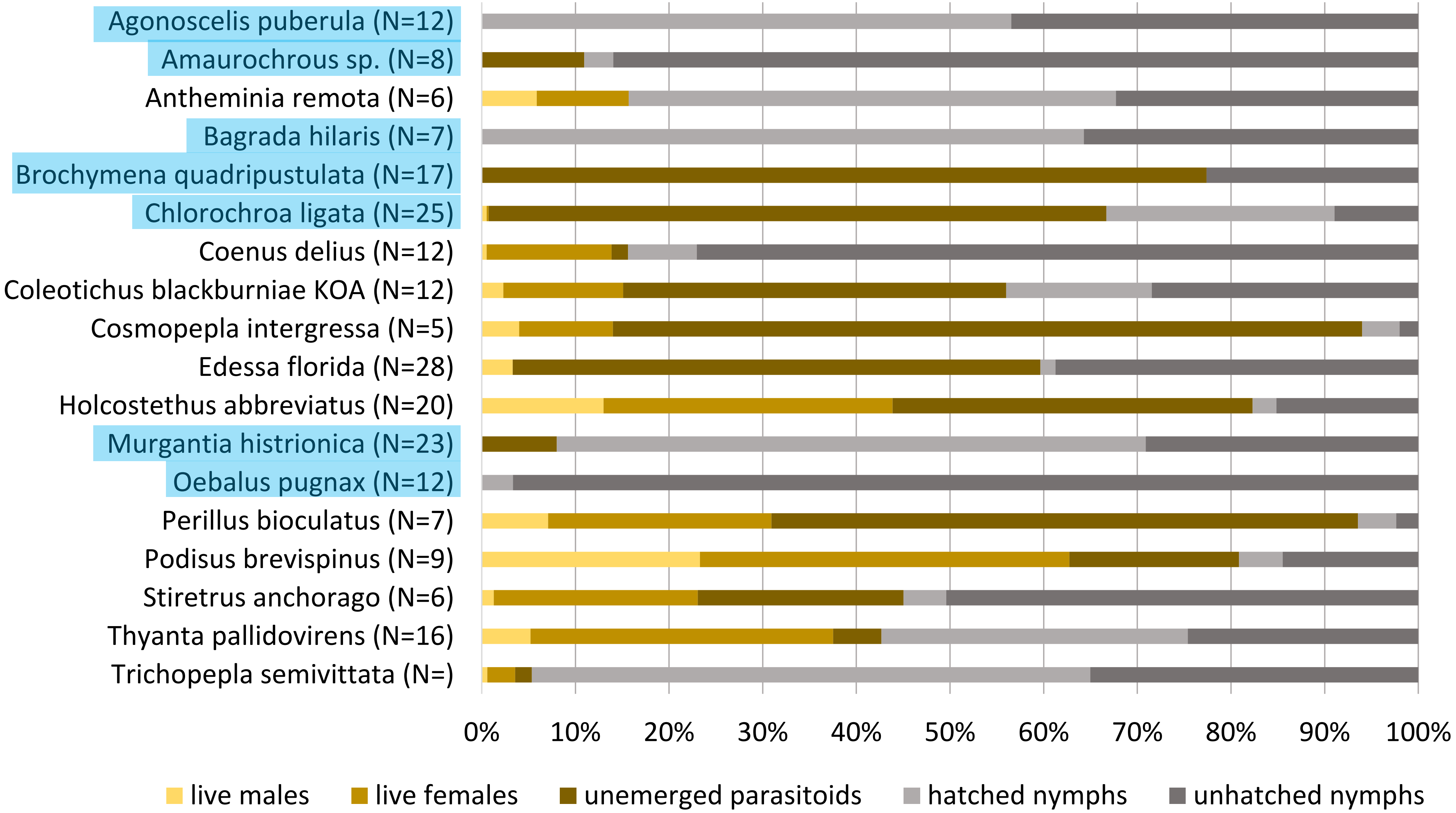
If parasitism on non-target is recorded

Choice Test

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

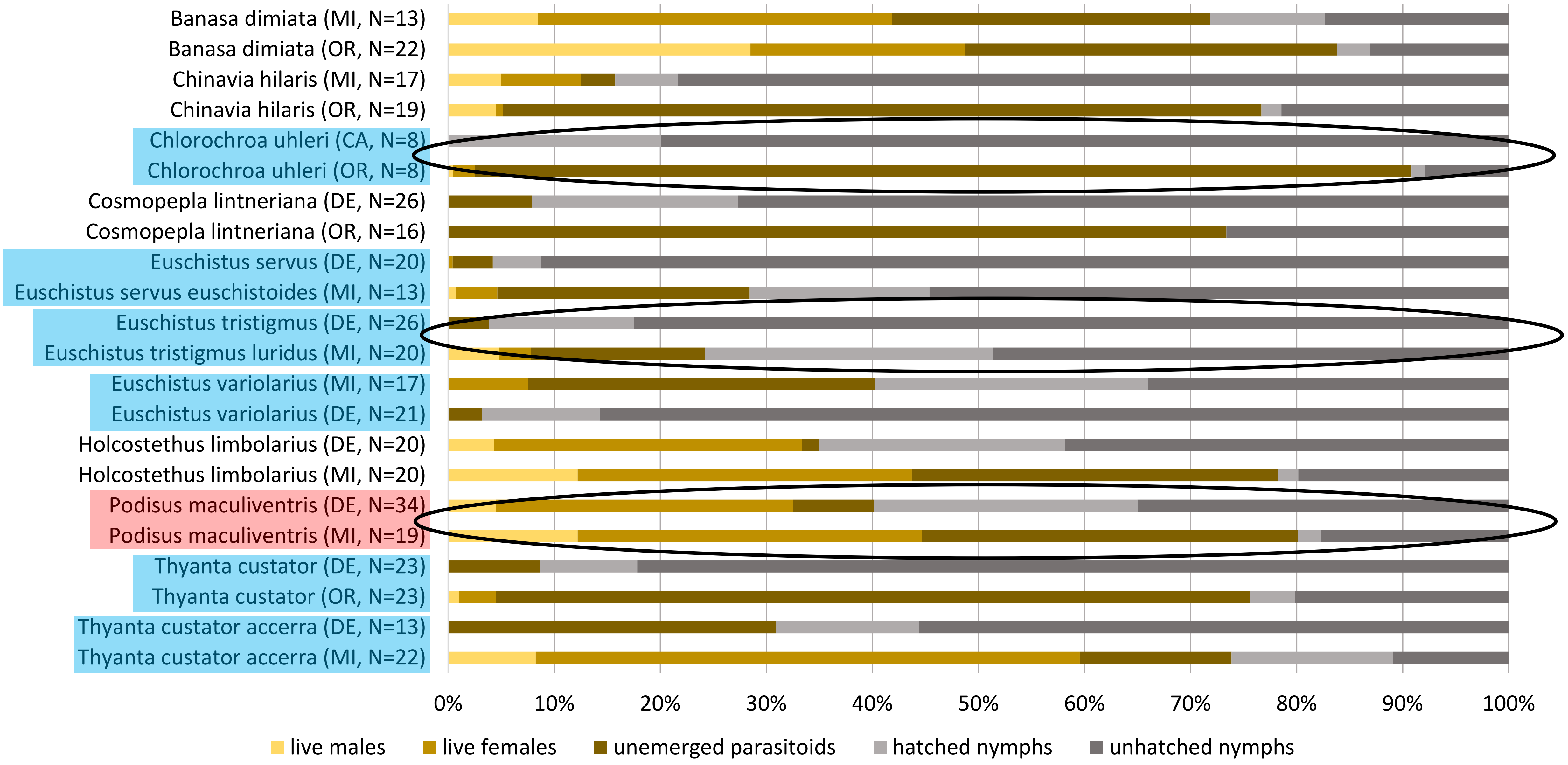
No-Choice Test Outcome - Part 1

(as of 2015)



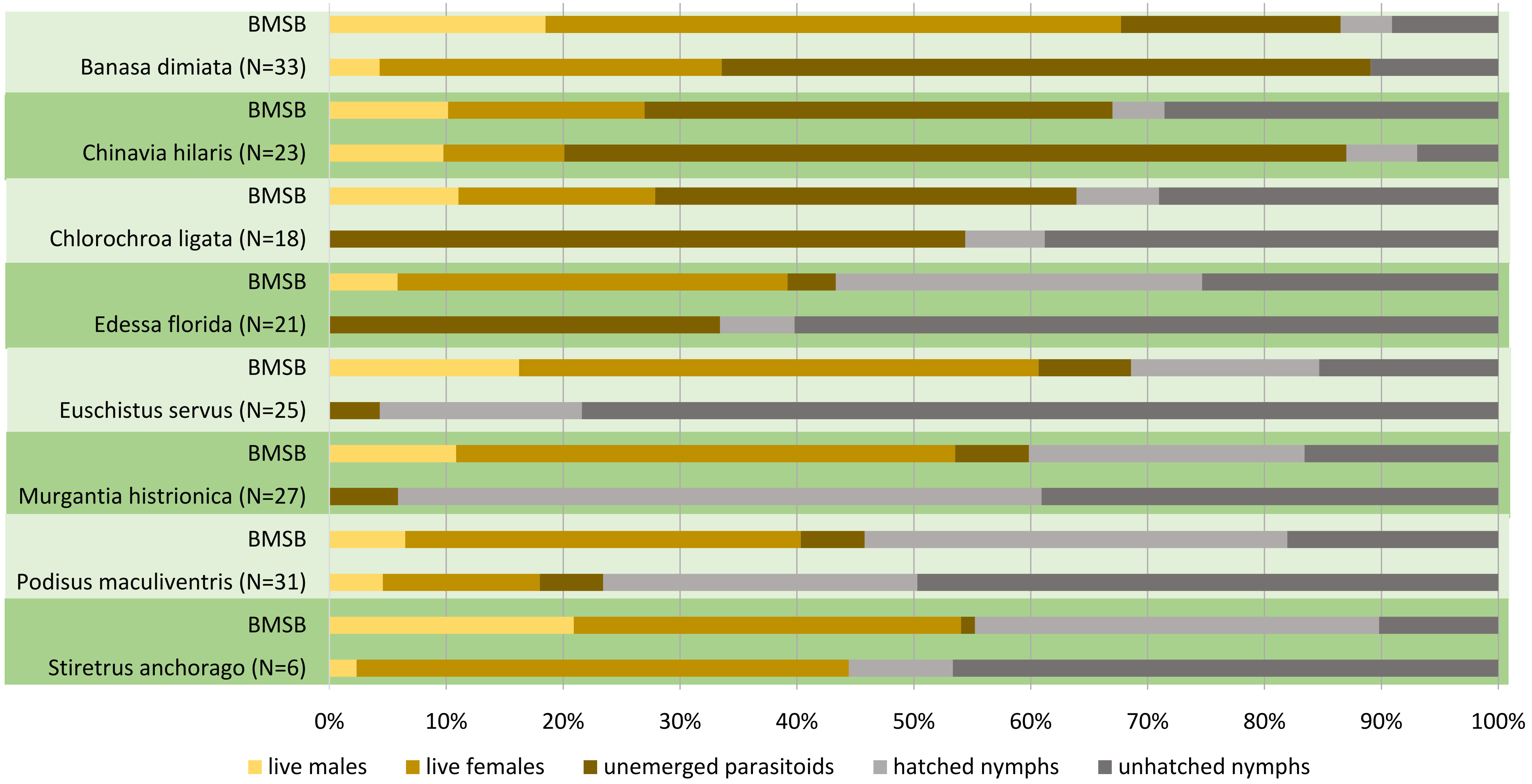
No-Choice Test Outcome - Part 2

(as of 2015)



