Hidden host mortality from *Trissolcus japonicus:* Conventional and molecular evaluation of non-target risk to native pentatomids

Jim Hepler [1], Kacie Athey [2], David Enicks [3], Paul K. Abram [4], Tara D. Gariepy [5], Elijah J. Talamas [6] and Elizabeth Beers [3]

[1] **Appalachian Fruit Research Station, United States Department of Agriculture, Agricultural Research Service, 2217 Wiltshire Road, Kearneysville, WV, USA; james.hepler@usda.gov**

[2] Crop Sciences, University of Illinois at Urbana Champaign, 1101 W Peabody Drive, Urbana, IL 61801, USA; kathey@illinois.edu

[3] Tree Fruit Research and Extension Center, Washington State University, 1100 N Western Avenue, Wenatchee, WA 98801, USA; daencks@gmail.com; ebeers@wsu.edu

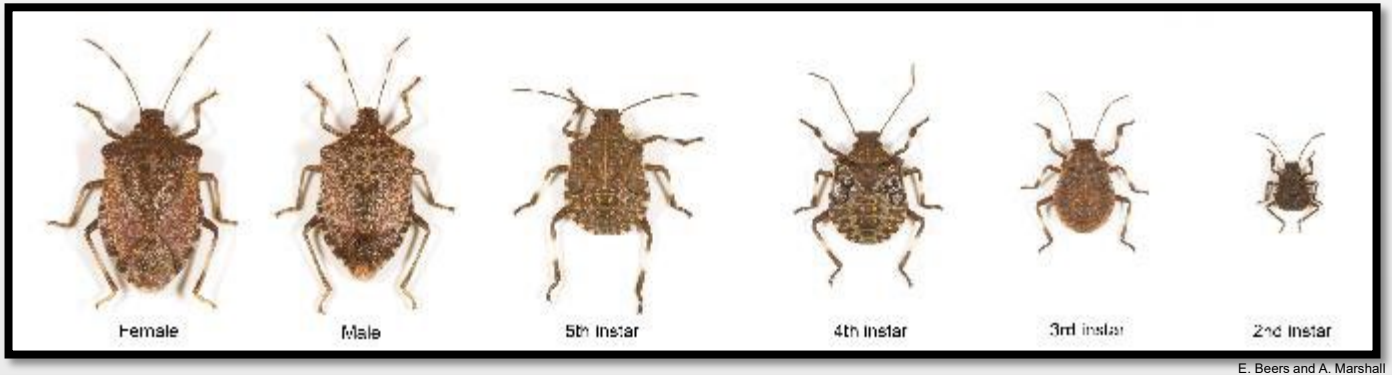
[4] Agassiz Research and Development Centre, Agriculture and Agri-Food Canada, 6947 Highway 7, PO Box 1000, Agassiz, BC V0M 1A2, Canada; paul.abram@canada.ca

[5] London Research and Development Centre, Agriculture and Agri-Food Canada, 1391 Sandford Street, London, ON N5V 4T3, Canada; tara.gariepy@canada.ca

[6] Florida Department of Agriculture and Consumer Services, Division of Plant Industry—The Doyle Conner Building, 1911 SW 34th Street, Gainesville, FL 32614-7100, Canada; Elijah.Talamas@FDACS.gov

The brown marmorated stink bug (BMSB), *Halyomorpha halys* Stål (Hemiptera: Pentatomidae)

- Large stink bug native to China, Korea, and Japan
- Now found across the United States
- Major pest of diverse specialty and row crops



E. Beers and A. Marshall

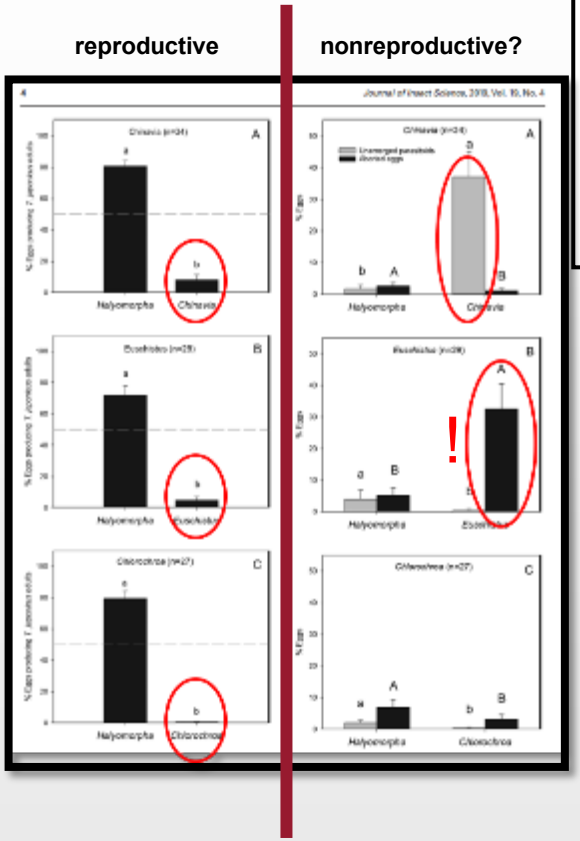
Trissolcus japonicus (Hymenoptera: Scelionidae), the primary parasitoid of BMSB

