Distribution and impact of *Trissolcus japonicus* and status of petition to release quarantined populations

Kim Hoelmer  
USDA-ARS  
Beneficial Insects Introduction Unit  
Newark, DE

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Fate of naturally laid BMSB eggs

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- Parasitized
- Predation - Chew
- Predation - Suck
- Nymph - unhatched
- Nymph - hatched
Trissolcus japonicus
“samurai wasp”
Trissolcus japonicus
(Hym.: Scelionidae)

- solitary egg parasitoid
- high % of eggs in mass attacked
- 2 - 3 weeks / generation
- multiple generations/season
- female-biased sex ratio
- 65 to 90% BMSB parasitism in Asia

Egg load over lifespan

% Survival

Time (d)

Female longevity

Time (d)

C. Dieckhoff/ARS
Summary – *T. japonicus* in Asia:

- *T. japonicus* is the dominant parasitoid species on BMSB throughout the season on different host plants.

- Other species (e.g., *Anastatus*, *Ooencyrtus*, tachinid flies) are of minor importance in limiting BMSB.

- Ecological host range of *T. japonicus* contains other species in these habitats, *e.g.* *Plautia* and *Dolycoris*.

- *T. japonicus* is an oligophagous species, thus non-target attacks is likely of other stink bugs, risk-benefit analysis needed for classical biocontrol.
Why is a Risk Assessment Needed?

NAPPO (and APHIS) Guidelines for Petitions for First Release of Arthropod Pest Biological Control Agents:

**General Requirements**
1. Proposed Action
2. Target Pest Information
3. Biological Control Agent Information
4. Host-Specificity Testing
5. Environmental and Economic Impacts of Proposed Release
6. Post-Release Monitoring
Distribution of adventive *Trissolcus japonicus* (as of December, 2017)
Parasitized egg masses recovered from sentinel egg canopy transects

2016:
- 135 egg masses deployed
- 4.4% (n = 6) of egg masses parasitized
- 2.2% (n = 3) of egg masses parasitized by *T. japonicus* (mid and upper canopy)

2017:
- 105 egg masses deployed
- 2.86% (n = 3) of egg masses parasitized
- 0.95% (n = 1) of egg masses parasitized by *T. japonicus* (upper-canopy)

<table>
<thead>
<tr>
<th>Canopy Location</th>
<th>Total # egg masses</th>
<th># Egg masses yielding <em>T. japonicus</em></th>
<th># Egg masses yielding other parasitoids</th>
<th># Egg masses previously parasitized</th>
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<tbody>
<tr>
<td>Upper</td>
<td>13</td>
<td>3</td>
<td>0</td>
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<td>Middle</td>
<td>28</td>
<td>7</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Lower</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>1</td>
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Numerically, but not significantly, greater levels of parasitism at mid-canopy (Fisher’s exact test, df = 6; p=0.27)
1. **Assembly:**
   Sticky traps attached to 4.8m poles

2. **Deployment:**
   At mid-canopy for 7 days

3. **Processing:**
   Parasitoids removed and identified
Egg mass fate

Unparasitized masses
86%

BIIRU Newark 2017
Sentinel Egg masses (placed on foliage)

Emerged Parasitoid Species Composition

- Trissolcus japonicus: 18%
- Anastatus sp. (males only): 20%
- Ooencyrtus sp.: 6%
- Trissolcus brochymenae: 5%
- Trissolcus euschisti: 6%
- Anastatus mirabalis: 4%
- Anastatus pearsalli: 7%
- Anastatus reduvii: 34%

N placed = 572
N parasitized = 80
(14.0% of total)
N with 2 or more parasitoid spp. emerged = 8
**COI** (barcode gene) insights:

- Genetic diversity of *T. japonicus* in Asia is structured in six major lineages
- Lineage 6 is the most widely distributed in Asia
- All U.S. adventive populations belong to lineage 6
- No significant variability among U.S. adventive populations

PCoA of 115 specimens recovered in U.S. & haplotyped
Principal coordinate analysis (PCoA) of haplotype diversity of 23 microsatellite markers in *T. japonicus* (through 2015)
Redistributions of *Trissolcus japonicus* within states

Over 60 egg clusters placed on 16 farms in 27 sites in 6 NY State counties in 2017 (Peter Jentsch)
APHIS policy on redistribution of *T. japonicus*

- APHIS regulates movement (including redistribution) *between* state lines but not *within* States.

