Quo Vadis Japonicus? Unfortunate non-target effects of parasitoids on native stink bugs

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Research

OXFORD

Trissolcus japonicus (Hymenoptera: Scelionidae) Causes Low Levels of Parasitism in Three North American Pentatomids Under Field Conditions

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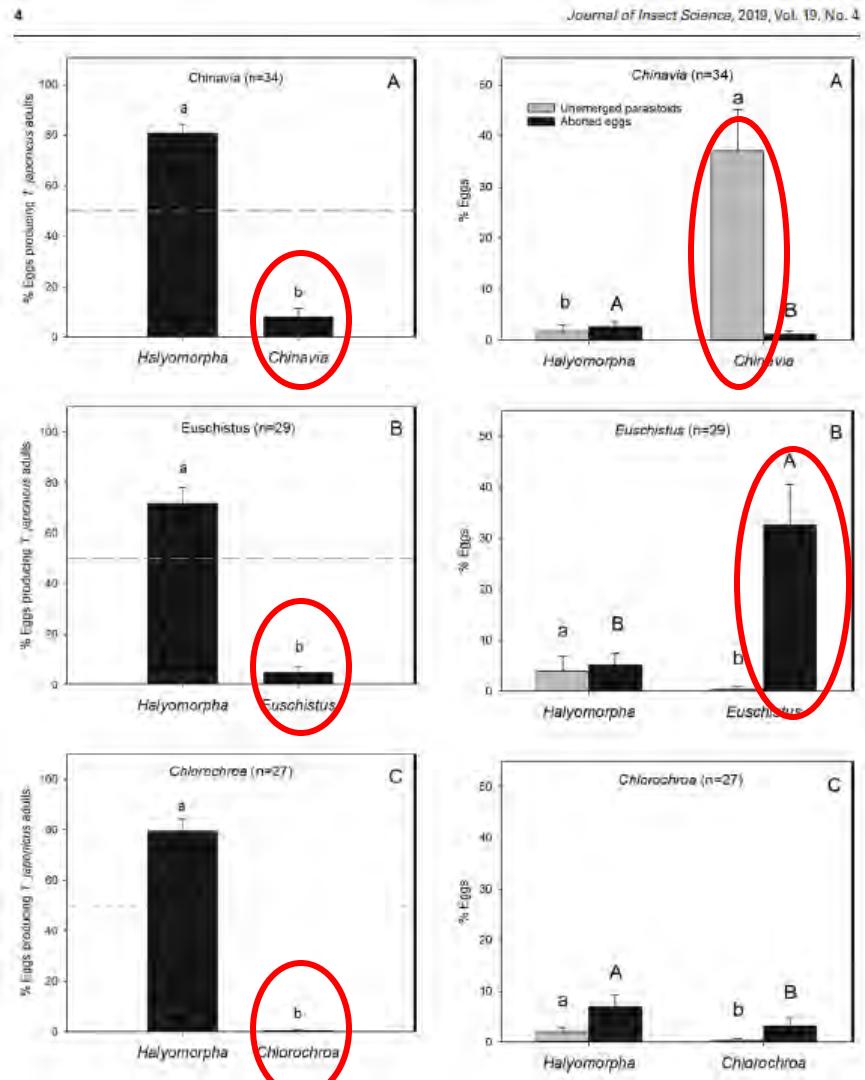
Abstract

Trissolcus japonicus (Ashmead), an Asian parasitoid of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), was first detected in North America in 2014. Although testing in quarantine facilities as a candidate for classical biological control is ongoing, adventive populations have appeared in multiple sites in the United States, Canada, and Europe. Extensive laboratory testing of *T. japonicus* against other North American pentatomids and *H. halys* has revealed a higher rate of parasitism of *H. halys*, but not complete host specificity. However, laboratory tests are necessarily artificial, in which many host finding and acceptance cues may be circumvented. We offered sentinel egg masses of three native pentatomid (Hemiptera: Pentatomidae) pest species (*Chinavia hilaris* (Say), *Euschistus conspersus* Uhler, and *Chlorochroa ligata* (Say)) in a field paired-host assay in an area with a well-established adventive population of *T. japonicus* near Vancouver, WA. Overall, 67% of the *H. halys* egg masses were parasitized by *T. japonicus* during the 2-yr study. Despite the 'worst case' scenario for a field test (close proximity of the paired egg masses), the rate of parasitism (% eggs producing adult wasps) on all three native species was significantly less (0.4–8%) than that on *H. halys* eggs (77%). The levels of successful parasitism of *T. japonicus* of the three species are