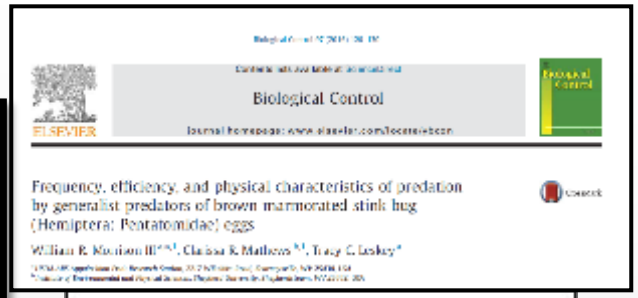
Trissolcus japonicus (Hymenoptera: Scelionidae)

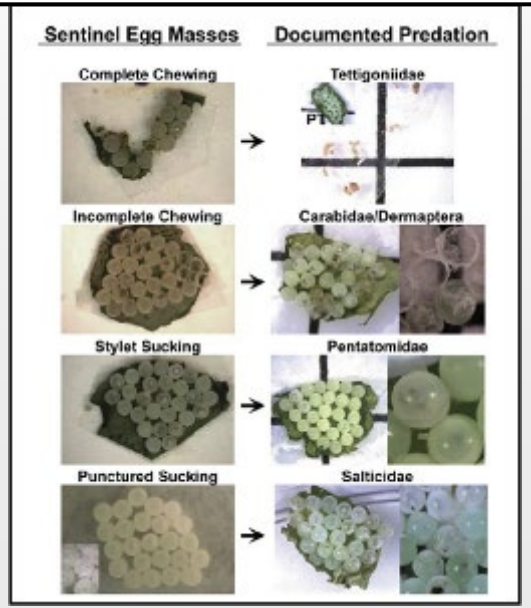
- Main parasitoid of BMSB in its native range
- Several adventive populations in North America
  - Found in Vancouver, WA in 2015
- Under consideration as a classical biocontrol agent of BMSB
- Redistribution process regulated by USDA-APHIS (Animal and Plant Health Inspection Service)
  - Requires assessments of potential ecological impacts, i.e., potential parasitism of native/nontarget hosts
- Mostly quarantine studies, but field conditions more reliable

Evaluating Impact of Parasitism on BMSB: Morphological Approach

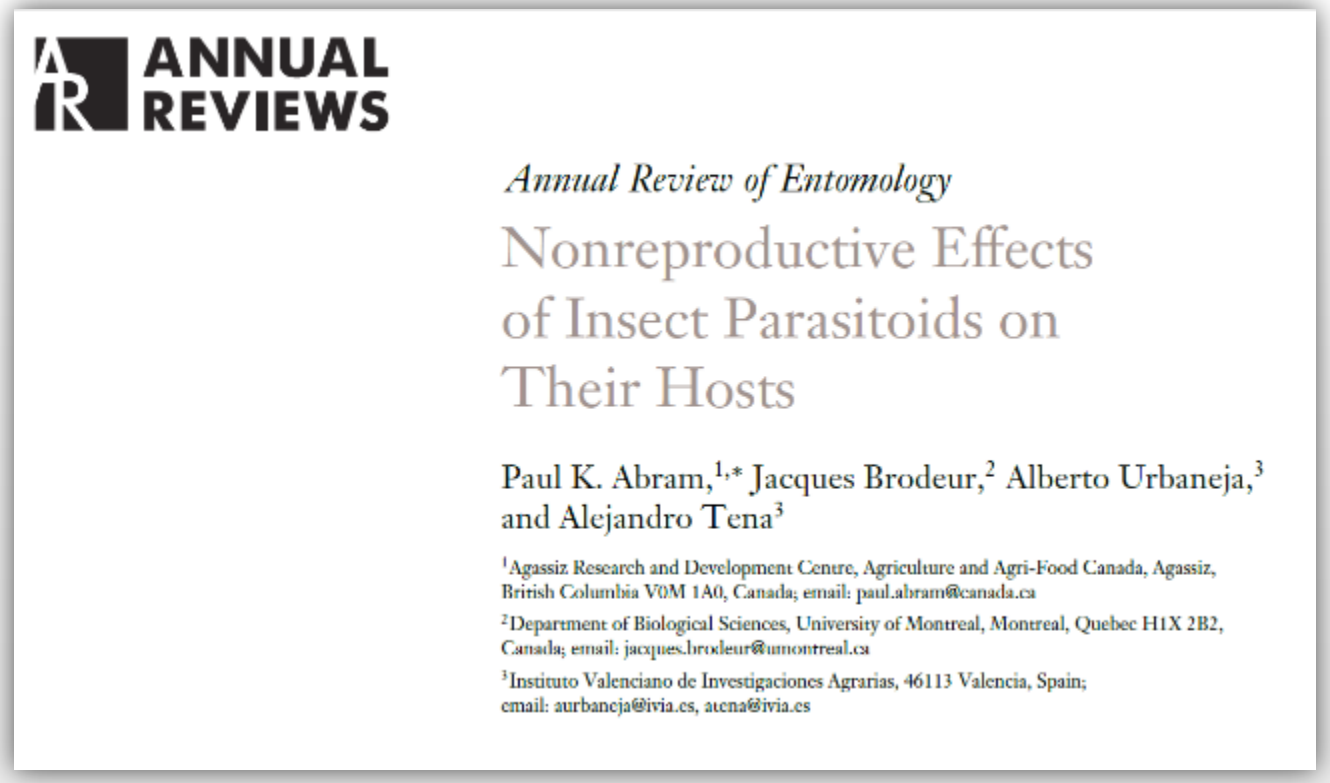
- Morphological classifications of egg fate
  - Predation (Morrison et al. 2016)
  - Parasitoid emergence, successful hatching, and range of other egg outcomes (Waterworth, unpublished)
    - Used by Milnes and Beers (2019) to evaluate parasitism in field conditions in Vancouver, WA
    - Reproductive effects (= wasp emergence) easy to quantify
    - Substantial mortality unexplained by morphology
      - › nonreproductive effects?