- What is their policy about moving established adventive *T. japonicus* between states?
  - APHIS Permits Group has discussed permitting the movement of “feral” *T. japonicus* and made preliminary notes for a proposal to articulate what policy would be.
  - However, it was not finished or taken “up the line” for official approval.

- Further response from APHIS is pending…
Status of Petition for field release of *T. japonicus* (Beijing population)

- Non-target attack laboratory data required addition research to evaluate the effect of environment and parasitoid behavior on attack of non-targets

- Researchers in the U.S. and in Canada are proceeding with (hopefully) concurrent Petitions for Field Release
  - Time line: submission by this spring?
  - Concurrent approach may strengthen the case for approval
What is the impact of attack on non-target species?

- Host egg killed from stinging, but without oviposition, or partial (but unsuccessful) development of parasitoid

- Host egg killed with *occasional* full development and emergence of adult parasitoid

- Viable offspring, but mostly male

- Reproductive females produced

- *Exotic enemy* displaces a *native enemy*
T. japonicus Behavioral Assays
MSc thesis research of Sean Boyle, Univ. Delaware

Experimental Design

60 mm petri dish arena

Ethovision XT 8.0 (Noldus Technologies)
### Mean Residence Time

<table>
<thead>
<tr>
<th>Plant Substrate</th>
<th>Red maple</th>
<th>Soybean</th>
<th>Apple</th>
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<tbody>
<tr>
<td>H. halys</td>
<td>P &lt; 0.0001</td>
<td>A</td>
<td></td>
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<tr>
<td>Podisus</td>
<td>B</td>
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<td>Control</td>
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</table>

- **H. halys** with **Red maple** is significantly different from the others at P < 0.0001.
- **Podisus** with **Soybean** is significantly different from the others at P < 0.0009.
- **Control** with **Apple** is significantly different from the others at P < 0.0003.
Mean Linear Walking Velocity

<table>
<thead>
<tr>
<th>Plant Substrate</th>
<th>Red maple</th>
<th>Apple</th>
<th>Soybean</th>
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<tr>
<td>Kairomone</td>
<td>H. halys</td>
<td>Podisus</td>
<td>Control</td>
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<td>H. halys</td>
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<td>Podisus</td>
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<td>Control</td>
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* P < 0.0229

** P < 0.0229

P < 0.0229
Experimental Set-Up: No-choice tests

Kairomone contamination of *P. vulgaris* leaf surfaces with 2 gravid female stink bugs

Attach *H. halys* or *P. maculiventris* egg mass to contaminated plant

24 h exposure of *H*- or *P*-strain *T. japonicus* females (mated, naïve, 3-5 days old)
Searching in cage arena for egg mass

Single BMSB or Pmac Egg Mass exposed inside cage arena

Parent female *T. japonicus* reared from either BMSB or Pmac

*Percent of Egg Masses Attacked*

*BMSB* and *Pmac* egg masses attacked by *T. japonicus* reared from BMSB or Pmac host.
Fate of BMSB & Pmac eggs attacked by different *T. japonicus* parental strains

(T. japonicus parental host: exposed egg mass species)
Parasitoid Size
(using right hind tibia length as indicator)

- BMSB-host wasps possessed 30% longer HLT ($P < 0.0001$)
- BMSB-host wasps weighed over twice as much as Podisus-parent wasps ($P < 0.0001$)
- Strong positive correlation between $T. japonicus$ HTL length and weight ($R^2=0.957; P < 0.0001$)
Continuing research with *T. japonicus*

- What is the distribution of *T. japonicus*?
  - Continue deploying sticky traps farther afield
- Do Tj prefer to forage on some host plants compared with others?
  - Lab and semi-field assays
    - Host plant effects on % parasitism and attack rates
    - Response of Tj to host plant volatiles
    - Mark-release-recapture
- Where does Tj overwinter?
- Is Tj attacking non-targets?
Implications of adventive populations

- What will be impact of competition with indigenous parasitoids and predators? Will native natural enemies be affected negatively?