Milnes and Beers 2019

- Reproductive effects very clear
- Non-reproductive effects less clear

- Aborted eggs ~ “black goo”



Reproductive vs Non-reproductive 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]

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

Molecular Approach: DNA Barcoding

Gariepy, T., T. Haye, and J. Zhang. 2014. A molecular diagnostic tool for the preliminary assessment of host–parasitoid associations in biological control programmes for a new invasive pest. Mol. Ecol. 23: 3912-3924.

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.

- Unpublished CO1 barcode data
- Access to Barcode of Life
- Budget for sequencing

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DOI: 10.1111/mec.14868

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]

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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. *Teleno-*

Is the egg half empty, or half full?

- Scelionid primer approach expensive (all samples sequenced—Gariepy method)
- Most of our parasitoids likely to be *T. japonicus*
- Needed a species-level primer (first pass), then scelionid

Primer has been tested in a time series; can detect *T. japonicus* egg in a *H. halys* egg immediately after it is laid, plus all stages right up to and after adult eclosion.

- TJ-164F: 5'-TATTGTAACTTCACATGCATTTATTATAATC-3'
- TJ-395R: 5'-AAATTCCTGCTATATGTAGGGAAAAAATA-3'
- ~200 bp amplicon

Dr. Kacie Athey

Gariepy Lab

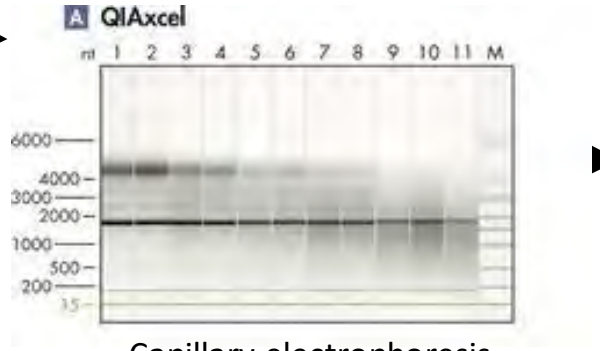
DNA Extraction

Chelex

PCR Primer

Scelionidae

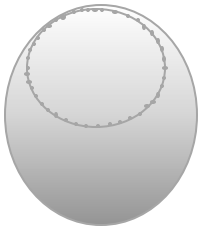
PCR Product Visualization

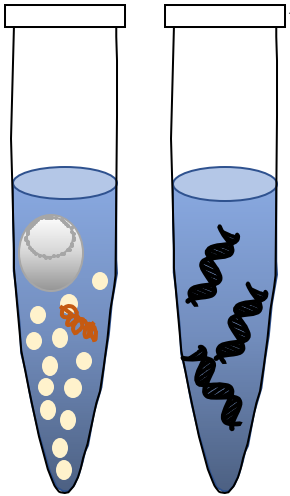


Capillary electrophoresis

Sequence Confirmation

BOLD SYSTEMS



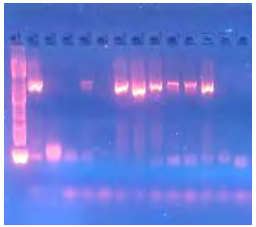


Beers Lab

Chelex

Trissolcus japonicus

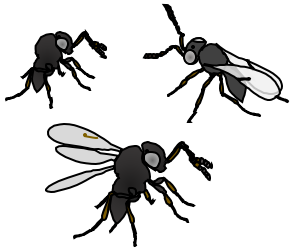
reamplification



Agarose electrophoresis

Negative for *T. japonicus*

Scelionidae



Graphic: Kacie Athey

Stink Bug Species for SEMs

https://www.odaguides.us/

https://www.odaguides.us/

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

Linking Egg Fate Dissections with PCR Diagnosis

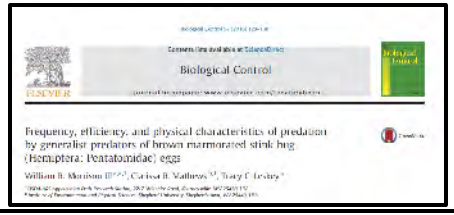
BMSB SCRI2 - Assessment of Stink Bug Egg Natural Enemies Year: 2018 **PI: Dr. Beers**