Reproductive vs. Nonreproductive Effects of Parasitoids



ANNUAL REVIEWS

Annual Review of Entomology

Nonreproductive Effects of Insect Parasitoids on Their Hosts

Paul K. Abram,[1,*] Jacques Brodeur,[2] Alberto Urbaneja,[3] and Alejandro Tena[3]

[1]Agassiz Research and Development Centre, Agriculture and Agri-Food Canada, Agassiz, British Columbia V0M 1A0, Canada; email: paul.abram@canada.ca

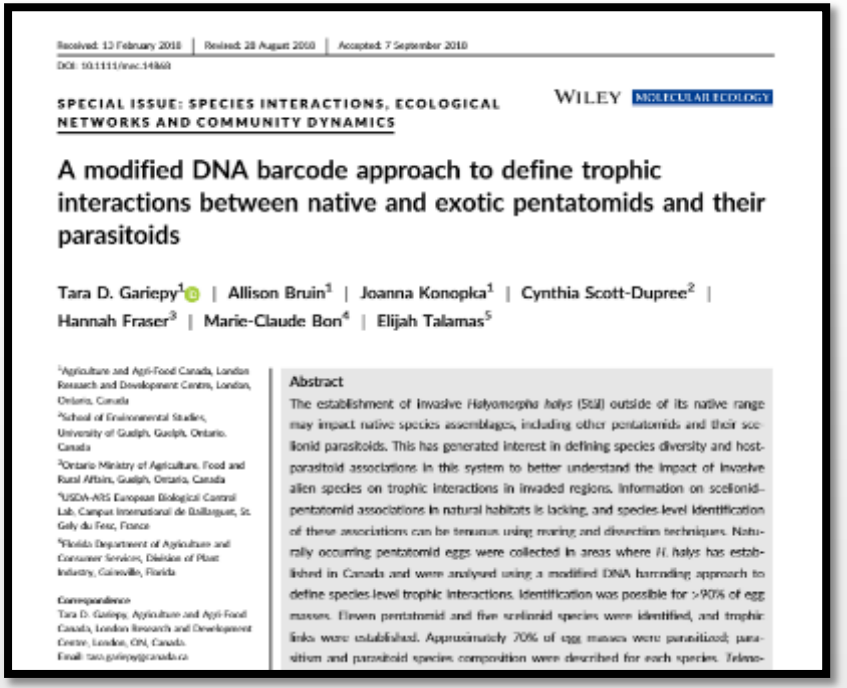
[2]Department of Biological Sciences, University of Montreal, Montreal, Quebec H1X 2B2, Canada; email: jacques.brodeur@umontreal.ca

[3]Instituto Valenciano de Investigaciones Agrarias, 46113 Valencia, Spain; email: aurbancja@ivia.es, atena@ivia.es

"These effects are widespread and can cause greater impacts on host populations than successful parasitism or host feeding."

Molecular Approach: DNA Barcoding

- Molecular assessments of parasitoid impact
  - DNA barcoding
  - Allows detection of nonreproductive parasitoid impact
  - Not practical without similar access to unpublished barcode data, lab facilities, and funding



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SPECIAL ISSUE: SPECIES INTERACTIONS, ECOLOGICAL NETWORKS AND COMMUNITY DYNAMICS

WILEY MOLECULAR ECOLOGY

A modified DNA barcode approach to define trophic interactions between native and exotic pentatomids and their parasitoids

Tara D. Gariepy[1] | Allison Bruin[1] | Joanna Konopka[1] | Cynthia Scott-Dupree[2] | Hannah Fraser[3] | Marie-Claude Bon[4] | Elijah Talamas[5]

[1]Agriculture and Agri-Food Canada, London Research and Development Centre, London, Ontario, Canada
[2]School of Environmental Studies, University of Guelph, Guelph, Ontario, Canada
[3]Ontario Ministry of Agriculture, Food and Rural Affairs, Guelph, Ontario, Canada
[4]USDA-ARS European Biological Control Lab, Campus International de Baillarguet, St. Gely du Fesc, France
[5]Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida

