

## *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae) emerges in North America

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### Abstract

*Trissolcus japonicus* (Ashmead) is an Asian egg parasitoid of the brown marmorated stink bug, *Halyomorpha halys* (Stål). It has been under study in U.S. quarantine facilities since 2007 to evaluate its efficacy as a candidate classical biological control agent and its host specificity with regard to the pentatomid fauna native to the United States. A survey of resident egg parasitoids conducted in 2014 with sentinel egg masses of *H. halys* revealed that *T. japonicus* was already present in the wild in Beltsville, MD. Seven parasitized egg masses were recovered, of which six yielded live *T. japonicus* adults. All of these were in a wooded habitat, whereas egg masses placed in nearby soybean fields and an abandoned apple orchard showed no *T. japonicus* parasitism. How *T. japonicus* came to that site is unknown and presumed accidental.

### Keywords

*Trissolcus japonicus*, *Halyomorpha halys*, *Trissolcus*, Scelionidae, biological control, egg parasitoid

## Introduction

The invasive brown marmorated stink bug, *Halyomorpha halys* Stål (Heteroptera: Pentatomidae), first identified in the U.S. in the Allentown area, Pennsylvania, in 2001 (Hoebeke and Carter 2003), has spread from the east to the west coast and has now been found in 41 states, with 13 states on both coasts reporting significant agricultural damage in addition to its nuisance status as an invader of buildings during the coldest months of the year (Northeastern IPM Center 2014a). The species is native to north-eastern Asia where it is widespread in China, Taiwan, South Korea, and Japan (Lee et al. 2013). In its native and introduced ranges *H. halys* feeds on a wide variety of economically important fruit crops (Lee et al. 2013), and not surprisingly it has become a significant agricultural pest in the U.S. (Leskey et al. 2012). Over 120 different host plants in numerous families have been recorded in Asia and North America (Lee et al. 2013, Northeastern IPM Center 2014b). In Asia, *H. halys* feeds on many tree fruit species, soybeans, and numerous woody tree and shrub hosts (Lee et al. 2013), although its importance as a pest tends to be sporadic and regionally localized. In the U.S., however, chemical control strategies have not prevented it from causing economic damage in fruit and vegetable crops (Rice et al 2014).

Several species of *Trissolcus* (Hymenoptera: Scelionidae) are reported to attack eggs of *H. halys* in Asia (Arakawa and Namura 2002, Yang et al. 2009), with high rates of parasitism noted (Yang et al. 2009). Beginning in 2007, collections of *Trissolcus* by the USDA/ARS Beneficial Insect Introduction Research Unit (ARS/BIIRU) from China, South Korea and Japan were brought to quarantine laboratories in the U.S. for evaluation as potential biological control agents of *H. halys* in North America. Taxonomic studies and genetic characterization of these collections determined that two species, *T. japonicus* (Ashmead) and *T. cultratus* (Mayr) predominated in the Asian collections obtained from *H. halys* (authors' unpublished data).

## Materials and methods

A sentinel egg mass study to assess indigenous egg parasitoids of *H. halys* was conducted in three field habitats during summer 2014 on the Beltsville Agricultural Research Station (BARC) North Farm in Beltsville, MD. All *H. halys* eggs used in this experiment came from a colony established at the USDA/ARS Invasive Insect Biocontrol and Behavior Laboratory (ARS/IIBBL) in 2011 with *H. halys* adults collected in Beltsville, MD. Insects in this colony are reared in growth chambers at 25 °C, 40–60% humidity and a 16L:8D light cycle. They are provided with certified organic green beans, hulled sunflower seed, whole buckwheat seed, and water *ad libitum*. Sentinel egg mass treatments, fresh and frozen in identical numbers, were deployed weekly in three different habitat types, as follows.

## Sentinel egg mass treatments

“Fresh” egg masses ( $\leq 24$ -hours-old) were laid by colony insects on paper towels. “Frozen” egg masses were identical to “fresh” but were then immediately frozen at  $-80^{\circ}\text{C}$  for 2 minutes. Frozen eggs were included to assess parasitism by species that would oviposit in *H. halys* but be killed by defenses of living fresh eggs (personal communication, Tim Haye, CABI Bioscience, Delémont, Switzerland). Both types of egg masses were then pinned, with the underlying piece of paper towel, to the underside of leaves of various vegetation via a sewing pin, at each of the three sites.

## Habitat characteristics

To gain insight into the effect of habitat on parasitoids of *H. halys*, we stationed each type of egg mass in each of three contrasting habitats: soybean field, apple orchard, and second-growth woods, on the BARC North Farm, Beltsville, MD.