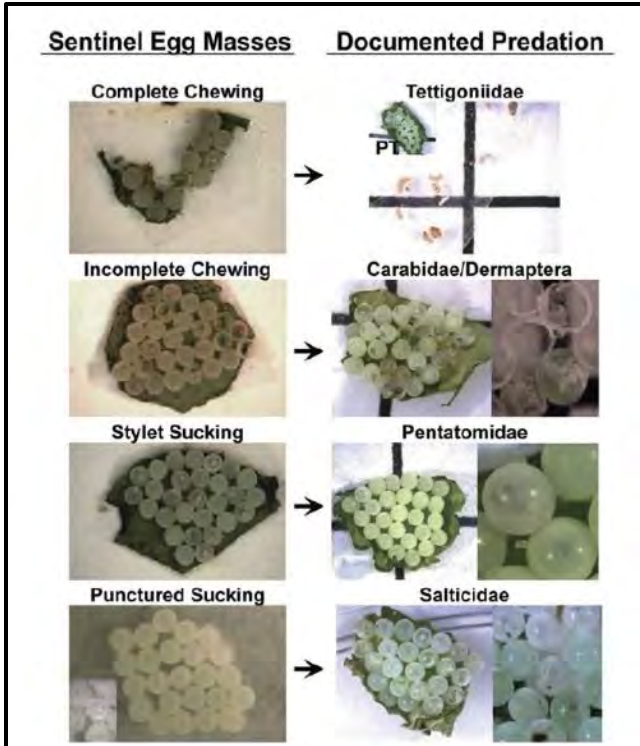
Data recorder name: Joshua Mines

Egg Mass #	Loc	Habitat	Plant species	SB sp	Egg Type	N latitude	W longitude	Date Out	Date In	ColFed #Eggs	Intact #Eggs	Assessments (7-21 days post deployment) sum = Intact Hatch	CP	ICP	SS	P, NP	HF	Par	Sunk	Un, W	Un, D	Mis	6 wk Dissection BG	UNE pa	Nym
1	Vancouve	riparian	vine maple	Halyomorpha h	H	45.610630	-122.602628	18-Jun-18	30-Jun-18	0	28	22	0	0	0	0	0	0	0	5	0	1	5	0	0
2	Vancouve	riparian	vine maple	Halyomorpha h	H	45.610630	-122.602628	18-Jun-18	30-Jun-18	0	25	13	0	0	0	0	0	0	2	8	0	2	8	0	0
3	Vancouve	riparian	vine maple	Halyomorpha h	H	45.610630	-122.602628	18-Jun-18	30-Jun-18	0	28	27	0	0	0	0	0	0	0	1	0	0	0	0	1
4	Vancouve	riparian	vine maple	Halyomorpha h	H	45.610630	-122.602628	18-Jun-18	30-Jun-18	0	28	26	0	0	0	0	0	0	0	0	2	0	0	0	2
5	Vancouve	riparian	vine maple	Halyomorpha h	H	45.610630	-122.602628	18-Jun-18	30-Jun-18	0	28	0	0	0	0	0	0	25	0	0	0	3	0	0	0
6	Vancouve	riparian	vine maple	Halyomorpha h	H	45.610630	-122.602628	18-Jun-18	30-Jun-18	0	28	25	0	0	0	0	0	0	1	2	0	0	2	0	0
7	Vancouve	riparian	vine maple	Halyomorpha h	H	45.610630	-122.602628	18-Jun-18	30-Jun-18	0	28	25	0	0	0	0	0	0	0	0	2	1	0	0	2
8	Walla Wa	managed urban	crab apple	Halyomorpha h	H	46.066280	-118.319683	20-Jun-18	24-Jun-18	0	28	21	0	4	0	0	0	0	0	3	0	0	0	0	3
9	Walla Wa	managed urban	crab apple	Halyomorpha h	H	46.066280	-118.319683	20-Jun-18	24-Jun-18	0	28	22	0	2	0	0	0	0	1	3	0	0	0	0	3
10	Walla Wa	managed urban	crab apple	Halyomorpha h	H	46.066280	-118.319683	20-Jun-18	24-Jun-18	0	28	27	0	0	0	0	0	0	0	1	0	0	1	0	0