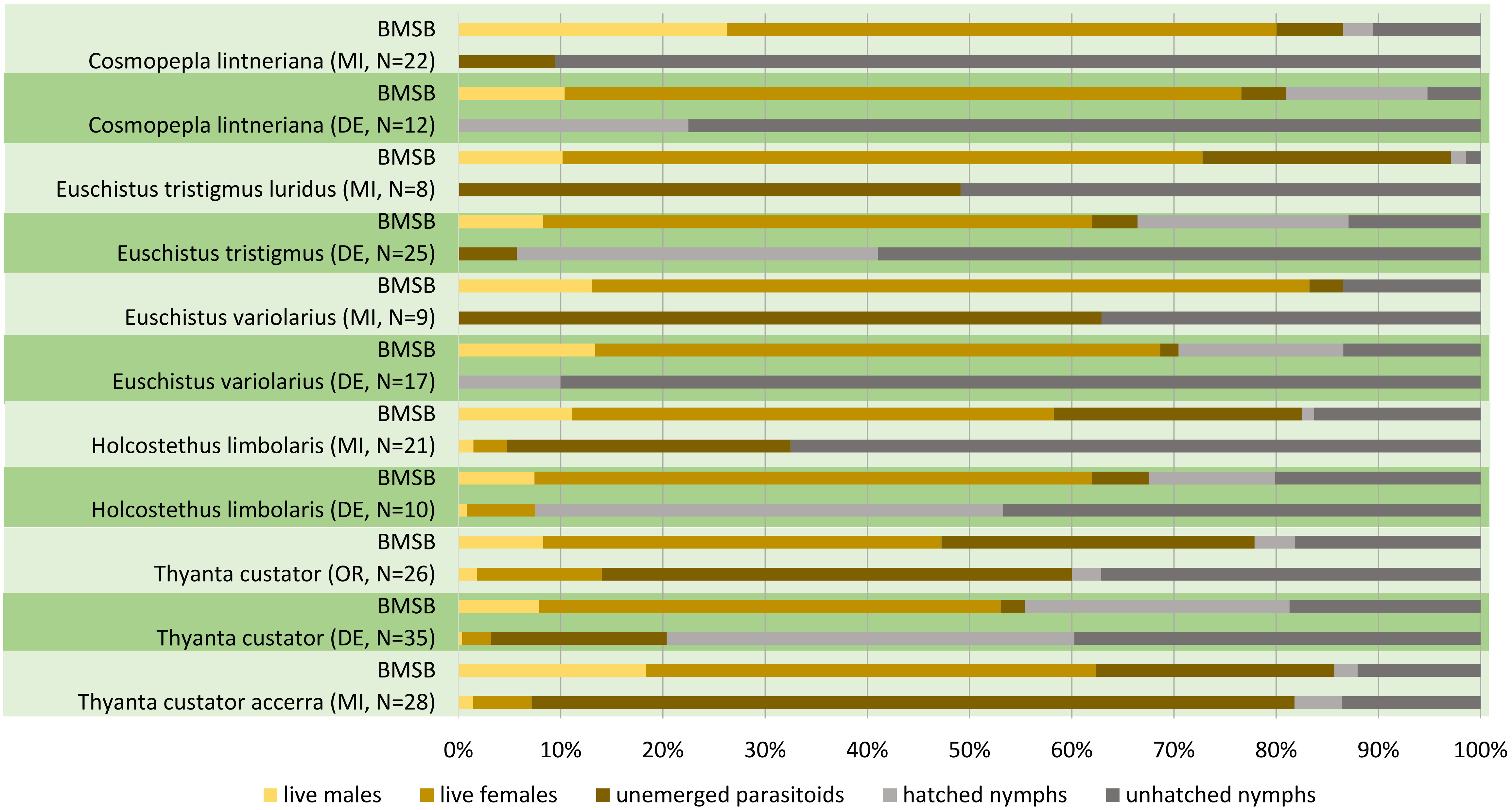
Choice Test Outcome - Part 1

(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

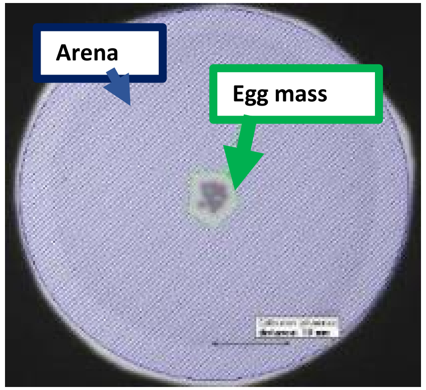
Role of parasitoid physiology & experience

- 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

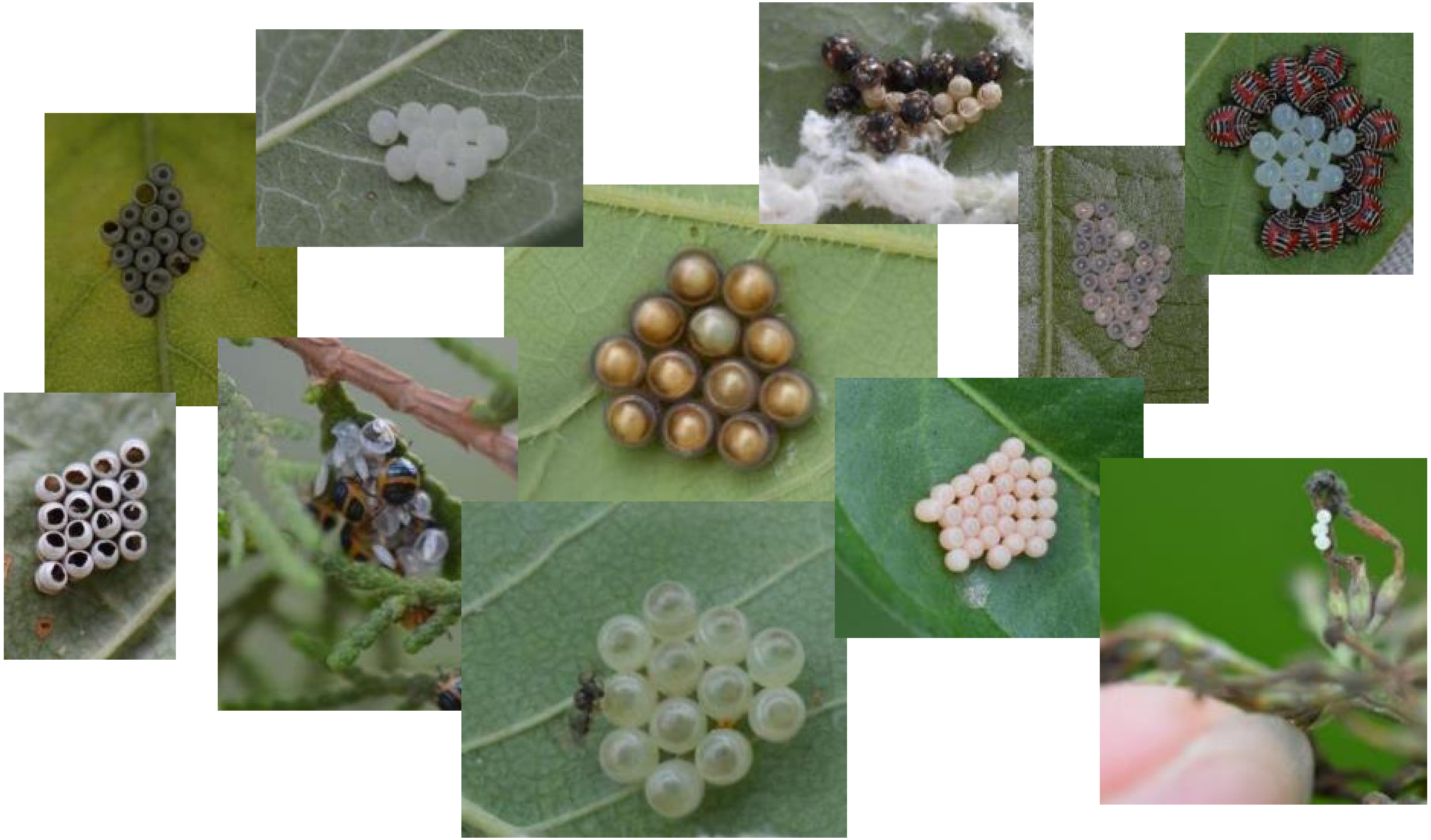


- **Ongoing**
  - Searching behavior
  - Oviposition behavior
  - Host choice
  - …