Correspondence
Tara D. Gariepy, Agriculture and Agri-Food Canada, London Research and Development Centre, London, ON, Canada.
Email: tara.gariepy@canada.ca

Abstract

The establishment of invasive *Halyomorpha halys* (Stål) outside of its native range may impact native species assemblages, including other pentatomids and their scelionid parasitoids. This has generated interest in defining species diversity and host-parasitoid associations in this system to better understand the impact of invasive alien species on trophic interactions in invaded regions. Information on scelionid-pentatomid associations in natural habitats is lacking, and species-level identification of these associations can be tenuous using rearing and dissection techniques. Naturally occurring pentatomid eggs were collected in areas where *H. halys* has established in Canada and were analysed using a modified DNA barcoding approach to define species-level trophic interactions. Identification was possible for >90% of egg masses. Eleven pentatomid and five scelionid species were identified, and trophic links were established. Approximately 70% of egg masses were parasitized; parasitism and parasitoid species composition were described for each species. Telenomus

Gariepy, T. D., A. Bruin, J. Konopka, C. Scott-Dupree, H. Fraser, M. C. Bon, and E. Talamas. 2019. A modified DNA barcode approach to define trophic interactions between native and exotic pentatomids and their parasitoids. Mol. Ecol. 28: 456-471.

Trissolcus japonicus Primer Development

- Development of *T. japonicus*-specific primer circumvents limitations of barcoding approach
  - *T. japonicus* is the dominant parasitoid at field site
  - Allows detection of *T. japonicus* DNA in pentatomid eggs from time of wasp oviposition through 6+ weeks post-emergence

Dr. Kacie Athey

Objective: Evaluate nontarget parasitism by the exotic egg parasitoid *Trissolcus japonicus* on BMSB and native pentatomids using morphological and molecular methods, with an emphasis on nonreproductive effects.

BMSB and Nontarget Stink Bug Species

https://www.odaguides.us/

- ***Chinavia hilaris***
- ***Euschistus conspersus***
- ***Podisus maculiventris***
- ***Halyomorpha halys***

https://www.odaguides.us/

Combining Morphological and Molecular Assessments of Parasitoid Impact

- Sentinel egg masses (SEMs) deployed at site in Vancouver, WA, with known population of wild *T. japonicus*
- Fresh egg masses mounted on cardstock and deployed on undersides of *Paulownia* tree leaves
  - Exposed for 3-4 days, then recovered and held for >6 weeks to allow wasp emergence
- BMSB egg masses and native egg masses deployed alternately (temporal separation to simulate natural presentation) from mid-July though late August 2019

After 6 weeks…

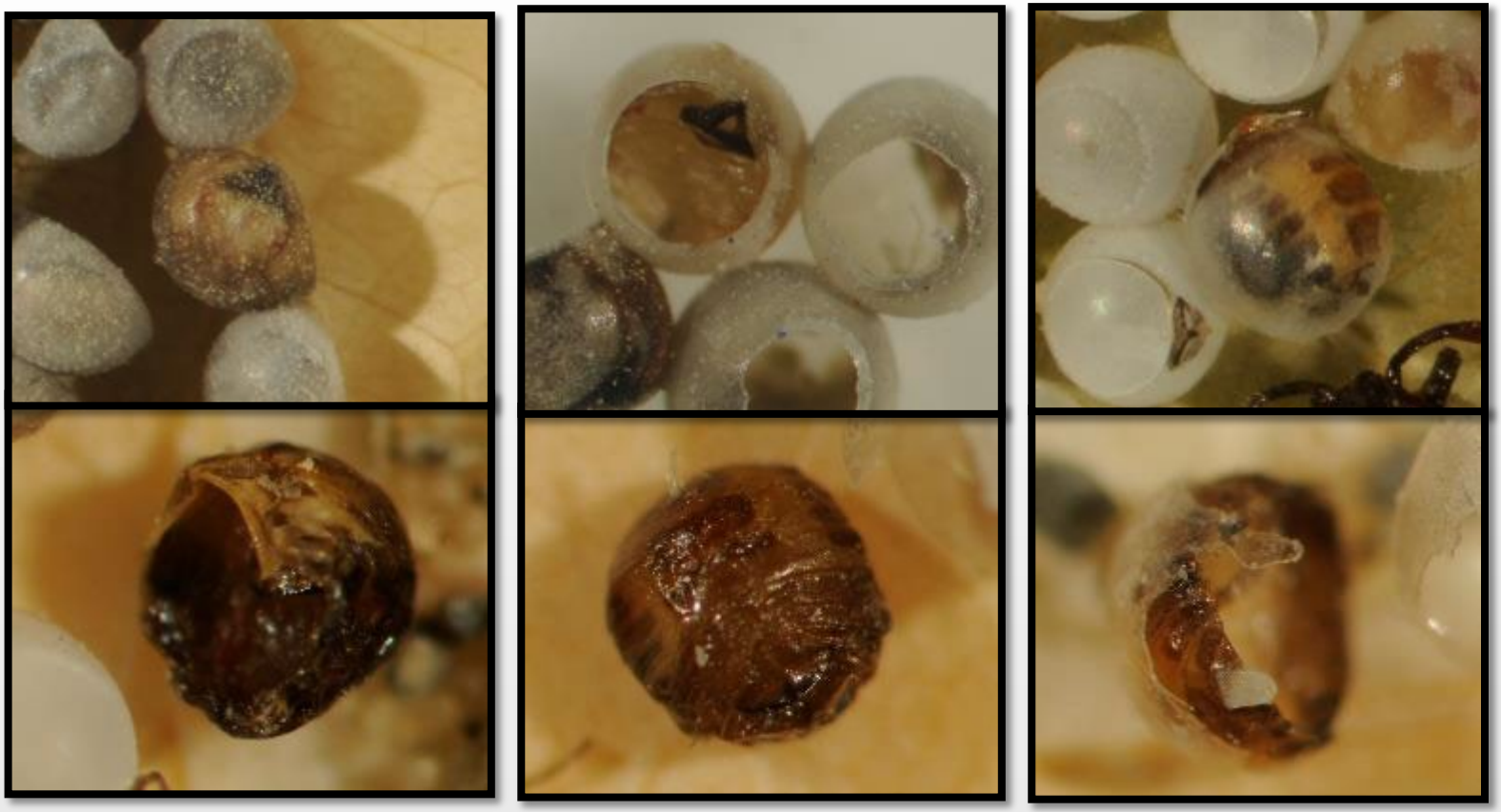
Egg masses photographed and eggs numbered for unambiguous ID