Hatch = hatched eggs
CP = complete chew, full removal of individual eggs and accompanying substrate
ICP = incomplete chew, remnants composed of irregular pieces of egg shell often w/ an outline of original egg mass remaining
SS = stylet sheath present, sucking predation
P, NP = punctured egg, no stylet sheath present (ex. spider feeding)
HF = host feeding spot w/ tube from *Anastatus* females

Par = emerged parasitoid
Sunk = sunken egg
Un, W = unhatched egg, white or light color (nothing visible)
Un, D = unhatched egg, dark color
Mis = Missing eggs or egg mass (no visible evidence of chewing on any eggs or card (= substrate) as in **CP or ICP**)
(See Morrison et al. 2016 for descriptions of egg feeding syndromes) (hyperlink to paper)

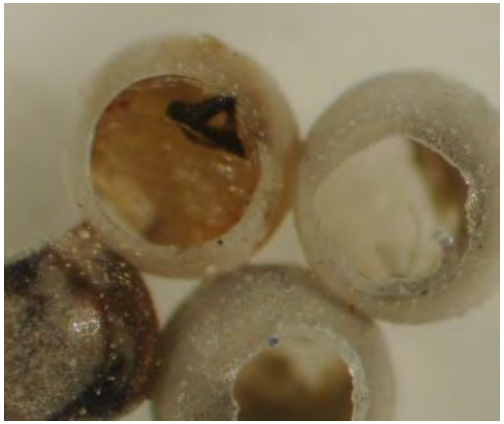


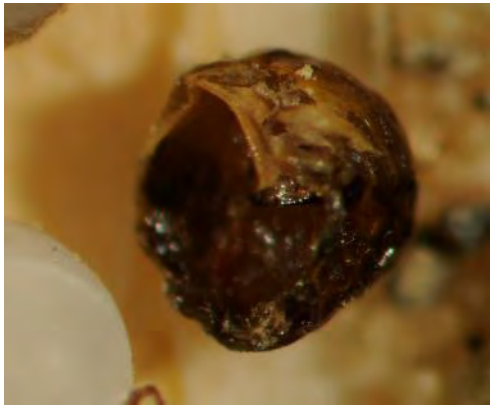
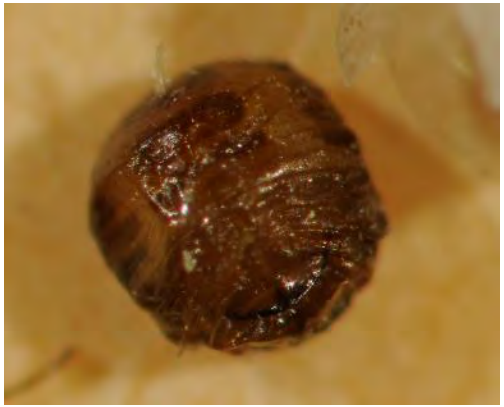
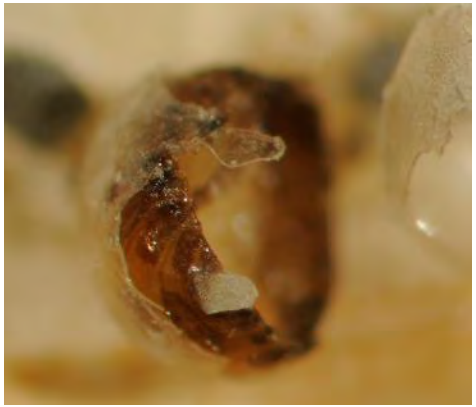