Olfactometer Studies

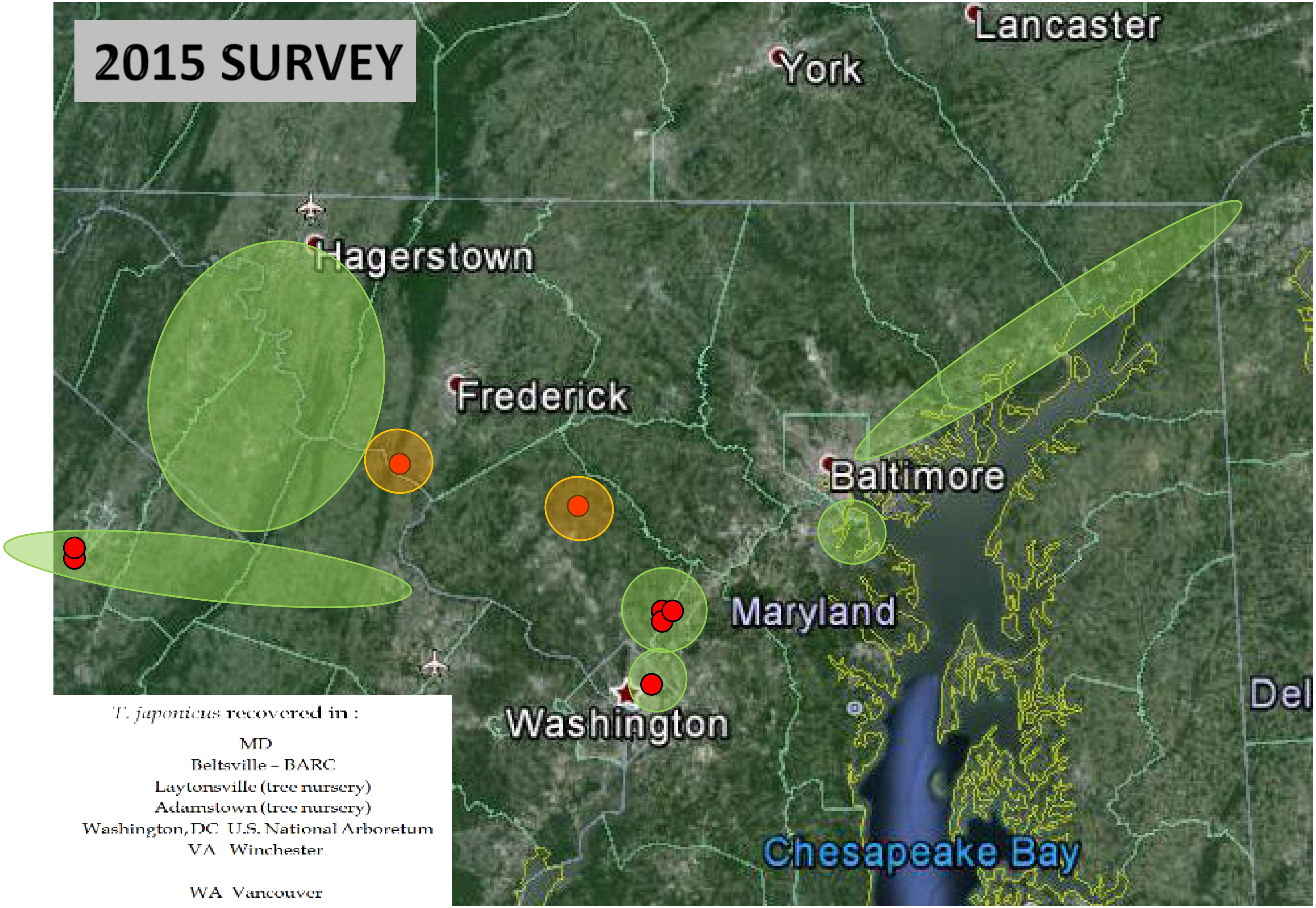
(FL, MI) - **ongoing**

Ecological Host Range of *T. japonicus* in Asia



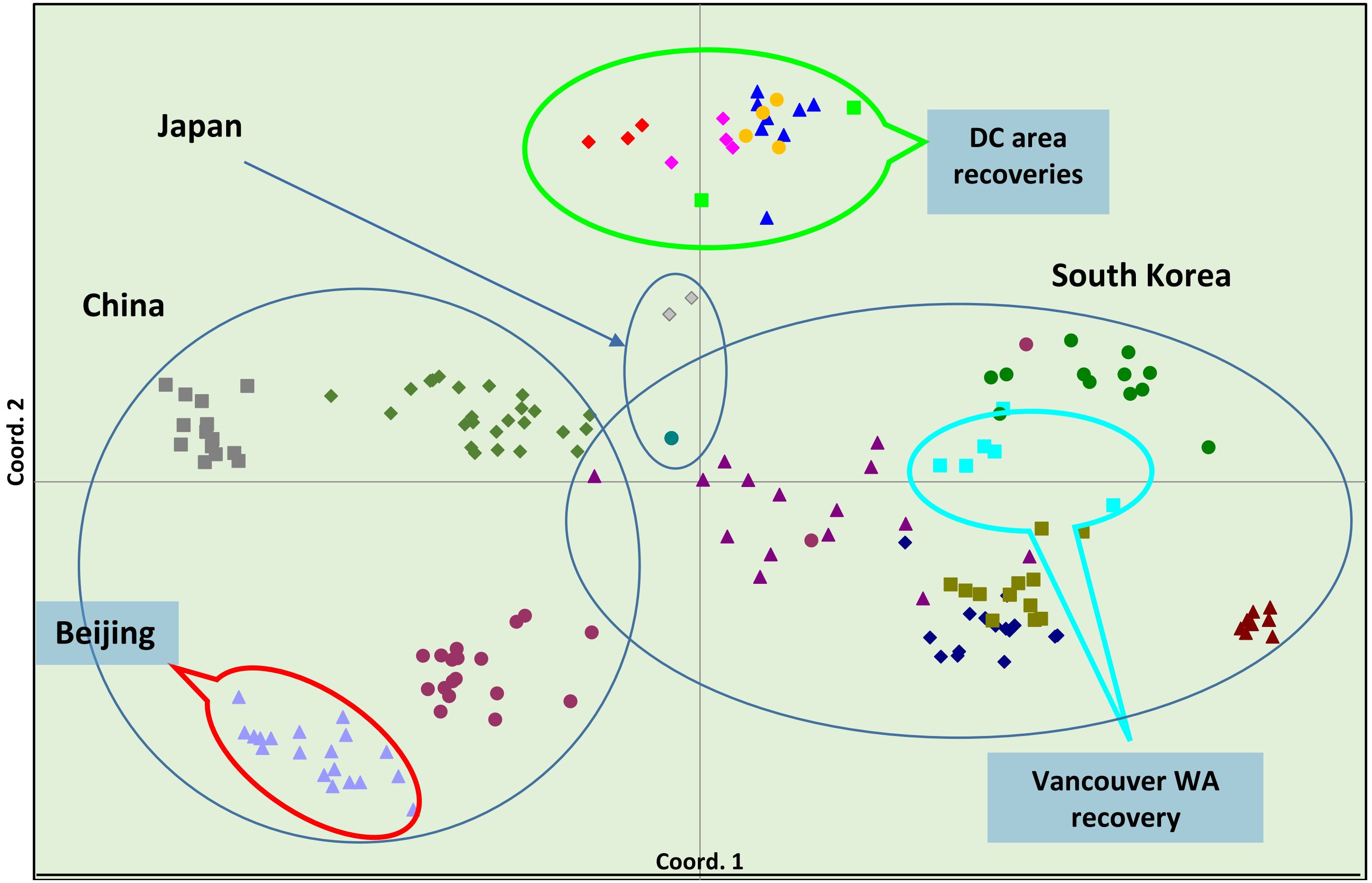
Recovery of *Trissolcus japonicus* in Maryland in 2014

a potential game changer?


2015 SURVEY
Lancaster
York
Hagerstown
Frederick
Baltimore
Maryland
Washington
Del
Chesapeake Bay
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

<u>Take home message:</u>

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

<u>Quarantine Host Range Evaluations:</u>

- 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)

<u>Adventive *Trissolcus japonicus*</u>:

- 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

USDA United States Department of Agriculture

National Institute of Food and Agriculture

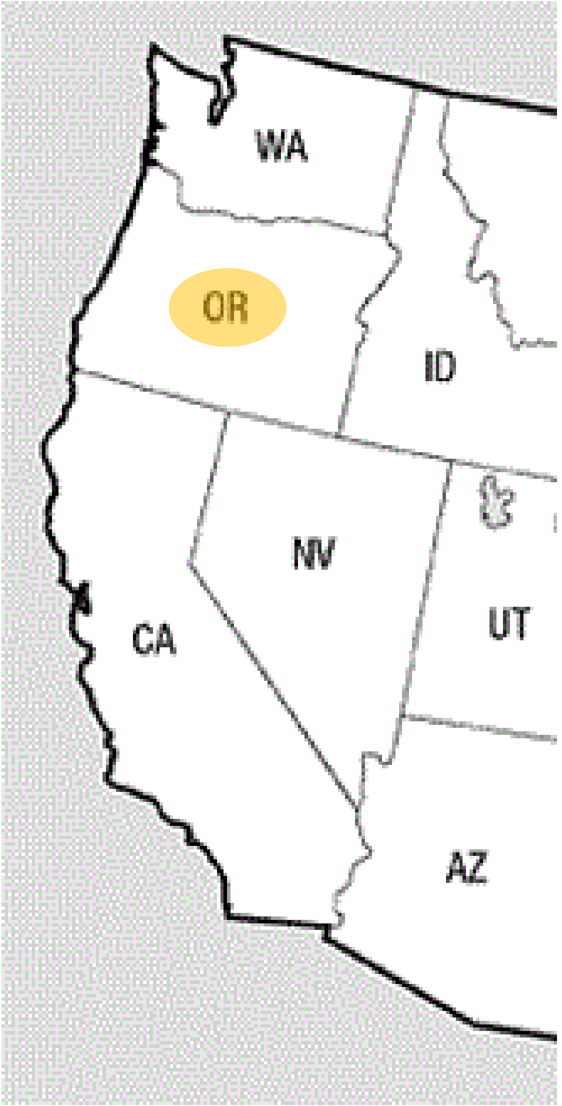
Specialty Crop Research Initiative
Grant #2011-01413-30937

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
OSU Oregon State University
NC State University

StopBMSB.org

Survey area and Collaborators



<u>Delaware</u> – USDA-ARS:
K. Hoelmer, K. Tatman & C. Dieckhoff

<u>Maryland</u> – University of Maryland:
P. Shrewsbury & A. Jones

<u>North Carolina</u> – NC State University:
J. Walgenbach & E. Ogburn

<u>Oregon</u> – USDA-ARS Corvallis:
Jana Lee

<u>Pennsylvania</u> – Penn State University:
D. Biddinger & N. Joshi

<u>Virginia</u> – Virginia Tech:
T. Kuhar, D. Pfeiffer & T. Trope

Native Biocontrol of BMSB In PA

D. Biddinger & N. Joshi
Penn State University Entomology

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

Biddinger 2014

Objective: Survey and Identify native natural enemies of BMSB

<u>Sentinel BMSB</u> egg mass placement (lab-reared) - DE, NC

<u>Naturally laid (wild) BMSB</u> egg masses –
DE, MD, NC, VA

Habitats surveyed

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

Paulownia

Ailanthus sp.

Other species

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

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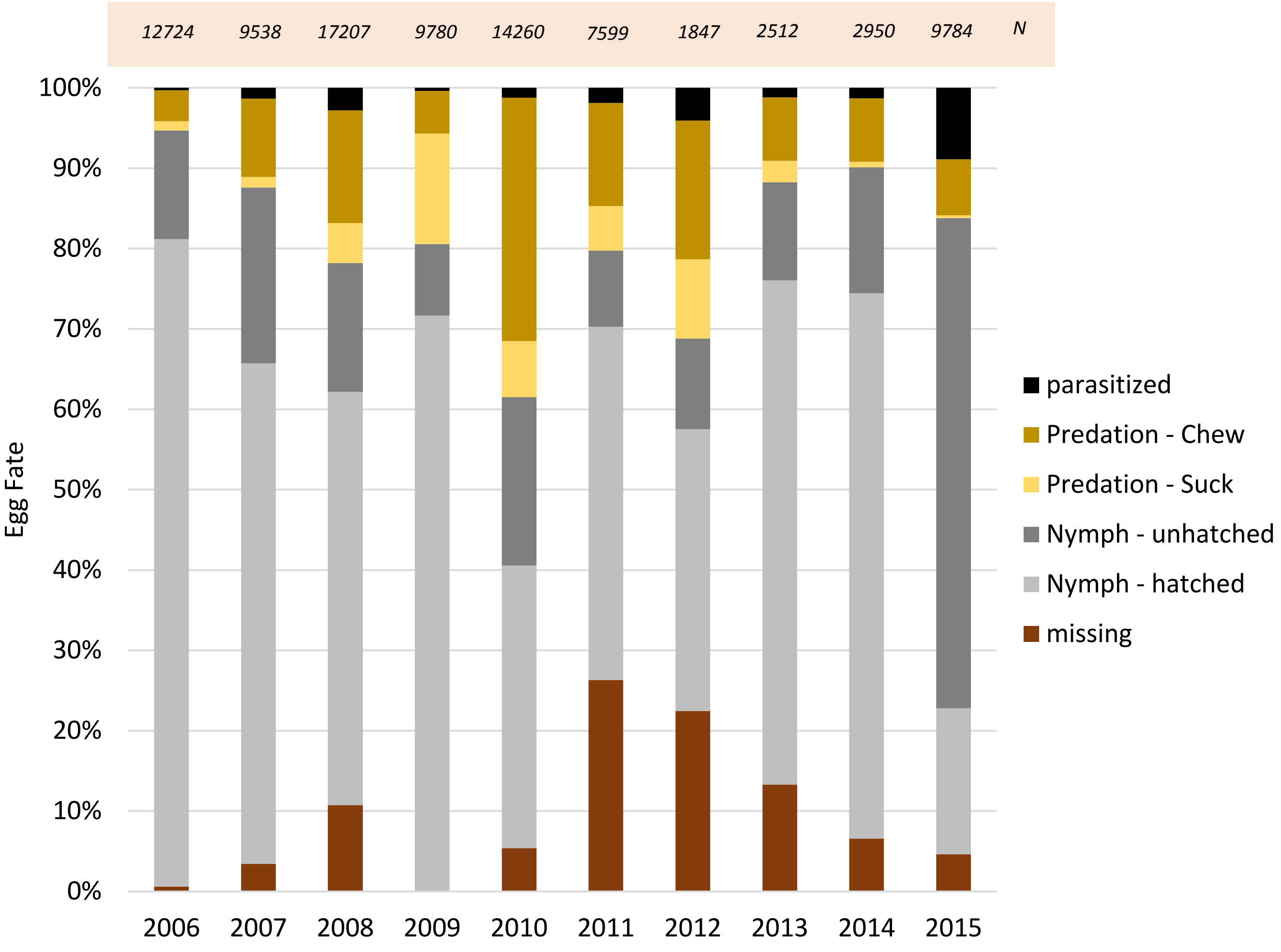
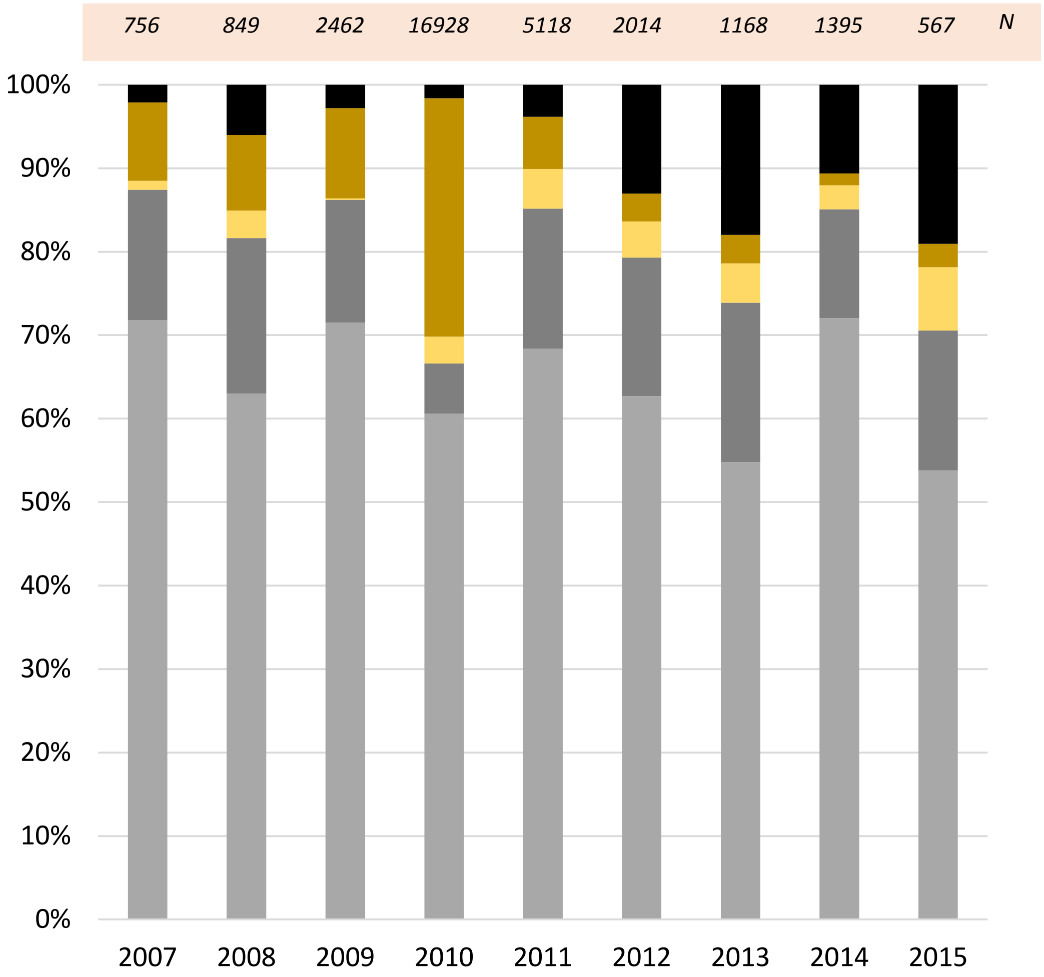
Fate of sentinel and naturally laid **BMSB** eggs





