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First report of *Trissolcus japonicus* parasitizing *Halyomorpha halys* in North American agriculture

Joe M. Kaser¹, Clement Akotsen-Mensah¹, Elijah J. Talamas², and Anne L. Nielsen^{1,*}

The invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is a polyphagous agricultural pest that feeds on over 170 plant species, including many cultivated fruits, vegetables, row crops, ornamentals, and wild host plants (Leskey & Nielsen 2018). Native to Asia, the earliest record of *H. halys* in North America is from 1996 in Allentown, Pennsylvania, in the eastern USA (Hoebeke & Carter 2003). It has since spread throughout much of North America, causing widespread economic harm (Rice et al. 2014; Leskey & Nielsen 2018). In 2014, an Asian egg parasitoid, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), was found successfully parasitizing laboratory reared, field deployed (sentinel) *H. halys* egg masses in Beltsville, Maryland, USA (Talamas et al. 2015; Herlihy et al. 2016). Several other field populations have since been reported in the mid-Atlantic and Pacific Northwestern regions of the US (10 states and Washington, DC, Hoelmer personal communication), which likely represent multiple independent introductions of the parasitoid (Milnes et al. 2016; Lara et al. 2016; Hedstrom et al. 2017). It is unclear through what pathways *T. japonicus* entered North America, but genetic data indicate that the parasitoid did not escape from quarantine facilities where it is being studied as a potential classical biological control agent of *H. halys* (Bon et al. 2017).

The recent introductions of *T. japonicus* in North America may increase biological control of *H. halys*. However, to our knowledge, *T. japonicus* has yet to be documented parasitizing *H. halys* within North American cultivated crops. All published recoveries of adventive *T. japonicus* in North America have occurred in non-agricultural, largely woody habitat despite surveys in crops (Talamas et al. 2015; Cornelius et al. 2016a, b; Herlihy et al. 2016; Ogburn et al. 2016; Hedstrom et al. 2017; Morrison et al. 2018) that has led to concern that introduced strains of *T. japonicus* may have limited biological control potential in North America. During the invasion process, genetic bottlenecks can affect life history characteristics, such as ecological host range (i.e., the number of host species that a parasitoid is able to complete development on in the field), and may limit the ability of introduced biological control agents to attack pests across the same breadth of habitat as in their native range (Hufbauer 2002).

We conducted surveys in 2 commercial apple and 3 peach orchards in southern New Jersey, USA (apple: Monroeville, Gloucester County [39.6877°N, 75.1875°W] and Richwood, Gloucester County [39.7355°N, 75.1748°W]; peach: Richwood, Gloucester County [39.7355°N, 75.1748°W], Glassboro, Gloucester County [39.7085°N, 75.1331°W], and Salem, Salem County [39.5665°N, 75.4248°W]) that had previous pest issues with *H. halys*. On each farm, contiguous

blocks were selected ranging from 2.0 to 9.7 ha of peach or apple, and assigned to 1 of 2 management regimes: Integrated Pest Management - Crop Perimeter Restructuring (IPM-CPR) or grower standard. There were 3 IPM-CPR blocks and 1 grower standard block replicated on 4 orchards for peach and 2 orchards for apple. Blocks within the IPM-CPR (Crop Perimeter Restructuring; described in detail in Blaauw et al. [2015]) management protocol applied insecticides only to the orchard border plus the first full row for *H. halys* management. In peach, this began at 100 DD₁₄ and continued weekly until harvest. In apple, border-based management was initiated when a cumulative threshold of 10 adult *H. halys* were found in any aggregation pheromone-baited *H. halys* trap (Short et al. 2016) and then continued until harvest. All IPM-CPR blocks per farm had a companion grower standard block of a minimum of 2.0 ha, managed according to recommendations from Rutgers University Fruit Management Guidelines (NJAES 2017). In each block, sentinel *H. halys* egg masses < 24 h old were sourced from the New Jersey Department of Agriculture, Trenton, New Jersey, USA, and deployed on orchard trees at 3 time points in apples (11 Jul, 27 Jul, and 8 Aug 2017) and 4 time points in peach (20 Jun, 11 Jul, 27 Jul, and 8 Aug 2017). Sentinel egg masses were glued using Elmer's Multi-purpose Glue-ALL (Elmer's Products, Inc., High Point, North Carolina, USA) onto paper cardstock and deployed by attaching an egg mass card to the underside of a leaf using a paper clip. Egg masses were deployed on 2 border trees and 2 interior trees (about 6 trees = 18.3 m) in a transect, and placed between 2 and 3 m from the ground. Trees on the border were adjacent to a pheromone baited *H. halys* trap. There were 192 sentinel *H. halys* egg masses (5,458 individual eggs) deployed in peach, and 48 in apple (1,321 individual eggs) (Table 1). Egg masses were left in orchards for about 48 h, then returned to the laboratory where each egg mass was placed separately in closed plastic containers, placed in an incubator (25 °C, 60–70% RH, 16:8h (L:D) photoperiod), monitored until emergence of *H. halys* or parasitoids, and the species identified based on adult morphological characteristics. If parasitoids emerged from an egg mass, or a guarding female was found in association with the retrieved egg mass, after waiting > 1 mo, the remaining unhatched eggs were dissected and inspected for dead parasitoids (larvae or pharate adults) or *H. halys* nymphs.