Simplified Scheme of Egg Fate Classification

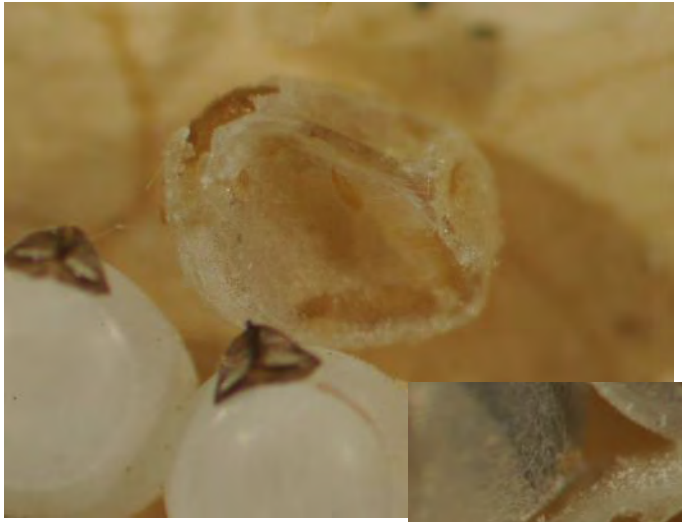
A	Stink bug nymph	Hatched/Emerged
B	Emerged parasitoid	Hatched/Emerged
C	Complete Chew	Predated
D	Incomplete Chew	Predated
E	Stylet Sheath	Predated
F	Punctured, but no stylet sheath (spider)	Predated
G	Host Feeding (Anastatus)	Predated
H	unemerged adult parasitoid	Unemerged/dissected
I	unemerged stink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