**Soybean field:** Two adjacent soybean (*Glycine max* (L.) Merr.) fields were used, the first one a 2.2 ha certified organic field ( $39^{\circ}01'47''\text{N}$ ,  $76^{\circ}55'41''\text{W}$ ) with abundant pigweed (*Amaranthus* sp.) used before 5 August 2014, and the second a 1.1 ha conventional soybean field ( $39^{\circ}02'01''\text{N}$ ,  $76^{\circ}55'39''\text{W}$ ) used thereafter.

**Orchard:** The orchard site ( $39^{\circ}01'28''\text{N}$ ,  $76^{\circ}56'22''\text{W}$ ) was an abandoned apple (*Malus domestica* Borkh.) orchard, 40 m by 43 m, with apple trees ~6 m tall, row spacing of 5 m, alleys mowed  $\sim 2\times$  per year, with native and non-native grasses, and additional woody vegetation growing in with the apple trees, primarily Japanese honeysuckle (*Lonicera japonica* Thunb.), bush honeysuckle (*Lonicera* sp.), feral Callery pear (*Pyrus calleryana* Decne.), blackberry (*Rubus* sp.), and multiflora rose (*Rosa multiflora* Thunb. ex Murr.). Overall canopy cover was  $<50\%$ . To the east and north were mowed hayfields, to the south, soybeans, and to the west, a gravel road bounded by a windbreak of arborvitae (*Thuja* sp.) and bush honeysuckle (*Lonicera* sp.).

**Woods:** The woods site was an open second-growth woods adjacent (within 20m) to the west bank of the channelized Little Paint Branch creek ( $39^{\circ}01'42''\text{N}$ ,  $76^{\circ}55'47''\text{W}$ ). Vegetation was native and nonnative, planted and invasive, with over 20 woody species within 10 m, dominated by basswood (*Tilia americana* L.), American holly (*Ilex opaca* Aiton), red maple (*Acer rubrum* L.), arborvitae (*Thuja* sp.), sycamore (*Platanus occidentalis* L.), mulberry (*Morus rubra* L.), Norway maple (*Acer platanoides* L.) tree of heaven (*Ailanthus altissima* (Mill.) Swingle), black cherry (*Prunus serotina* Ehrh.), feral Callery pear, Japanese honeysuckle (*Lonicera japonica*), bush honeysuckle (*Lonicera* sp.), grape (*Vitis* sp.), poison ivy (*Toxicodendron radicans* (L.) Kuntze), and dewberry (*Rubus hispidus* L.), with an herb layer including grasses, mugwort (*Artemisia vulgaris* L.) and yellow rocket (*Barbarea vulgaris* R.Br.).

## Egg deployment, collection, and evaluation

From 18 July through 15 September 2014, weekly, egg masses were exposed in their respective habitats for 72 hours, at which time they were returned to the laboratory and held to determine survival and rates of parasitism. Field exposures comprised a total of 39 egg masses per treatment (fresh or frozen) per habitat, for a total of 234 egg masses with 5864 individual *H. halys* eggs. All field-exposed egg masses were reared in plastic zip top bags in a growth chamber set at 25 °C, 40–60% humidity and a 16L:8D light cycle, and checked daily for emergence of *H. halys* or parasitoids. Any parasitoids that emerged were placed in 95% ethanol for identification. Parasitoid adults that were unable to emerge from the host eggs were extracted for identification. If this dissection was required, the egg mass was placed in a Petri dish, covered with gel hand sanitizer (62% ethanol, HDX<sup>®</sup>, Atlanta, GA) to prevent live wasps from flying or the eggs from bouncing away during dissection. Dissected wasps were then placed in 95% ethanol to await identification.

Fresh sentinel egg masses were placed in two other nearby locations (personal communications, M. Cornelius and M. Greenstone, ARS/IIBBL). One involved vegetable fields (<500 m from both our woods and soybean sites; summer squash and bell pepper, and additionally, tomato in 2014) during 4 June through 27 September, 2013, and 10 June through 26 September, 2014, with a total of 124 and 263 egg masses in these years respectively. A second site was sampled with 183 fresh sentinel egg masses from 4 June to 12 September, 2014, in experimental plots of ornamental trees and shrubs at the U.S. National Arboretum (located in Washington, DC, ca. 13 km SSW of the BARC recovery site). The Arboretum plots had a similar canopy structure to the BARC orchard plots. Deployment for the vegetable and ornamental habitats was for 72 hours and the egg masses were sourced from the ARS/IIBBL rearing as above.

## Statistical analysis

Differences for parasitism between egg treatments (fresh and frozen) and among the three habitats were assessed at the egg mass level (since this presumably reflects the choices of individual ovipositing females) using Fisher's exact probability tests for frequency (Lowry 2015).