The effect of management strategy on the number of parasitized egg masses was analyzed using a 2-sided Fisher's exact test (FET) (Sokal and Rohlf 1995), pooling border and interior trees. The analysis was conducted on a 2 × 2 contingency table of management regime (IPM-CPR vs. grower standard) and parasitism status (parasitized vs. unparasitized egg mass). The GPS locations of sentinel egg masses were

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Table 1. Sentinel egg masses deployed and number of exotic (*Trissolcus japonicus*) and native (*Trissolcus edessae*) parasitoids emerging as adults.

Crop	Treatment	No. of egg masses	No. of eggs	<i>Trissolcus japonicus</i> adults	Native adults
Peach	Grower Standard	48	1,383	0	0
Peach	IPM-CPR	144	4,075	74(3)	2(1)
Apple	Grower Standard	24	662	0	0
Apple	IPM-CPR	24	659	0	0

Numbers of sentinel egg masses deployed and total number of parasitoid adults successfully emerging are listed (number of egg masses parasitized). All egg masses parasitized by *Trissolcus japonicus* were deployed on either 20 Jun or 8 Aug 2017.

recorded in the field, and the distances of successfully parasitized egg masses to field edge and the nearest woody border were estimated in Google Earth Pro.

No successful parasitism was found on eggs placed in apple orchards. In peach, 4 (2.2%) of 185 retrieved egg masses were successfully parasitized (7 egg masses were not recovered from the field). All successful parasitism in peach occurred under IPM-CPR management; however, the effect of management regime was not statistically significant ($P = 0.57$, FET). In total, 74 *T. japonicus* adults emerged from 3 egg masses, with an average proportion parasitism of each successfully parasitized egg mass of $0.917 (\pm 0.083 \text{ SEM})$, and no emerging *H. halys*. One of the egg masses parasitized by *T. japonicus* was deployed on 20 Jun, and 2 were deployed on 8 Aug 2017. Egg masses parasitized by *T.*

japonicus had a distance to the nearest wooded border ranging from 19.7 m to 68.3 m (mean = 40.0 m, SEM = 15.3) (Fig. 1). Two adults of the native parasitoid *Trissolcus edessae* Fouts (Hymenoptera: Platygasteridae) emerged from a single egg mass, along with 1 *H. halys* nymph and 15 eggs without emergence. Dissection of the 15 unhatched eggs uncovered 8 dead parasitoid larvae, 1 dead pharate parasitoid adult, and 6 with no clear insect development. The egg mass was deployed on 11 Jul at an interior location, 27.8 m (6 trees) from the nearest orchard edge that interfaced a soybean field, and 117 m from the nearest wooded border. One native adult female parasitoid, *Telenomus astrictus* Johnson (Scelionidae), was captured guarding an egg mass, deployed on 8 Aug and collected on 10 Aug, at a border location in an IPM-CPR peach orchard, 19.9 m from the nearest wooded border.



Fig. 1. White and black circles indicate the locations of 2 sentinel egg masses that were parasitized by *Trissolcus japonicus* within a heterogeneous landscape at 1 of the field sites in New Jersey. Black lines indicate the nearest tree line, and the inset map of New Jersey indicates with a star where this farm was located.

Twenty-two *H. halys* nymphs successfully emerged from the guarded egg mass, and 6 eggs did not hatch. Dissection of the 6 unhatched eggs uncovered 4 dead parasitoid larvae, 1 dead partially developed *H. halys* nymph, and 1 with no clear insect development. Whereas *H. halys* nymphs and adults are highly mobile (Lee et al. 2014; Lee & Leskey 2015), it is not clear how far *T. japonicus* moves during its lifetime. In frequently disturbed agricultural habitat, *T. japonicus* may be able only to disperse from source habitats like a wooded field border.