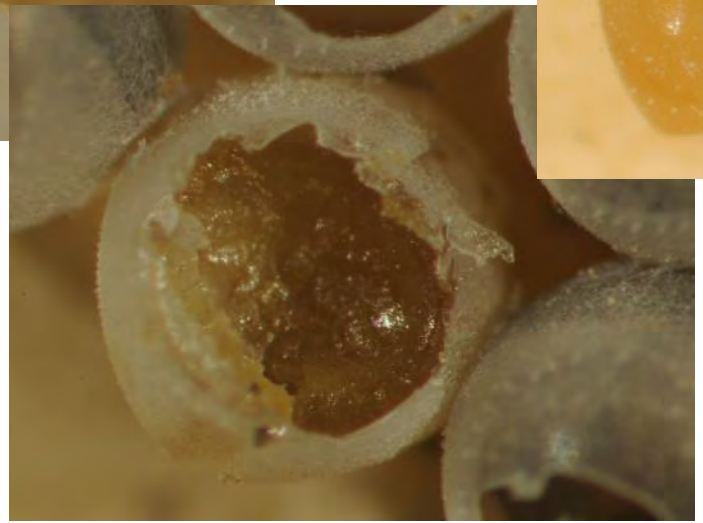
Egg Fate – Unemerged Stink Bugs

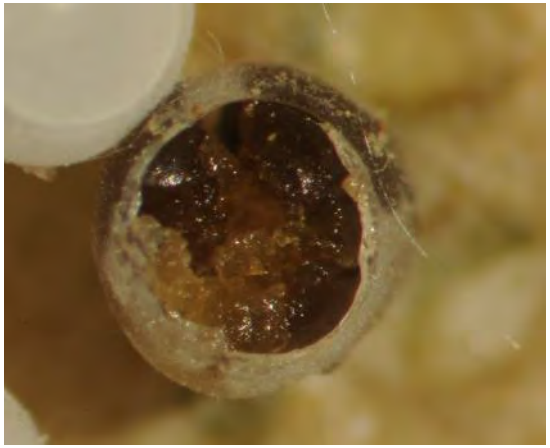




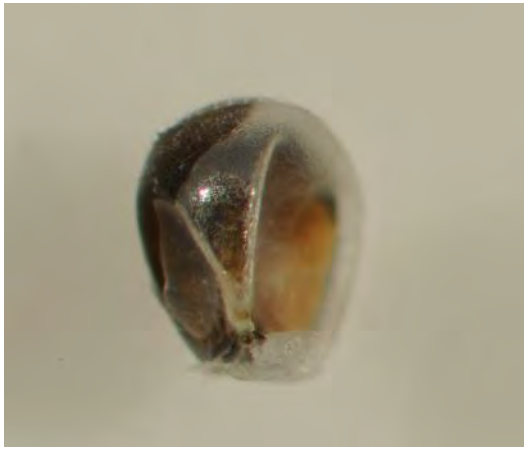
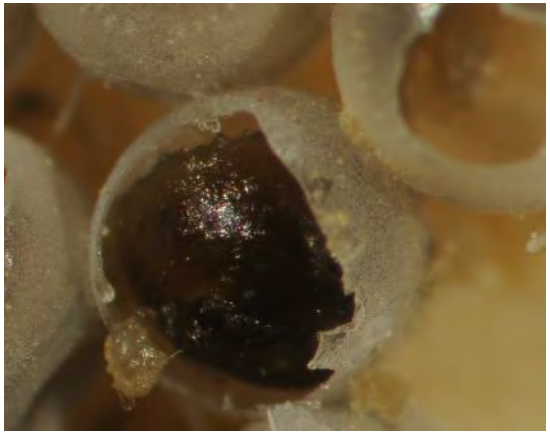
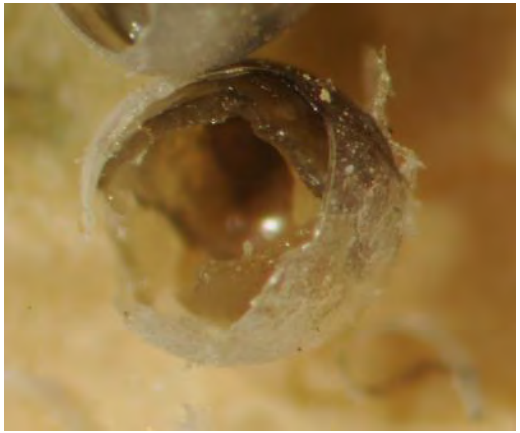
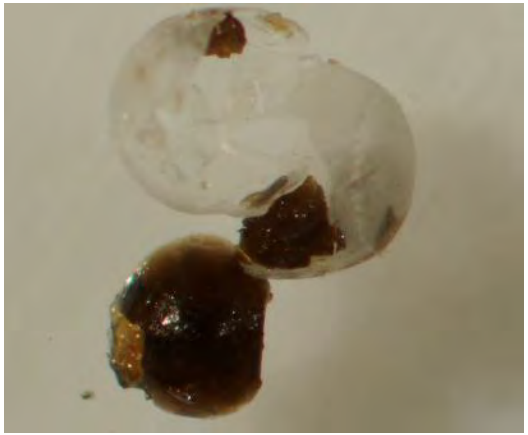
Egg Fate – No Development (not black)