## Species Identification

Species of *Trissolcus* were determined using the characters and identification key of Talamas et al. (2015). Images of *T. japonicus* collected in Beltsville, MD, are presented in Figures 1–5. To confirm morphological identifications, genomic DNA was extracted from the legs of 4 females, and one whole female specimen, using techniques summarized in Buffington et al. (2012). The 'barcode' region of cytochrome oxidase I (*COI*) and the internal transcribed spacer region 2 (*ITS2*) regions were amplified and



**Figure 1.** *Trissolcus japonicus*, female (USNMENT01059357), specimen preserved during emergence from BMSB egg. Scale bar in millimeters.<sup>1</sup>

sequenced in the Scheffer Laboratory (USDA/ARS, Systematic Entomology Laboratory) and compared with sequences obtained from populations of previously identified Asian *Trissolcus* from Asian field surveys (Bon et al., unpublished). Voucher specimens for all molecular data are deposited in the National Insect Collection of the National Museum of Natural History, Smithsonian Institution, Washington, DC.

## Photography

Images were captured using a Z16 Leica<sup>®</sup>™ lens with a JVC KY-F75U digital camera and Cartograph<sup>™</sup> software. Montage images were produced from image stacks with the program CombineZP<sup>™</sup>. Full resolution images are archived at the image database at The Ohio State University (<http://purl.oclc.org/NET/hymenoptera/specimage>) and MorphBank (<http://www.morphbank.net>).

## Results

The specimens of *T. japonicus* collected in Beltsville fall well within the concept of this species developed by EJT and MLB in their ongoing revision of *Trissolcus* from the





**Figures 2–5.** *Trissolcus japonicus*, female (USNMENT01059359) **2** lateral habitus **3** dorsal habitus **4** head, anterior view **5** head, anterodorsal view. Scale bars in millimeters.<sup>2</sup>

Palaearctic. They exhibit no morphological anomalies that would complicate confirmation of species identity based on morphology alone. The *COI* and *ITS2* sequences were identical among specimens sampled from the US population, and to sequences generated from *T. japonicus* populations from China, Japan, and South Korea. A complete molecular analysis of *Trissolcus* species is beyond the scope of this note, and will be published in a forthcoming paper (Bon et al., in prep.)

Of the 234 egg masses and 5864 total *H. halys* eggs deployed, 47 egg masses and 994 eggs (17.0%) were parasitized, and 35 egg masses yielded 756 fully-developed adults, but only 389 of these adults (51.5%) successfully emerged from the host eggs, for a total of 6.6% successful parasitism from all eggs exposed. *Trissolcus japonicus* parasitized a total of 7 egg masses, 6 successfully, yielding a total of 159 adults, 131 (82.4%) of which emerged successfully from host eggs, with an approximate 19:1 female:male ratio. *Trissolcus japonicus* parasitized 2 egg masses on 28 July (both fresh), 3 on 2 September (1 fresh and 2 frozen), and 2 on 9 September (both fresh). The other parasitoids recovered consisted of 3 scelionids native to North America, *Telenomus podisi* Ashmead, *Trissolcus euschisti* (Ashmead) and *Trissolcus brochymenae* Ashmead, and 1 species of *Anastatus* Motschulsky (Eupelmidae).

*Trissolcus japonicus* was found only in host eggs deployed in the woods habitat, and this pattern of occurrence was a significant departure from the null hypothesis of equal likelihood for parasitism in the three habitats (Fisher's exact test, Freeman-

Halton extension (2×3), parasitized versus unparasitized by *T. japonicus* in three habitats:  $P_A=0.00114$ ; total  $n=234$ ). Two of the 7 parasitized egg masses were in the frozen treatment (total of 43 adults, of which 39 emerged alive), and the remaining 5 from fresh egg masses (total of 116 adults, of which 92 emerged alive); this represents no significant difference between fresh versus frozen egg masses for *T. japonicus* (Fisher's exact test  $P_{1-tail}=0.215$ , NS (2×2 fresh versus frozen and parasitized versus unparasitized by *T. japonicus*, total  $n=78$ , woods habitat only)). The native parasitoids, as a group, also exhibited a significant habitat preference, with 14 of 29 parasitized egg masses occurring in the woods habitat (Fisher's exact test, Freeman-Halton extension (2×3), parasitized versus unparasitized by all native species in three habitats:  $P_A=0.035$ ; total  $n=234$ ). Natives were far more likely to develop to adulthood in frozen egg masses (26 egg masses with total 425 adults, of which 253 emerged alive), compared to the fresh hosts (only 3 egg masses with a total of 5 adults, all of which emerged alive); this frozen/fresh difference was highly significant (Fisher's exact test  $P_{1-tail}=0.0000022$ , (2×2 fresh versus frozen and parasitized versus unparasitized by all native species, total  $n=234$ , all habitats)).