To our knowledge, these are the first observations of successful parasitism of *H. halys* by *T. japonicus* on a cultivated crop in the US that occurred in 2 different orchards. In China, the percentage of each *H. halys* egg mass attacked by *T. japonicus* ranges between 20% and 80% of eggs, with a season-long average parasitism of each egg mass of 50% among field collected egg masses on fruit and forest trees (Yang et al. 2009; Lara et al. 2016). In northern China, *T. japonicus* was the dominant egg parasitoid attacking *H. halys* sentinel egg masses deployed in cultivated peach, mulberry (*Morus alba* L.; Moraceae), and jujube (*Ziziphus jujuba* Mill.; Rhamnaceae) trees (Zhang et al. 2017). However, novel environments for parasitoid-host interactions, as with *T. japonicus* and *H. halys* in the US, open up an array of questions. For a given host species, its risk of parasitism may vary in different ecological contexts (Cronin & Reeve 2005).

Despite the high success of *T. japonicus* in its native range within agricultural crops (Zhang et al. 2017), the paucity of parasitism in US commercial agriculture had raised concerns. Our results suggest 2 important findings. First, *T. japonicus* can successfully forage and parasitize in US crops. Peach is a preferred host plant of *H. halys* (Acebes-Doria et al. 2016) and produces *n*-tridecane volatiles to which female *T. japonicus* also respond (Zhong et al. 2017), perhaps enhancing foraging behavior. Second, despite differences in management styles between Asia and the US, our results suggest that *T. japonicus* may be compatible with the IPM-CPR tactic in peaches, as all parasitism occurred within these reduced input blocks. The IPM-CPR tactic targets about 25% of the orchard with insecticide and integrates mating disruption for lepidopteran pests, significantly reducing insecticide applications throughout the growing season (Blaauw et al. 2015). Further research on the impact of insecticides on foraging behavior and survivorship of *T. japonicus* is needed to balance managing injury caused by *H. halys* in tree fruit with enhanced biological control.

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Summary

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), the brown marmorated stink bug, an invasive agricultural pest in America and Europe, is reaching a global distribution. In the US, the first detection of *H. halys* was in the mid-1990s, and it has become a serious pest in multiple crop systems. In 2014, an exotic egg parasitoid, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), was documented parasitizing sentinel *H. halys* egg masses in a wooded habitat in Beltsville, Maryland, USA. The parasitoid has since been reported in several other locations in the eastern and western US, and its population appears to be expanding in geographic range. However, there have been no reports of *T. japonicus* parasitizing *H. halys* egg masses within cultivated crops in the US. Whereas attack of *H. halys* in non-agricultural

habitat may provide important biological control services in the landscape, if *T. japonicus* is not able to successfully forage for *H. halys* eggs within crops, its impact as a biological control agent may be limited. Here we report on successful parasitism of egg masses deployed in 2 peach orchards in New Jersey, USA. Egg masses were deployed as part of an experiment investigating the efficacy of an integrated pest management (IPM) strategy utilizing border insecticide sprays in apple and peach. While overall egg parasitism was low, the majority of successfully developing parasitoids (97.4% of total adult parasitoids emerging, and from 75% of successfully parasitized sentinel egg masses) were *T. japonicus*.

Key Words: Invasive species; biological control; parasitism; first occurrence; Scelionidae

Sumario

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), el chinche marmorino café, una plaga agrícola invasora en América y Europa, está alcanzando una distribución mundial. En los Estados Unidos, la primera detección de *H. halys* fue a mediados de la década del 1990, y se ha convertido en una plaga seria en sistemas de cultivos múltiples. En el 2014, se documentó un parasitoide exótico de los huevos, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), parasitando las masas centinelas de *H. halys* en un hábitat de bosque en Beltsville, Maryland, EE. UU. El parasitoide ha sido reportado en varios otros lugares en el este y el oeste de los EE. UU. y su población parece estar expandiendo su rango geográfico. Sin embargo, no ha habido informes de *T. japonicus* parasitando masas de huevos de *H. halys* dentro de cultivos en los EE. UU. Mientras que el ataque de *H. halys* en hábitats no agrícolas puede proporcionar un servicio importante de control biológico en el campo, si *T. japonicus* no es capaz de alimentarse con éxito de los huevos de *H. halys* dentro de los cultivos, su impacto como agente de control biológico puede ser limitado. Aquí informamos sobre el parasitismo exitoso de masas de huevos puestos en dos huertos de melocotones en Nueva Jersey, EE. UU. Las masas de huevos se implementaron como parte de un experimento que investiga la eficacia de una estrategia integrada de manejo de plagas utilizando aerosoles insecticidas de frontera en manzana y melocotón. Mientras que el total del parasitismo de los huevos fue bajo, la mayoría de los parasitoides desarrollándose con éxito (el 97.4% del total de parasitoides adultos que emergieron y del 75% de las masas de huevos centinela parasitados con éxito) fueron *T. japonicus*.

Palabras Clave: especies invasoras; control biológico; parasitismo; primera aparición; Scelionidae

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