- How will they impact non-target stink bugs and other spp.?

- Several states already proceeding to redistribute populations within their boundaries.

- Given the adventive populations, should preparations be continued for a Petition to Release the Beijing quarantine population?
### Comparison of parent female wasps reared from BMSB vs. SSB (*P. maculiventris*)

#### Parasitized Egg Masses

<table>
<thead>
<tr>
<th>Parental host species</th>
<th>Exposed egg mass species</th>
<th>$n$ parasitized (&gt; 50% parasitism)</th>
<th>% suitable egg masses</th>
<th>% Emerged parasitoids</th>
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<tbody>
<tr>
<td><em>H. halys</em></td>
<td><em>H. halys</em></td>
<td>18 (17)</td>
<td>94.4</td>
<td>84.8 ± 16.4</td>
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<tr>
<td><em>H. halys</em></td>
<td><em>P. maculiventris</em></td>
<td>6 (4)</td>
<td>66.7</td>
<td>69.2 ± 20.2</td>
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<tr>
<td><em>P. maculiventris</em></td>
<td><em>H. halys</em></td>
<td>22 (20)</td>
<td>90.9</td>
<td>73.7 ± 18.6</td>
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<tr>
<td><em>P. maculiventris</em></td>
<td><em>P. maculiventris</em></td>
<td>8 (3)</td>
<td>37.5</td>
<td>44.1 ± 26.5</td>
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Fate of Control BMSB Eggs in Field (within mesh cages)

- Hatched nymphs: 84%
- Unhatched nymphs: 9%
- Undeveloped: 5%
- Prior predation (in lab colony)

N = 115 egg masses with 3,125 eggs total
22 egg masses (19%) had 100% hatch of nymphs

Fate of BMSB Egg Controls Kept in Laboratory

- Hatched nymphs: 84%
- Unhatched nymphs: 10%
- Undeveloped: 4%
- Prior predation (in lab colony)

N = 62 egg masses with 1,677 eggs total
13 egg masses (21%) had 100% hatch of nymphs
Comparison of parent female wasps reared from BMSB vs. SSB (*P. maculiventris*)

Fate of host eggs in No-choice tests

- Egg mortality
- Hatched nymphs
- Emerged T.j

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<td>Egg mortality (%)</td>
<td>22.9</td>
<td>27.5</td>
<td>53.1</td>
<td>63.2</td>
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<tr>
<td>Hatched nymphs (%)</td>
<td>16.3</td>
<td>7.5</td>
<td>28.6</td>
<td>22.4</td>
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<tr>
<td>Emerged T.j (%)</td>
<td>60.8</td>
<td>65.0</td>
<td>18.3</td>
<td>14.4</td>
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Regional surveys (ongoing) to document the occurrence & impact of natural enemies:

- Overall low levels of parasitism
- Impact varies according to habitat
- Predation is often more important than parasitism

Studies in conservation biological control to increase impact of native predators and parasitoids

- Border plantings, trap crops, insectary plants

Will native natural enemies adapt to BMSB over time?

- Why are native parasitoids poorly adapted to BMSB?
- Can adaptation be enhanced via laboratory selection?
F1 progeny from ≤ 24 hr. old Delaware & Beijing *T. japonicus* females, each given 16 BMSB egg masses successively (a new egg mass every 48 hours). Delaware *T. japonicus* had ~89% parasitism rate (~28 eggs per egg mass) for the first 8 days (4 egg masses) which then tapered off, while the Beijing *T. japonicus* did not exceed 38% parasitism rate over any 8 day period.

(preliminary data from Zach Schumm, UD)