Sentinel BMSB eggs

Naturally laid BMSB eggs

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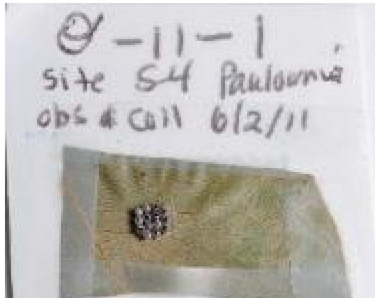
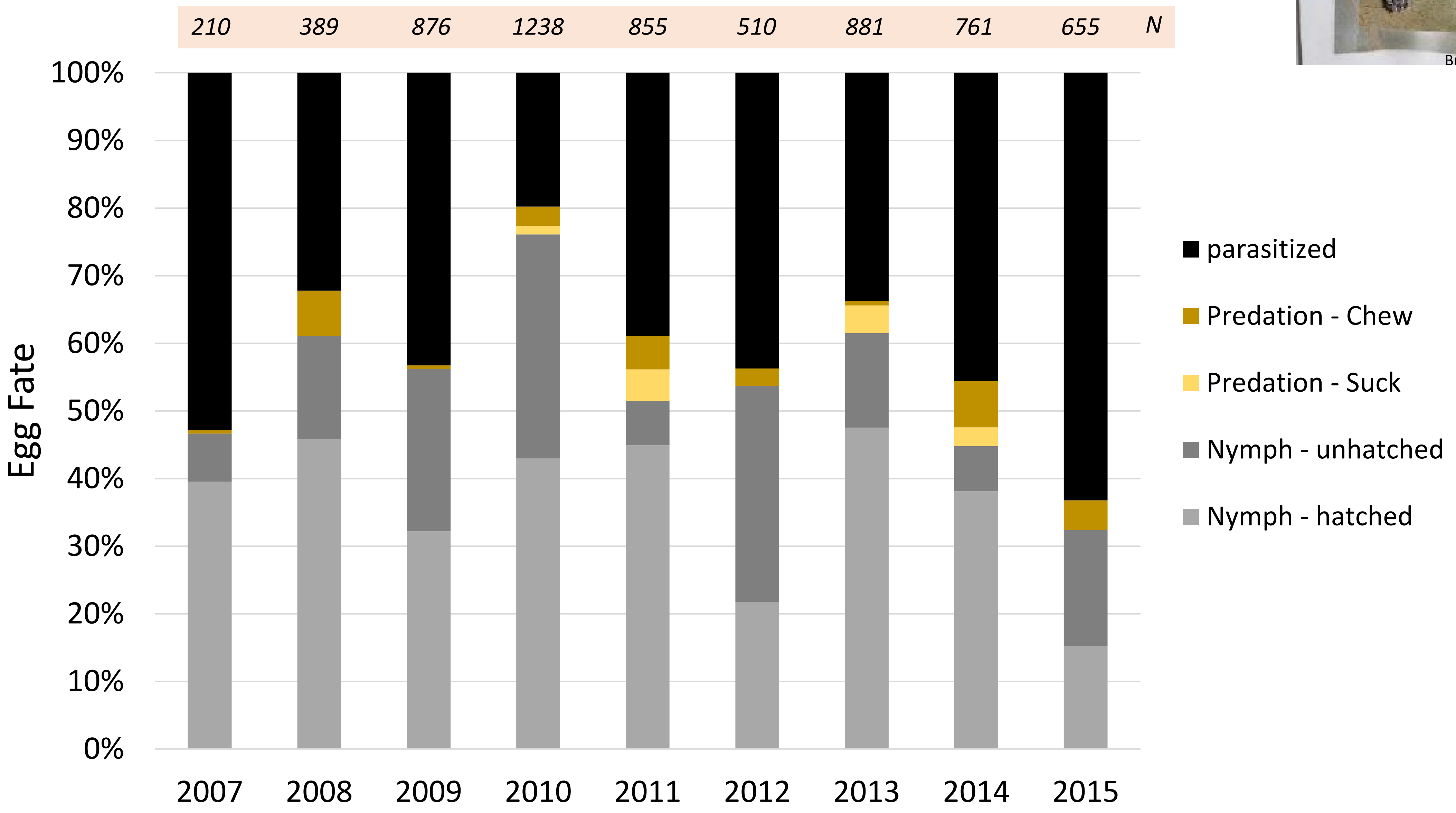
Fate of naturally laid non-BMSB eggs

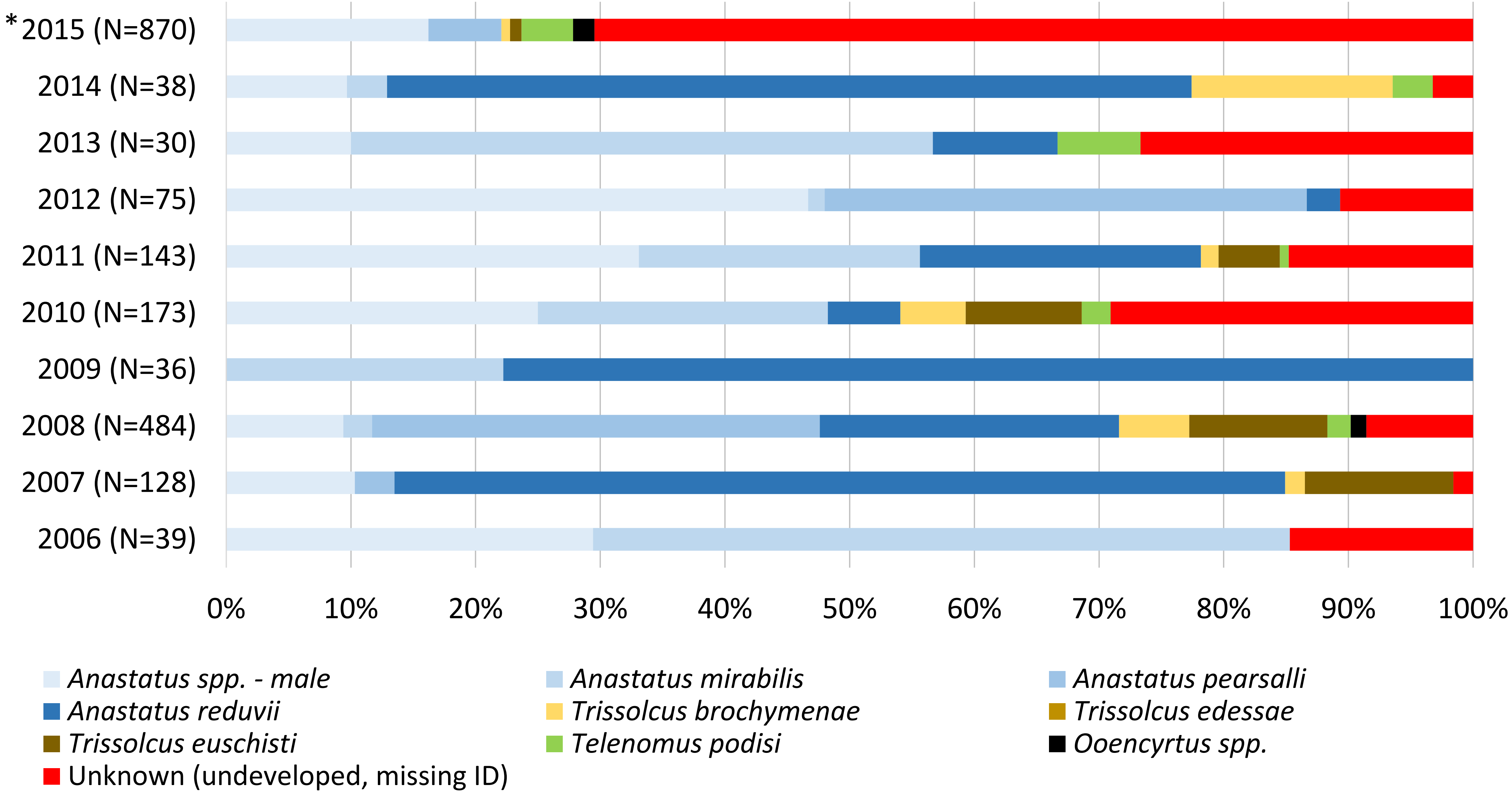


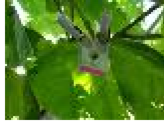
Brian T. Cutting



Delaware

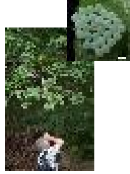
Species composition of parasitized sentinel **BMSB** eggs

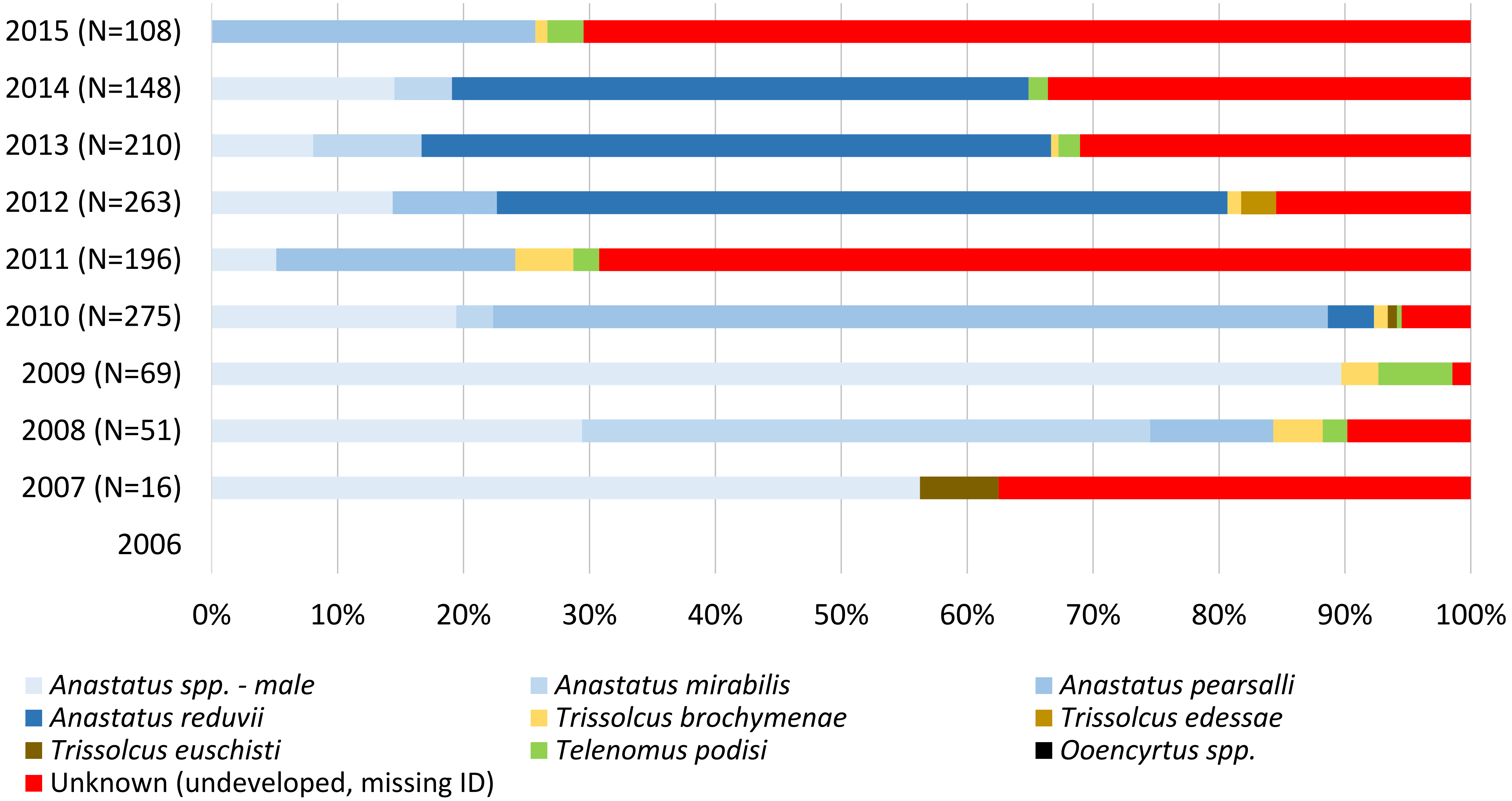




Delaware

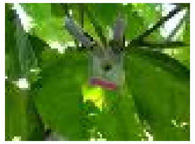
Species composition parasitized <u>naturally laid</u> **BMSB** eggs