*Trissolcus japonicus* made up 159 (27.0%) of the 589 parasitoid adults in all habitats, and 33.7% of emerged live adult parasitoids in all habitats. Within the woods habitat, the 159 *T. japonicus* were 41.4% of parasitoid adults recovered, and 42.5% of emerged live parasitoids. Among fresh egg masses only, a more realistic treatment for field performance than the frozen treatment, 5 of the 8 parasitized egg masses in the woods habitat were parasitized by *T. japonicus*, and 4 of them yielded a total of 92 live adults (96.8% of live parasitoids for fresh eggs in the woods habitat), as compared to only 5 live adults emerged for native parasitoids.

Sentinel fresh egg masses on nearby vegetables, and at the Arboretum site in Washington, DC, yielded 98 and 38 parasitized egg masses, respectively. Egg parasitoid recoveries (including females that were guarding egg masses) from the vegetable and Arboretum sites described above, and surveys in seven adjacent states collected during 2013 and 2014, were identified at ARS/BIIRU in Newark, DE, with no evidence of the presence of *T. japonicus*.

## Discussion

Although we do not know how *T. japonicus* arrived in Maryland (populations imported for laboratory evaluation have not yet been released from quarantine facilities pending completion of studies and issuance of permits), we may speculate that parasitized egg masses of *H. halys* could easily be transported long distances by air cargo (two busy international airports and a major maritime port are located within the Baltimore-Washington area) on the foliage of many different species of plants shipped from Asia to the U.S. Furthermore, *T. japonicus* is recorded from several other Asian pentatomids with wide host plant ranges, and those species and their host plants could also have been the source. It is not known how adult *T. japonicus* overwinter, but they

are capable of living for several months and it is conceivable that they survive host-free periods under bark or in soil litter, and could have arrived in this manner.

Should the occurrence of *T. japonicus* at a single site in Maryland be regarded as an established population? It is probably premature for this conclusion, as the identified samples were obtained only during a single field season in 2014, and numerous additional samples from other nearby and more distant sites have not yet evidenced any *T. japonicus*.

Surveys in subsequent years at this site and at progressively more distant locations will demonstrate whether *T. japonicus* has successfully overwintered in temperate North America and begun to disperse over a wider range. Of particular interest will be the habitats that are colonized if establishment occurs. Among the habitats selected for sentinel deployment at Beltsville, occurrence of *T. japonicus* was limited to the woods, where it was by far the most successful parasitoid in terms of emergence of live adults from fresh *H. halys* eggs. Does the initial discovery only in host eggs in wooded areas but not in nearby soybeans, vegetables, and an apple orchard, indicate a habitat preference? Again, it is probably too soon to be certain given the limited time frame and lack of knowledge regarding how *T. japonicus* arrived.

The host range of classical biological control candidates must first be evaluated and a high degree of host specificity (narrow host range) demonstrated in order to obtain regulatory permission for field release in the U.S. Release permits are unlikely to be obtained if there is significant risk of non-target impact. Information on physiological host range (ability to successfully develop in a host) is ideally accompanied by knowledge of ecological (realized) host range and parasitoid behavior, because maximum-challenge laboratory conditions often overestimate the likely impact and host range in the field (e.g., Haye et al. 2005, Jenner et al. 2014). Given that hundreds of pentatomid species occur in the U.S., some of which are predatory species, extensive host range testing of Asian *Trissolcus*, including *T. japonicus*, as candidate agents for *H. halys* has been in progress but is not yet completed.

Many species and/or geographic populations of non-target Pentatomoidea have been examined using no-choice (only non-target species offered) and choice (*H. halys* and non-target offered simultaneously) tests in quarantine facilities. Thus far, *T. japonicus* (Beijing, China, population) has shown an inability to attack or develop in some species tested, whereas other non-target species were attacked to varying degrees (authors' unpublished data, CD and KH). Results from both no-choice and choice tests have shown that *H. halys* is a preferred or superior host based on higher parasitism rates. Further laboratory studies are in progress to examine the influence of environment (e.g., host habitat) and parasitoid behavior on host selection.

It is possible that the Beltsville population of *T. japonicus* differs from populations under study in quarantine in some characteristics that influence their impact on *H. halys* and on other hosts. The introduction of *T. japonicus*, if it proves to have established and spreads beyond the Maryland site, will provide an opportunity to compare laboratory evaluation studies of host selection and specificity with performance in the field.



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## Endnotes

- 1 [www.morphbank.net/?id=852700](http://www.morphbank.net/?id=852700)
- 2 [www.morphbank.net/?id=852708](http://www.morphbank.net/?id=852708)