Egg Fate – Black Goo

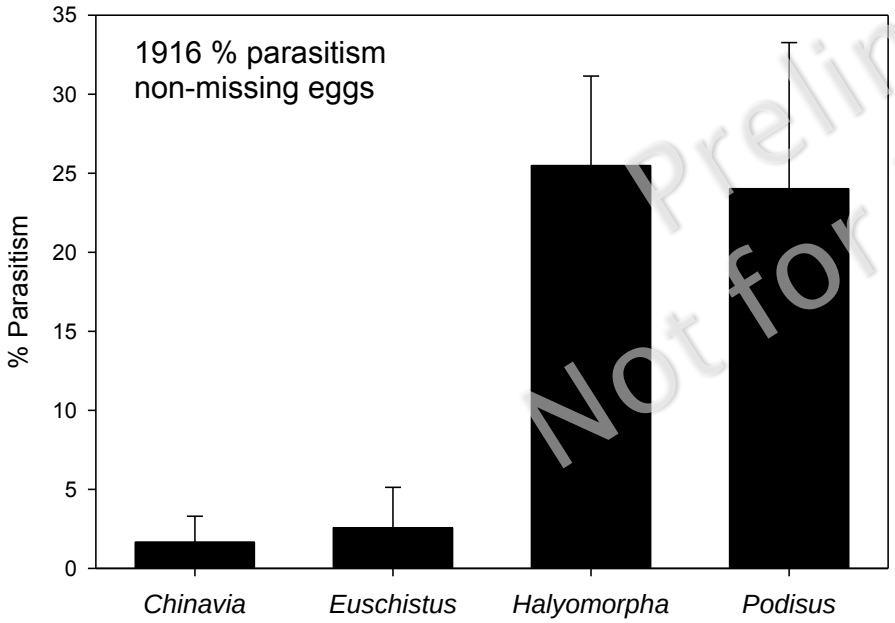


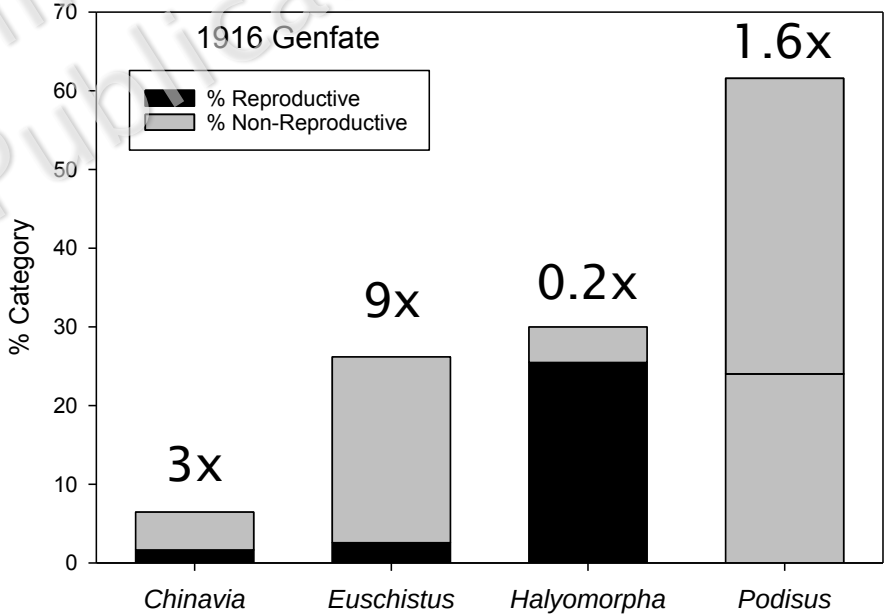
Eggs numbered for unambiguous ID

Reproductive vs Total Impact

Reproductive Impact



Total Impact



Podisus? Seriously?

Modified Table 1 from **Hedstrom et al. 2017**: No-choice tests with *T. japonicus* in the quarantine lab

Species	n masses	n parasitized	% parasitized	Adult parasitoids per egg mass
Chinavia hilaris	21	6	28.6%	5.6
Chlorochroa ligata	25	2	8.0%	0.8
Euschistus conspersus	27	0	0.0%	0
Podisus maculiventris	23	3	13.0%	8.5
Halyomopha halys	20	20	100.0%	56.7

Thoughts and Speculations

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 eggs PCR positive	% PCR positive
Chinavia	I	Unemerg SB	246	243	12	4.94
Halyomorpha	I	Unemerg SB	88	84	6	7.14
Podisus	I	Unemerg SB	29	28	16	57.14
Chinavia	J	Unhatch not black	21	17	6	35.29
Euschistus	J	Unhatch not black	27	27	10	37.04
Halyomorpha	J	Unhatch not black	88	85	11	12.94
Podisus	J	Unhatch not black	66	57	43	75.44
Chinavia	K	Black goo	2	2	1	50.00
Euschistus	K	Black goo	17	17	15	88.24
Halyomorpha	K	Black goo	63	59	16	27.12
Podisus	K	Black goo	22	19	10	52.63

Thoughts and Speculations

- Negative impact of *T. japonicus* highest in soybean systems where *C. hilaris* and *P. maculiventris* co-occur?

- Negative impact of *T. japonicus* less in tree crops, or where native SB oviposition period precedes peak egg abundance of BMSB?

Photo by J. Obermeyer

Acknowledgements

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Questions

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