NC State

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

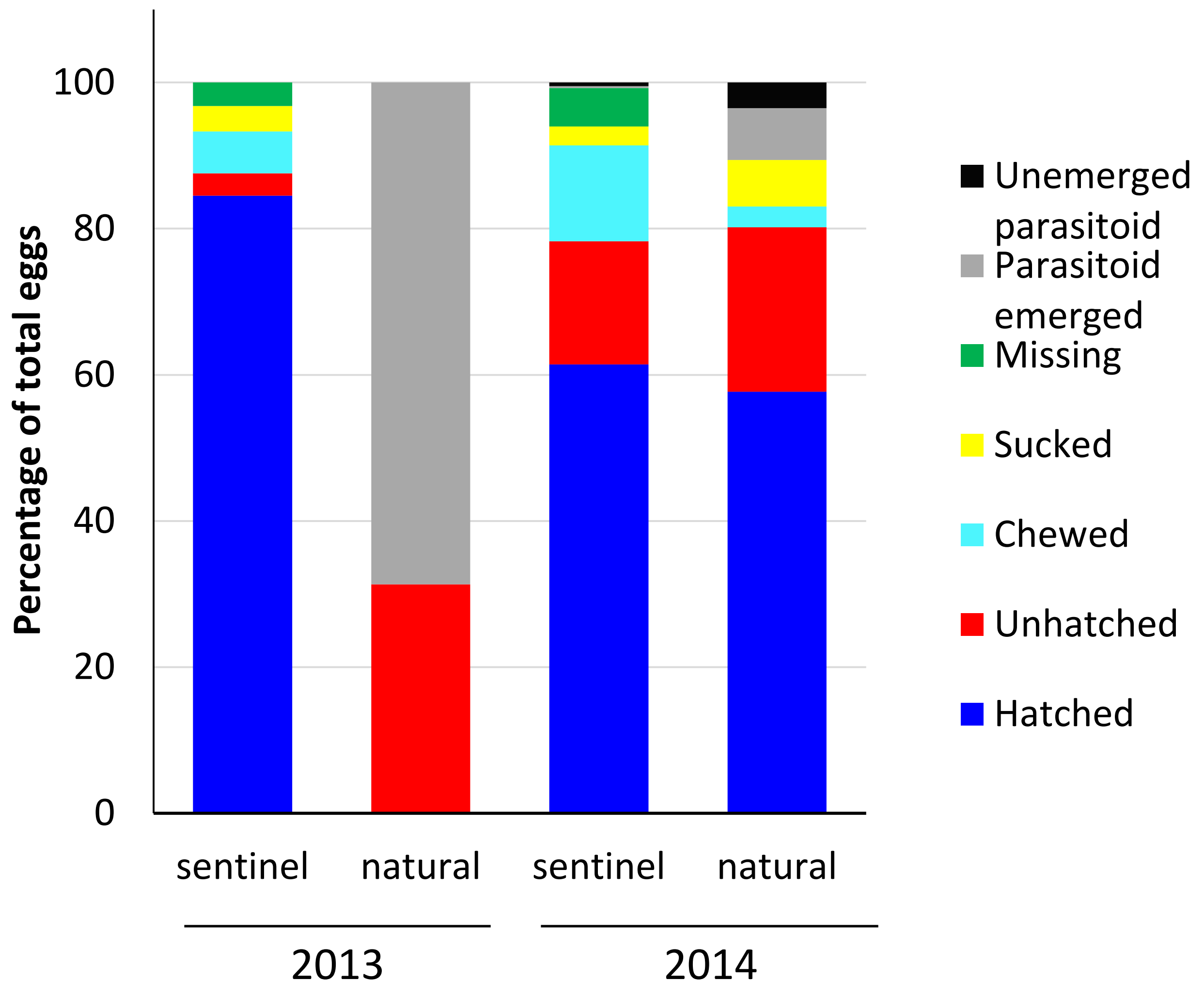


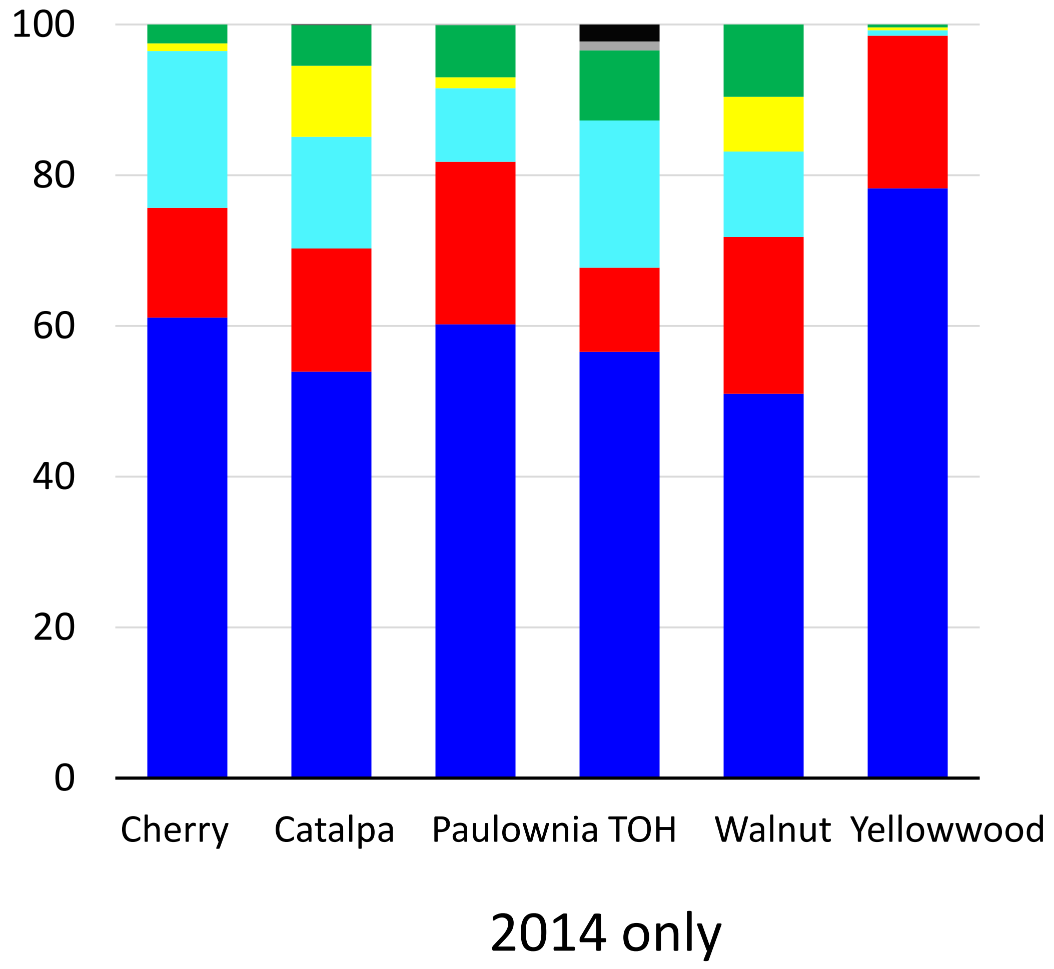
Parasitism rates of sentinel and naturally laid eggs recovered in 2013 and 2014 ranged from 0.26% to 27.54%

- 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.

NC State

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

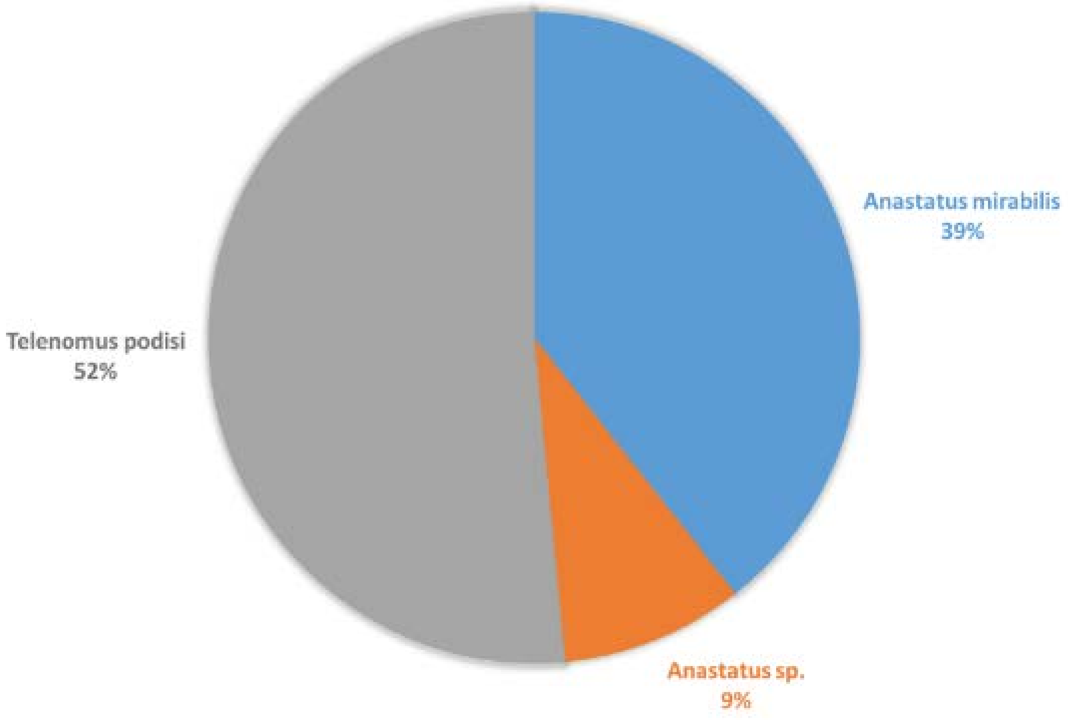




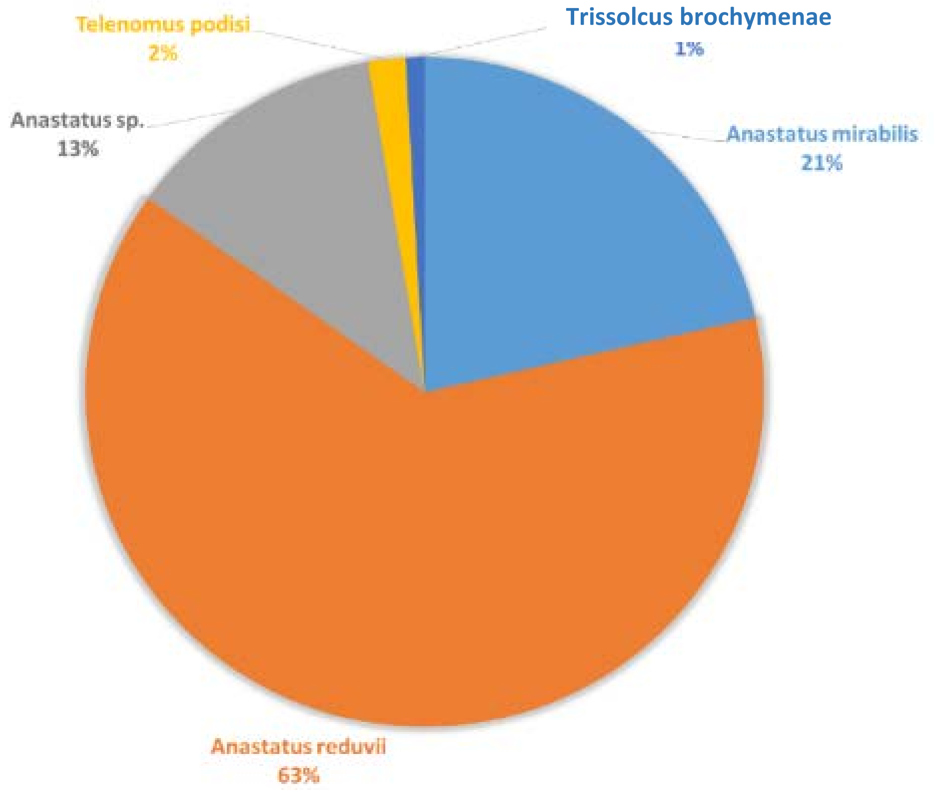
N C State

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



VA Tech

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.