Eggs Dissected and Assigned a Fate Code

Simplified scheme modified from Morrison et al. (2016) and Waterworth (unpublished)

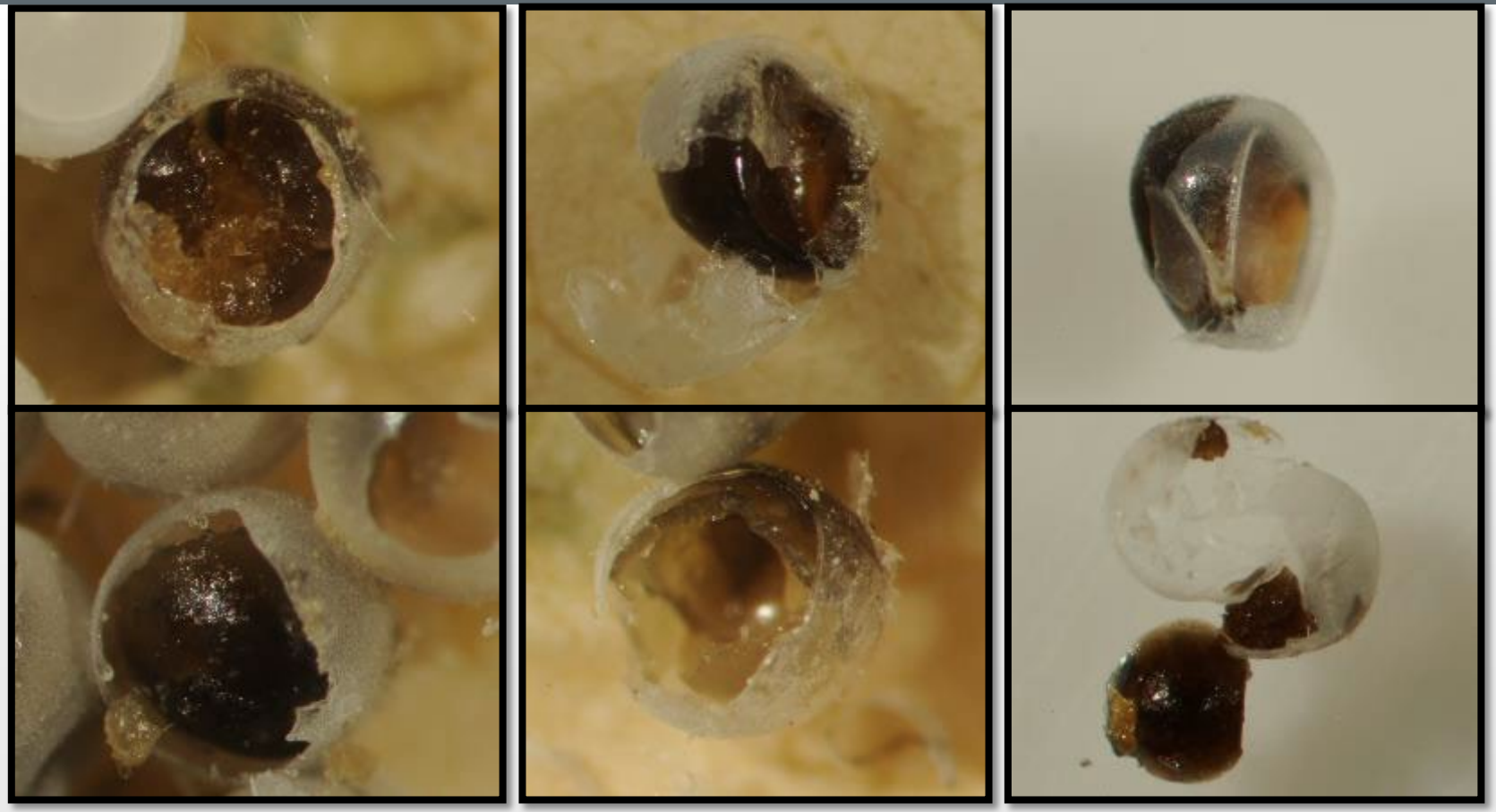
A	Stink bug nymph	Hatched/Emerged
B	Emerged parasitoid	Hatched/Emerged
C	Complete Chew	Predated
D	Incomplete Chew	Predated
E	Stylet Sheath	Predated
F	Punctured, but no stylet sheath (spider)	Predated
G	Host Feeding (Anastatus)	Predated
H	unemerged adult parasitoid	Unemerged/dissected
I	unemerged stink bug	Unemerged/dissected
J	no development, but not black	Unemerged/dissected
K	black goo	Unemerged/dissected