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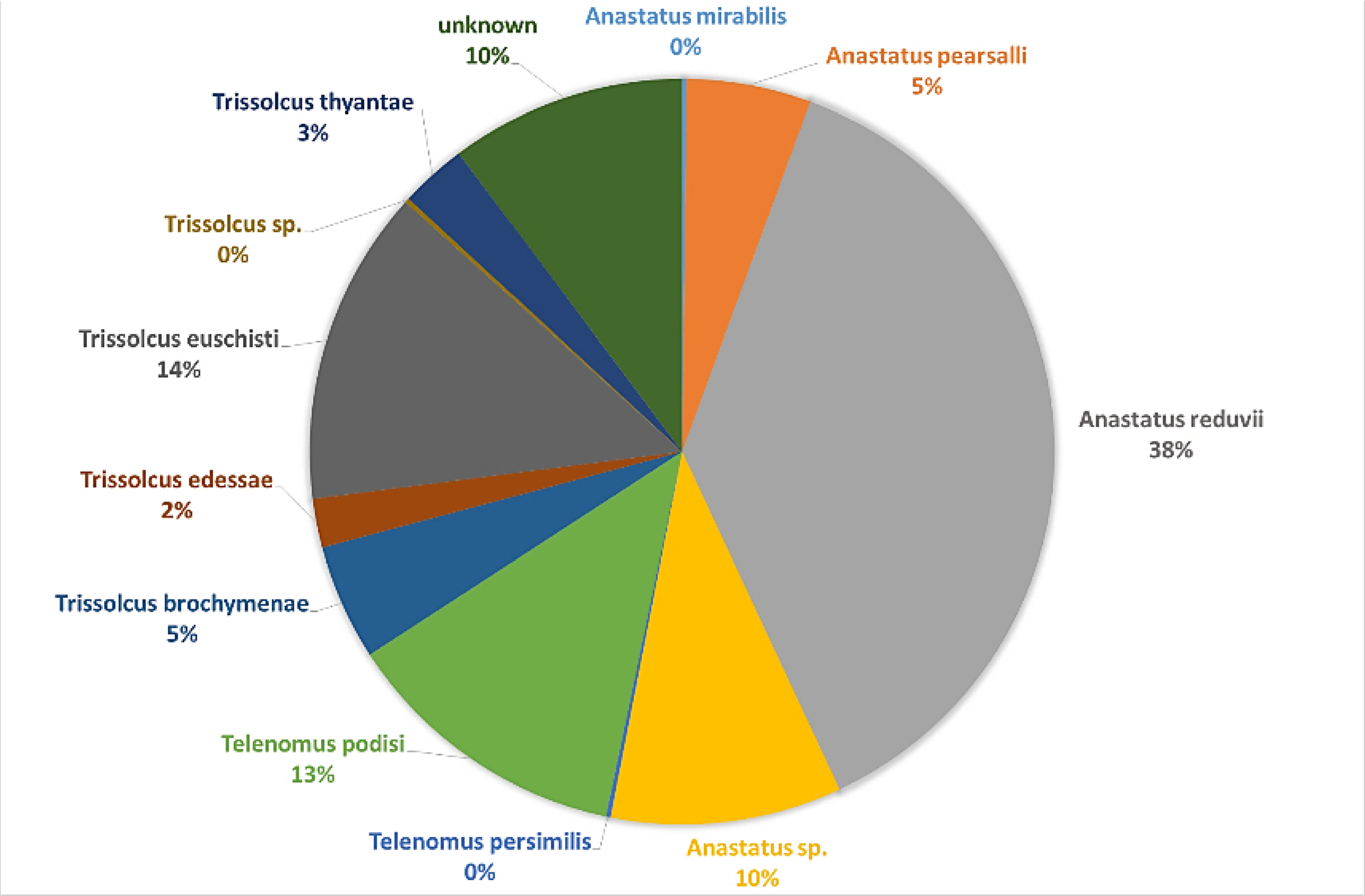
Species composition of parasitized naturally laid BMSB eggs in Virginia from 2011-2015



Maryland

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**

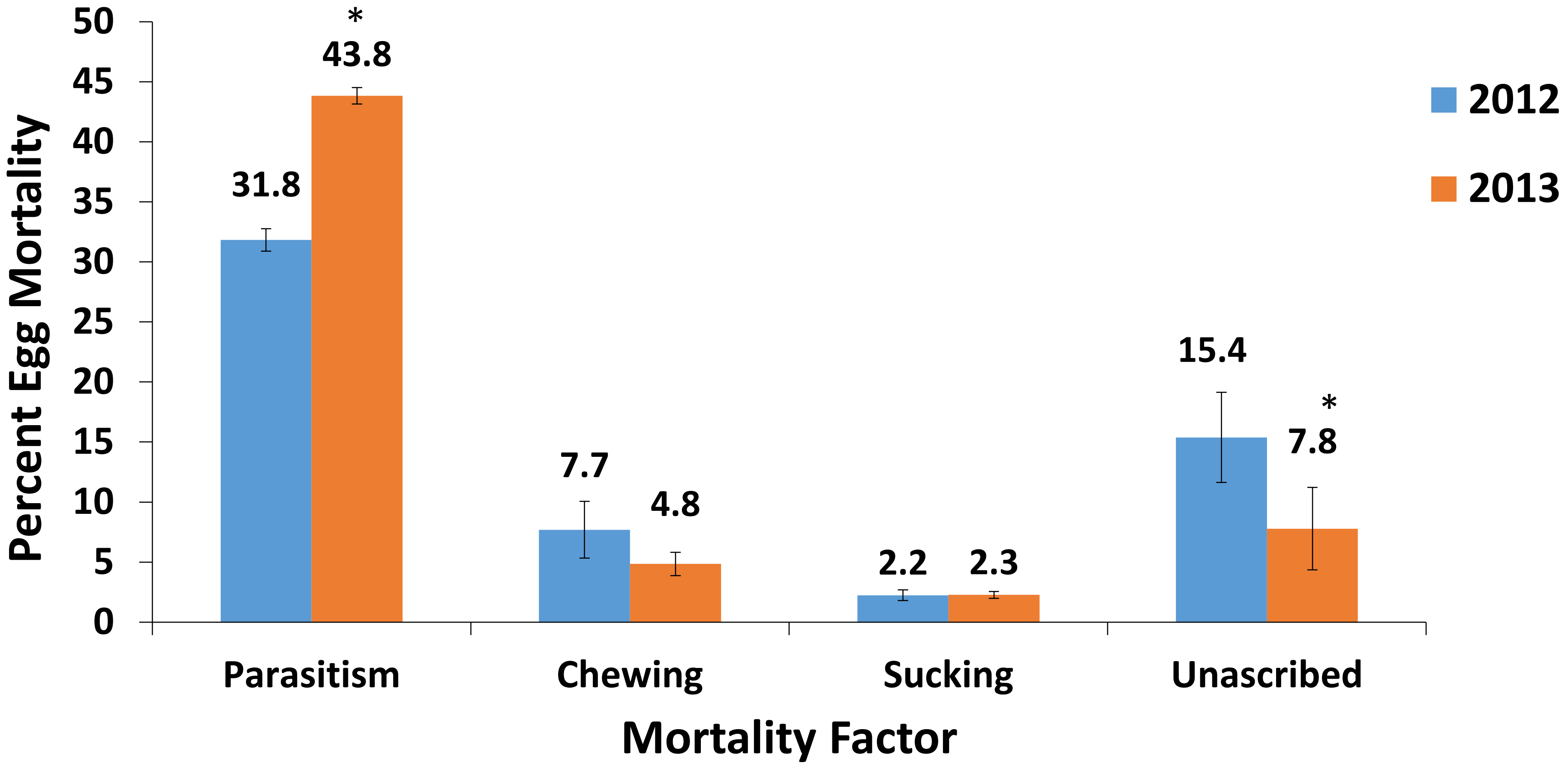
Photo by Steve Black, Raemelton Farm

Photo by Ruppert Nurseries

Maryland

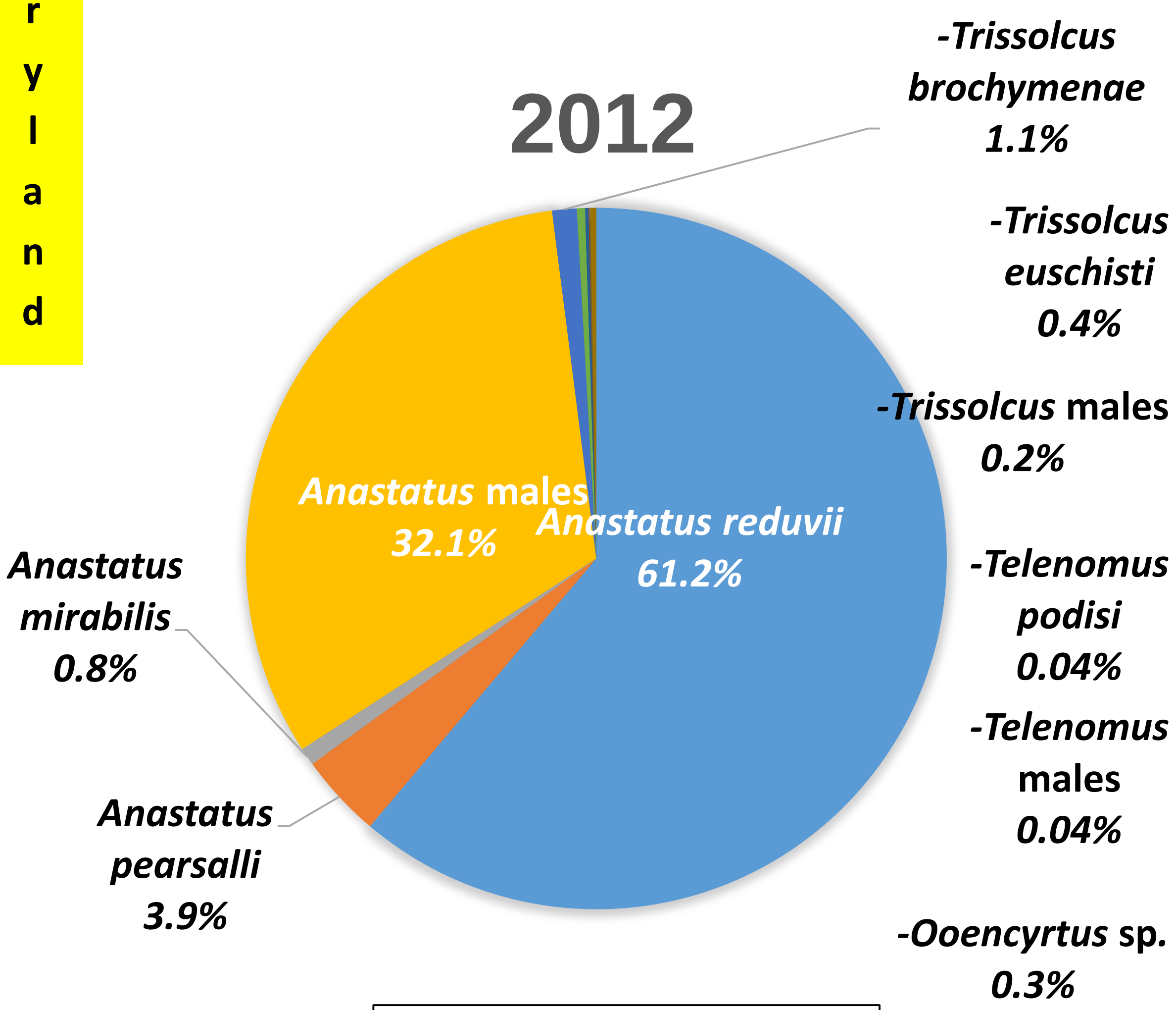
BMSB Egg Mortality Factors

Total Mortality ~ 58%



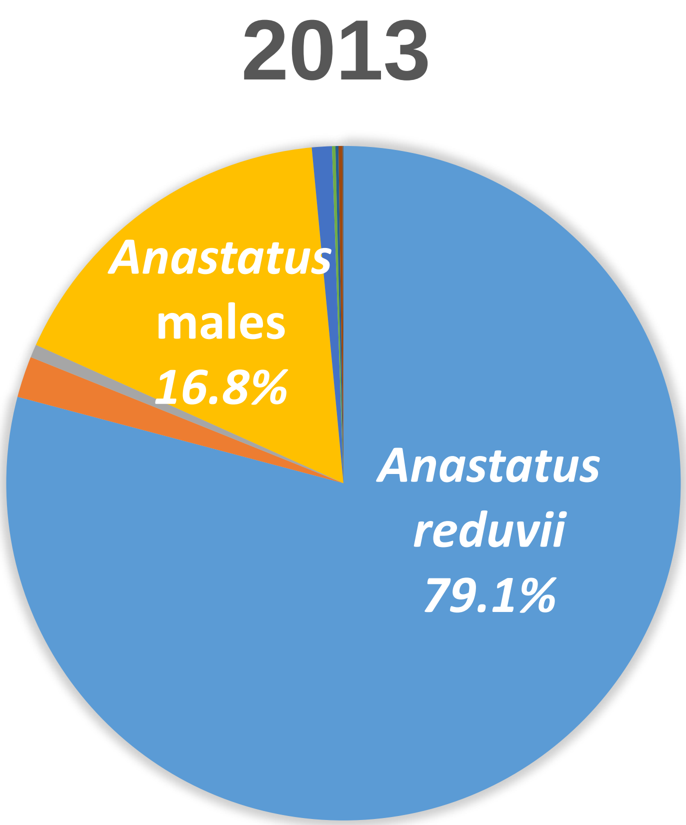
Maryland

Parasitoid species composition in Maryland, 2012-2013



5,638 parasitoids
24,124 eggs

1 : 2.05 M:F ratio



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 reduvii* 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

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- **Ruppert Nursery, Laytonsville, MD**
- **Many stakeholder cooperators, students, post-docs**

United States Department of Agriculture

National Institute of Food and Agriculture

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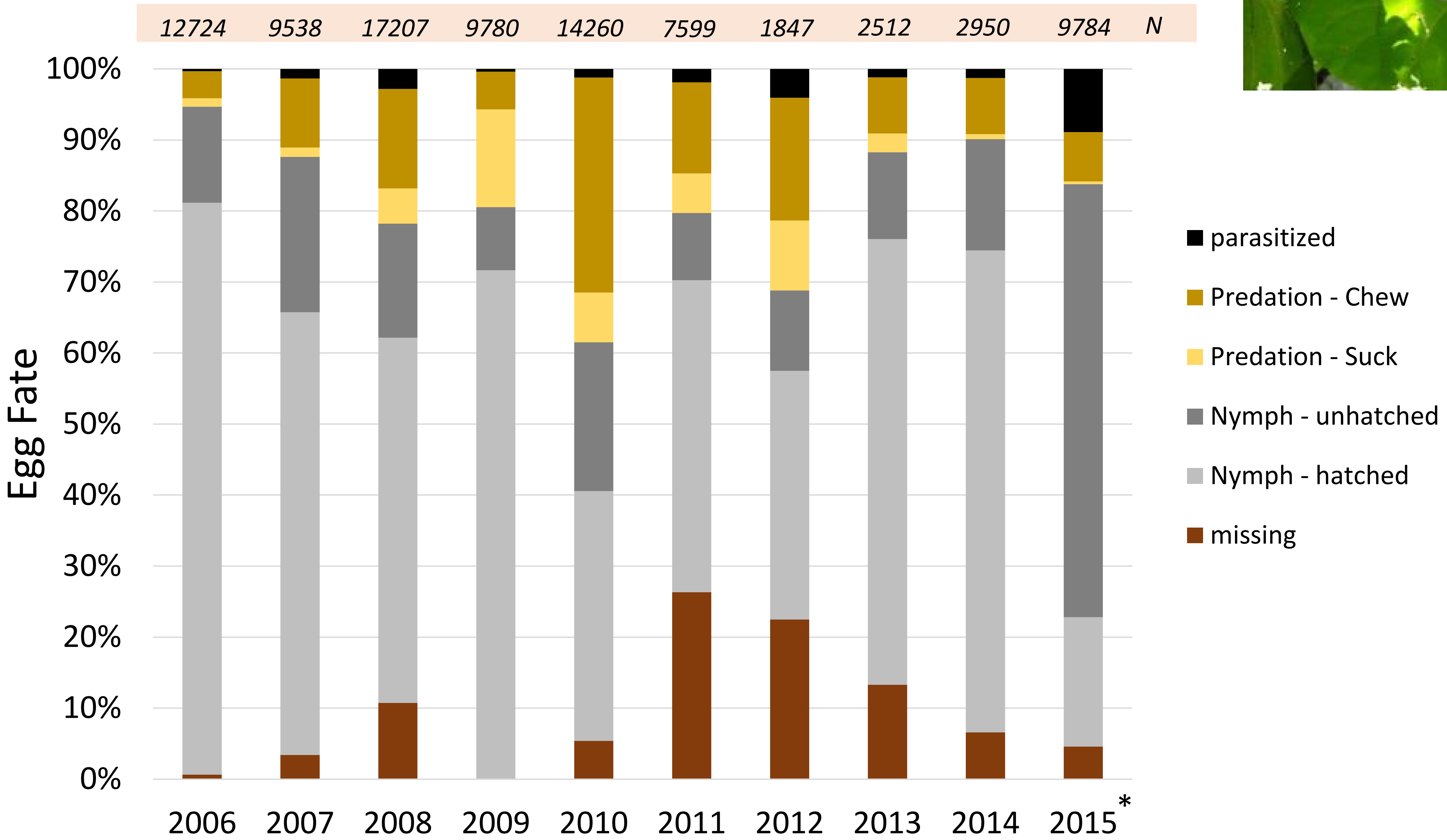
Fate of sentinel BMSB eggs



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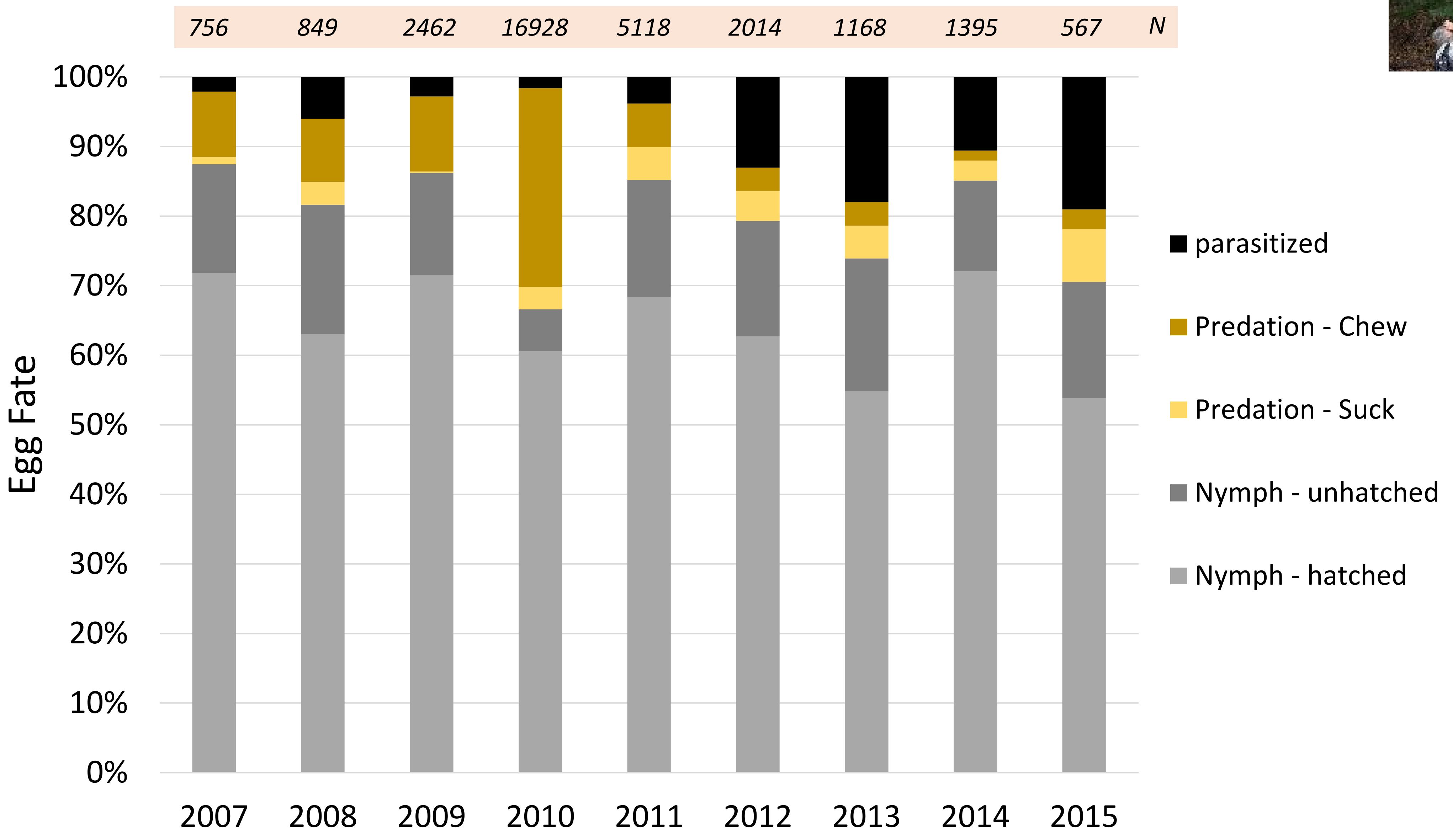
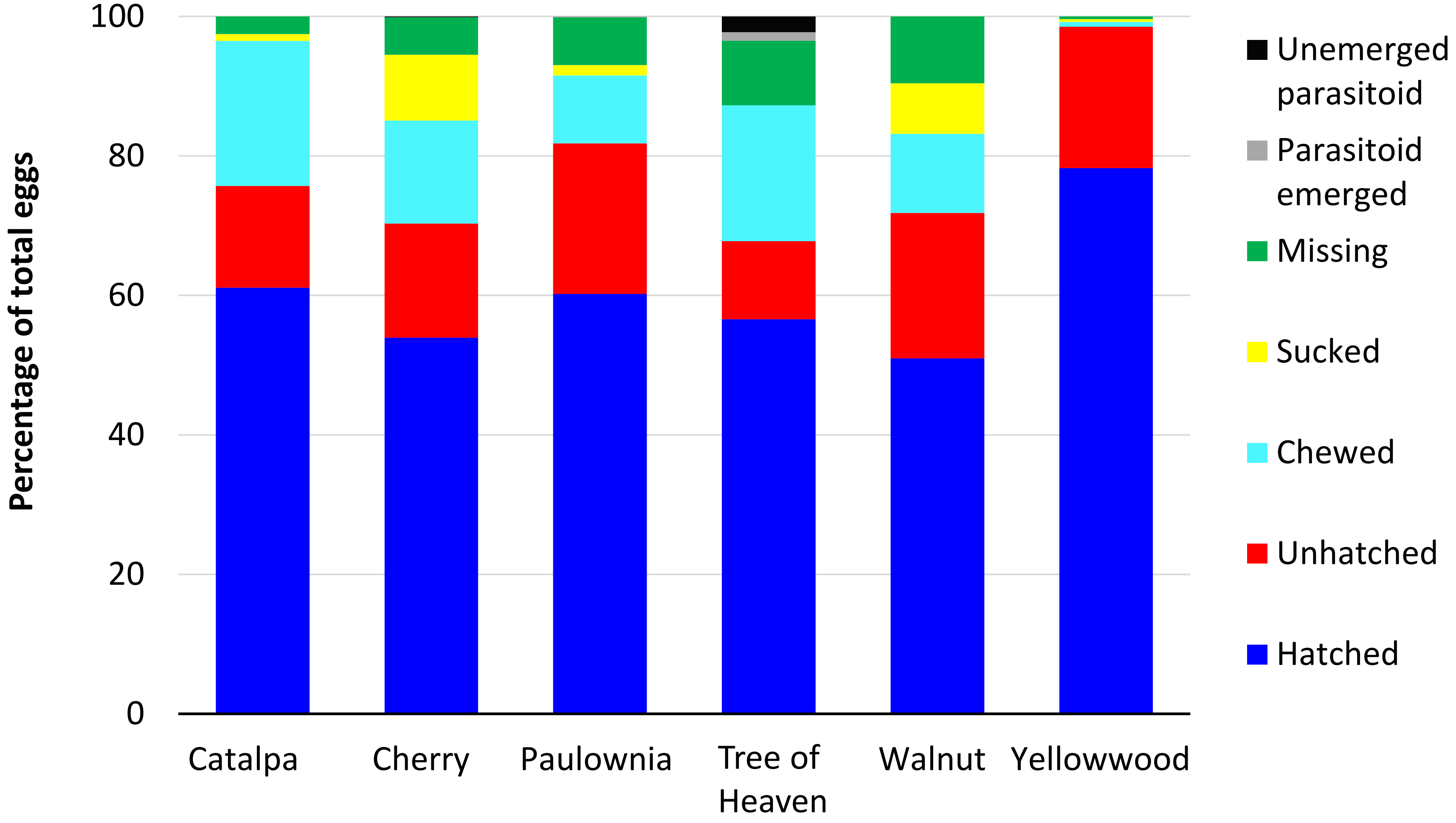
Fate of naturally laid **BMSB** eggs