Egg Fate I – Unemerged Stink Bugs



Egg Fate J – No Development (not black)

Egg Fate K – ‘Black Goo’



PCR Diagnosis of Sentinel Eggs

- All eggs assigned to codes C – K subjected to PCR analysis, plus a subsample of codes A and B

A	Stink bug nymph	Hatched/Emerged
B	Emerged parasitoid	Hatched/Emerged
C	Complete Chew	Predated
D	Incomplete Chew	Predated
E	Stylet Sheath	Predated
F	Punctured, but no stylet sheath (spider)	Predated
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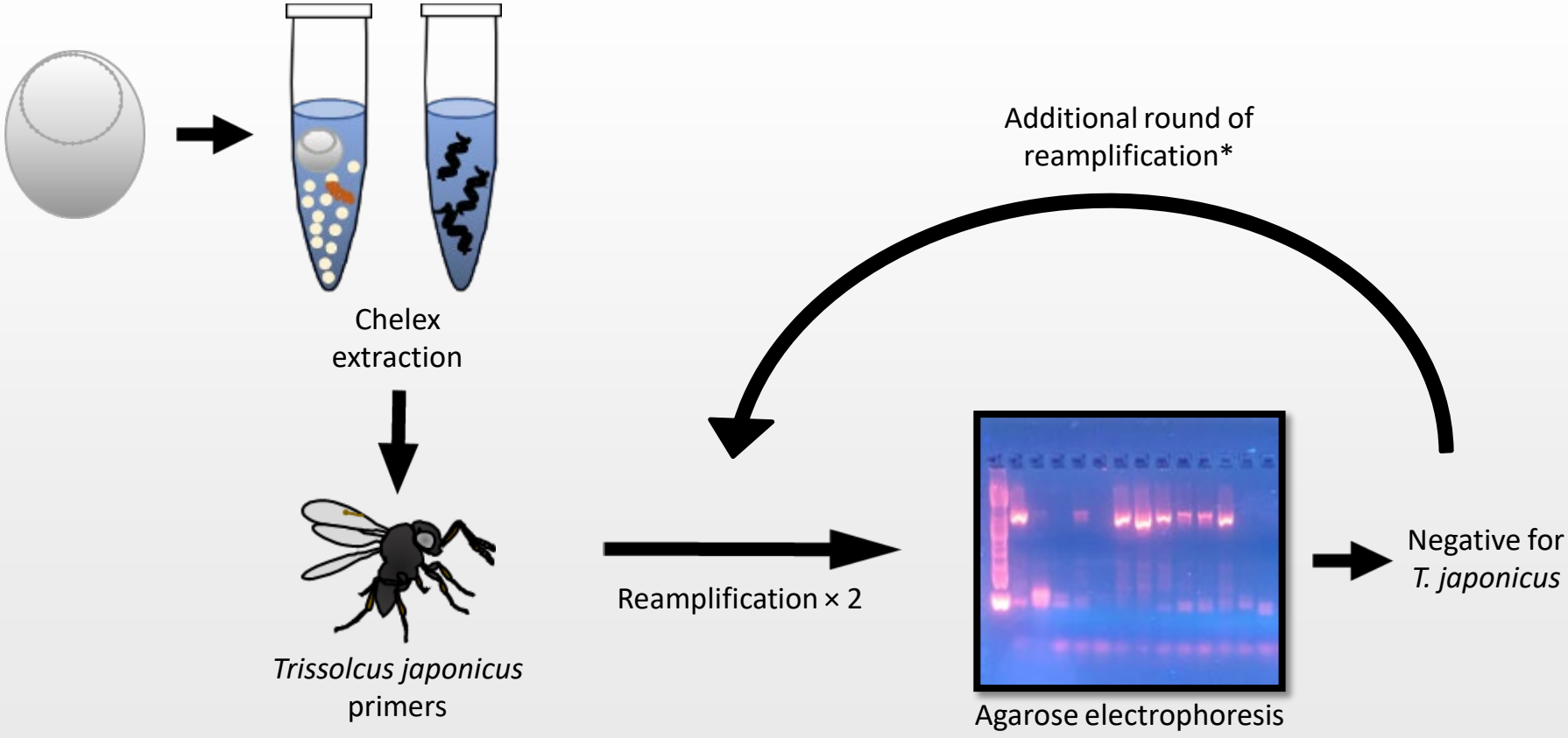
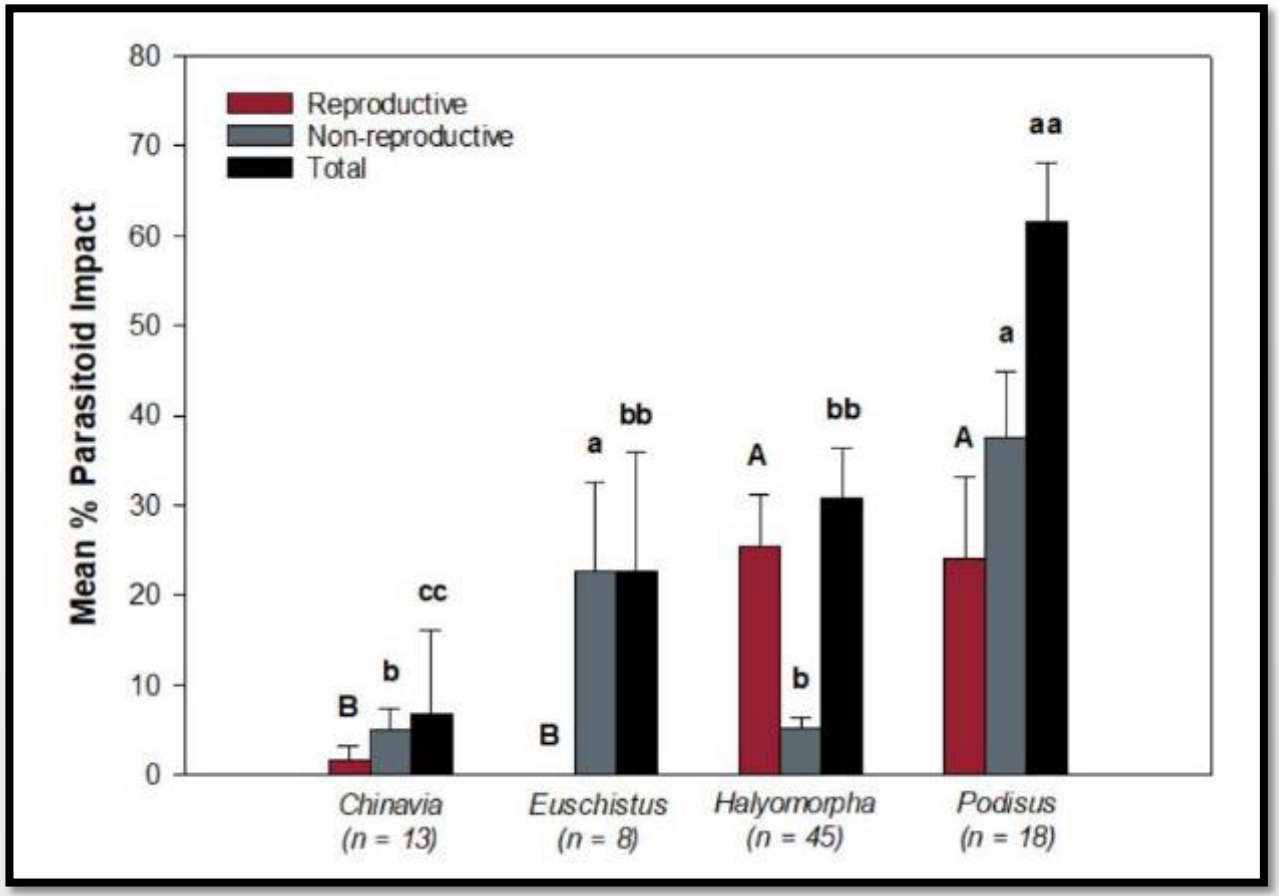
PCR Diagnosis Procedure