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



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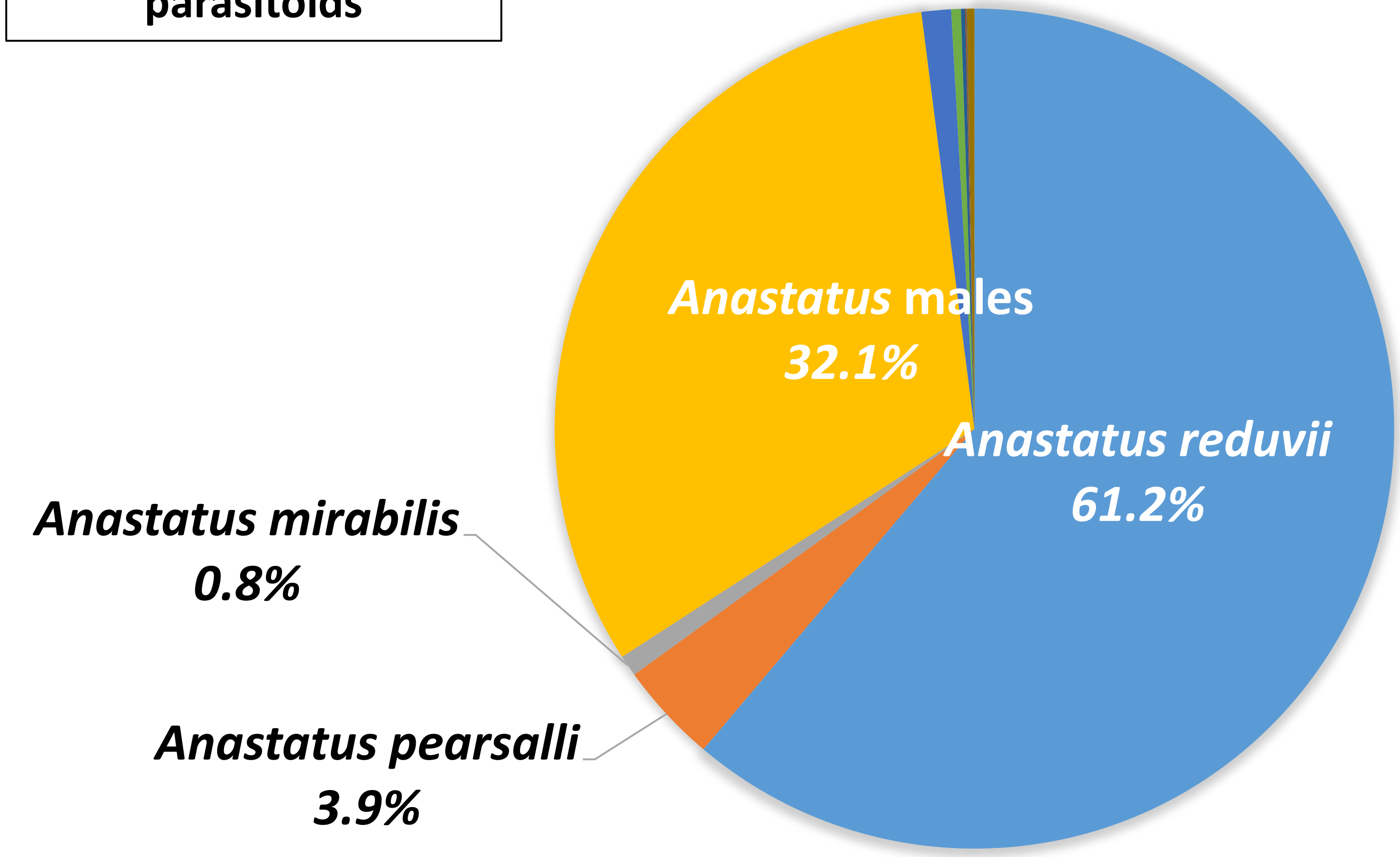
Parasitoid species composition in Maryland, 2012

2012

2012: 5,638 parasitoids



-Trissolcus brochymenae* *1.1%

-Trissolcus euschisti* *0.4%

-Trissolcus* males *0.2%

-Telenomus podisi* *0.04%

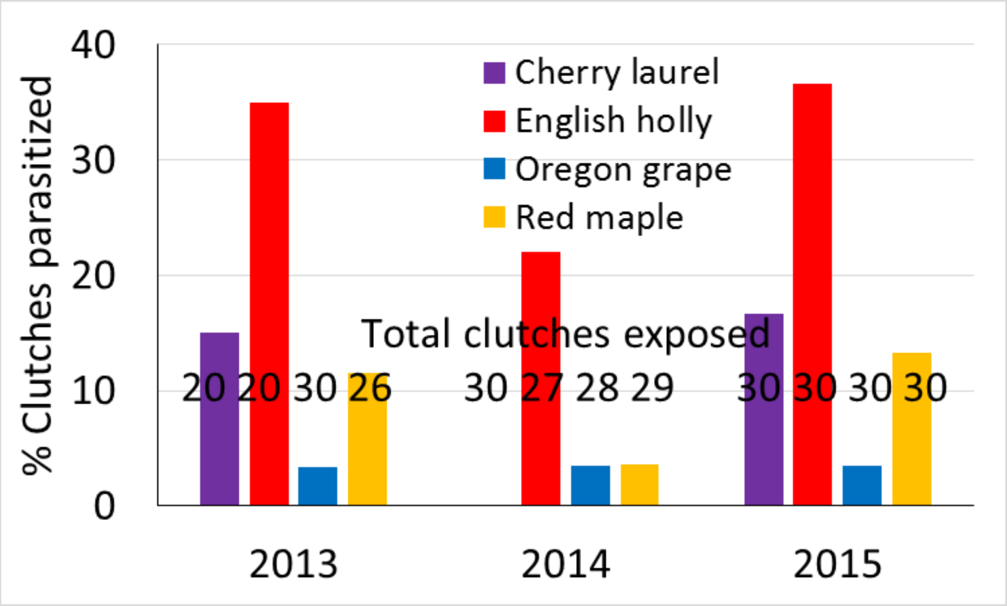
-Telenomus* males *0.04%

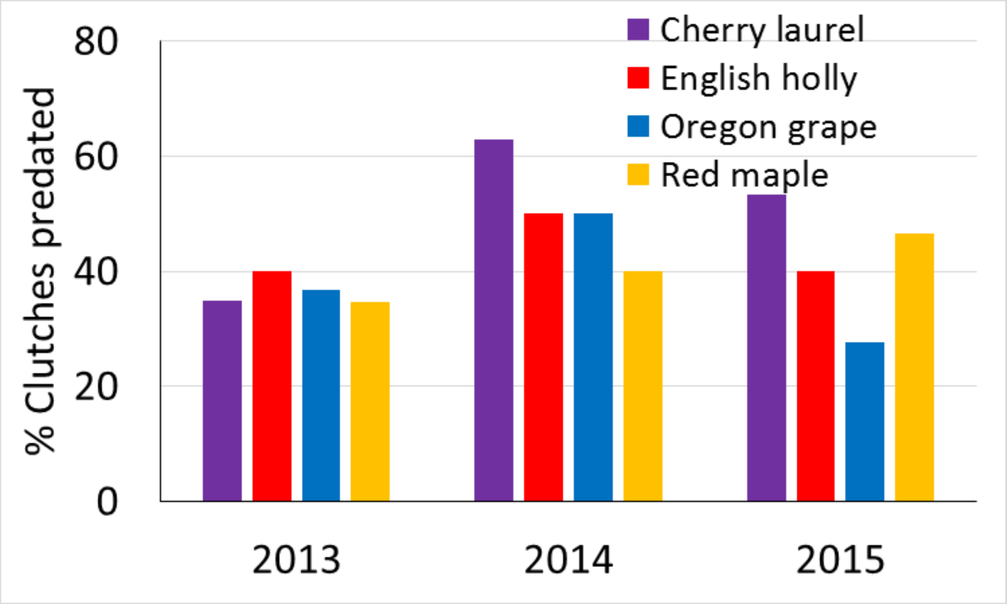
-Ooencyrtus* sp. *0.3%

***Anastatus* total: 98%**

Oregon

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



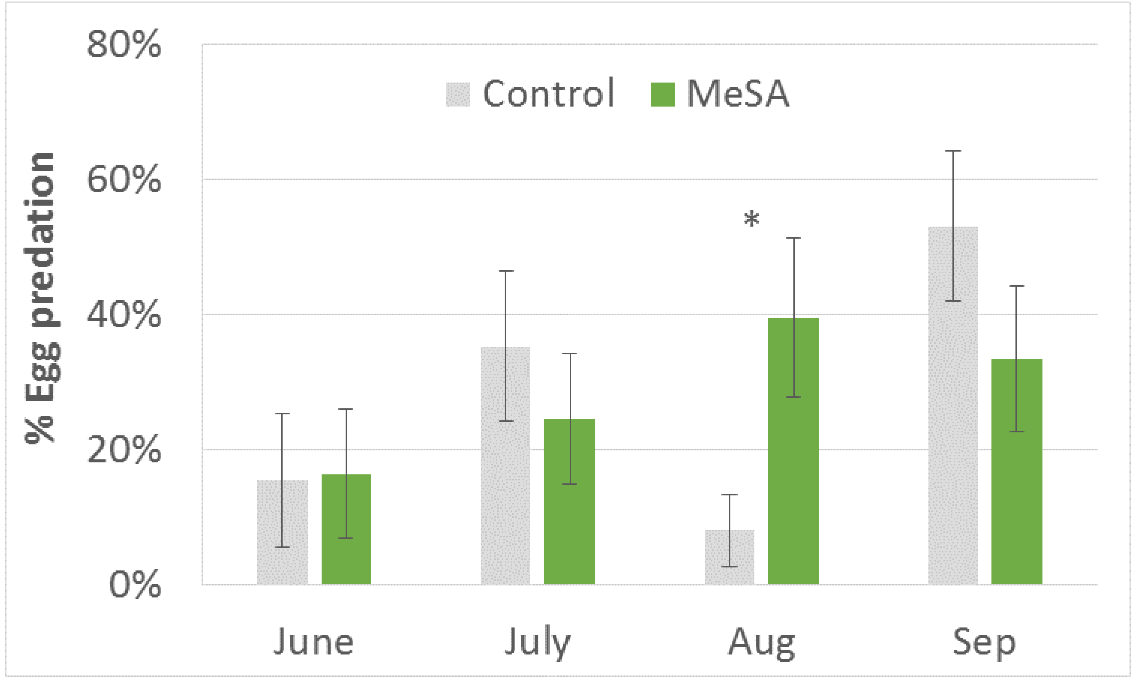


- 5 sites per host, with/out mesh cages at each site
- 1 wk exposure
- Monthly June – Aug/Sep
- **Frozen (!)** sentinel eggs

Lee, Cave-USDA Corvallis

Does Methyl salicylate enhance <u>egg</u> biocontrol through <u>predation</u>? (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

Skillman, Lee -USDA Corvallis