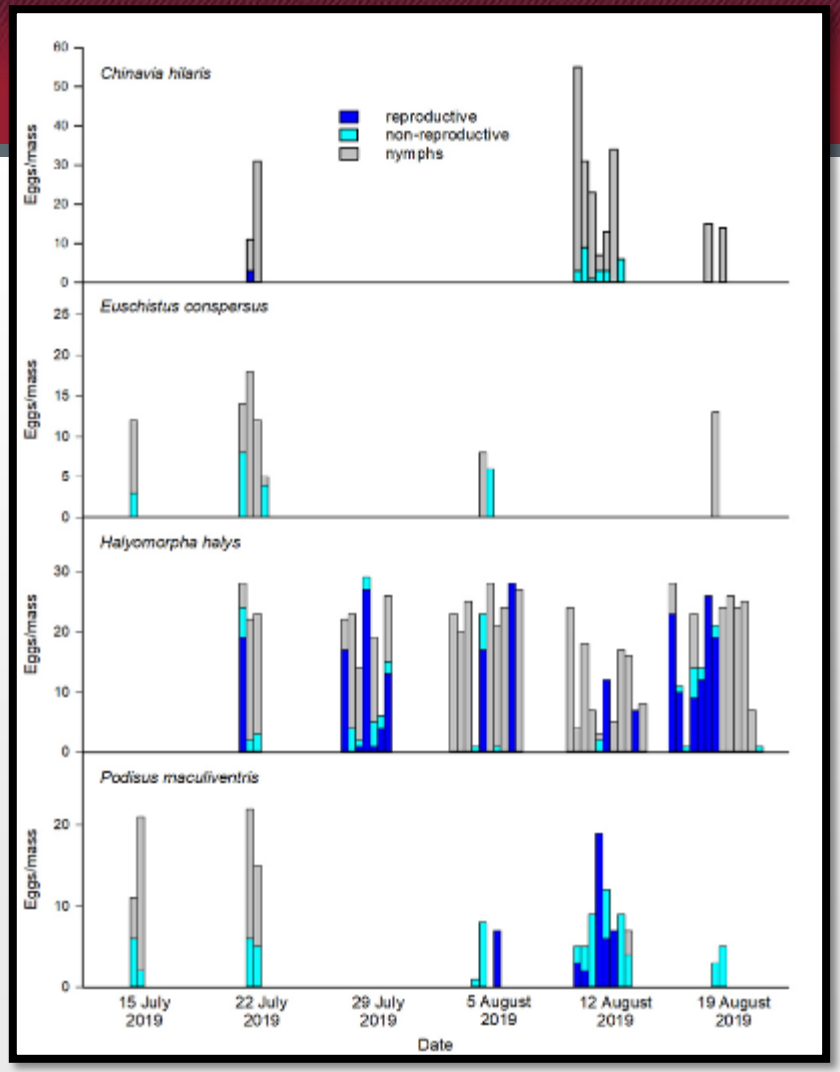
Data Analysis

- Data grouped into reproductive effects (emerged adult parasitoid) and nonreproductive effects (unemerged parasitoids and PCR-positive unemerged stink bugs, unhatched eggs with no development, unhatched eggs with black residue)
  - Assumes that *T. japonicus* activity caused the death of the egg when wasp DNA is present
  - *Reproductive effects and total parasitoid-induced mortality (='impact') analyzed as proportions with generalized analysis of variance model with binomial distribution and logit link.*

Reproductive, non-reproductive, and total impact of parasitism



*Reproductive, non-reproductive, and total impact analyzed separately



Occurrence of reproductive and nonreproductive effects during the study period. The colored portions in each bar represent the number of eggs in individual egg masses for each of four pentatomid species (*Chinavia hilaris*, *Euschistus conspersus*, *Halyomorpha halys*, and *Podisus maculiventris*) on a given date.

- Egg mass sizes variable
- No clear patterns in parasitoid activity vis a vis reproductive vs. non-reproductive effects
- Highlights constraints of colony egg production

Can we estimate non-reproductive effects from morphological characterization without the expense/trouble of PCR?

Trt	Fate	Fate Name	N eggs evaluated	N eggs PCR	N egg PC positiv	% PCR positive
Chinavia	I	Unemerg SB	246	243	1	4.94
Halyomorpha	I	Unemerg SB	88	84		7.14
Podisus	I	Unemerg SB	29	28	1	57.14
Chinavia	J	Unhatch not black	21	17		35.29
Euschistus	J	Unhatch not black	27	27	1	37.04
Halyomorpha	J	Unhatch not black	88	85	1	12.94
Podisus	J	Unhatch not black	66	57	4	75.44
Chinavia	K	Black goo	2	2		50.00
Euschistus	K	Black goo	17	17	1	88.24
Halyomorpha	K	Black goo	63	59	1	27.12
Podisus	K	Black goo	22	19	1	52.63

Conclusions

- The reproductive and non-reproductive impacts of *T. japonicus* parasitism activity varied substantially between stink bug species.
- *T. japonicus* readily developed and successfully emerged from *Podisus maculiventris* at rates comparable to in BMSB (24% vs. 25%).
- Nonreproductive impacts were substantial and were highest in the nontarget species *Euschistus conspersus* and *P. maculiventris* (23% and 36%, respectively); nonreproductive impacts must be considered when evaluating nontarget parasitism.
- Total parasitism impact was very high on *P. maculiventris*, causing an overall mortality rate of 61.6 % of deployed eggs vs. 30.7% of BMSB eggs.
  - Like other members of the Asopinae, *P. maculiventris* appears to be a highly suitable host for *T. japonicus.*

(Lara et al. 2019; Zhang et al. 2017; Haye et al. 2020; Charles et al. 2019; Botch and Delfosse 2018)

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Questions?

Jim Hepler
Ph.D., Entomology
Postdoctoral Researcher, Leskey Lab
USDA-ARS, Appalachian Fruit Research Station
2217 Wiltshire Rd
Kearneysville, WV 25430
Cell: 951-235-3017
Email: james.hepler@usda.gov