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Cover Page Footnote

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The Discovery of *Trissolcus japonicus* (Hymenoptera: Scelionidae) in Michigan

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Abstract

The invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a pest of growing economic importance in the United States, the control of which currently relies on pesticide applications. Biological control could provide sustainable and long-term control but classical biological control agents have not yet been approved at the federal level. Adventive populations of a potential biological control agent, the Samurai wasp, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), have been found in the United States, first in Maryland in 2014, expanding its range west to Ohio by 2017. *Trissolcus japonicus* is a highly effective parasitoid of *H. halys* eggs, but its redistribution and augmentative releases are restricted to states where it has been detected in the wild. To assess the presence of *T. japonicus* in Michigan and attack rates on *H. halys* by native natural enemies we deployed 189 *H. halys* egg masses at ten sites in lower Michigan between May and October in 2018. In addition, we deployed 51 native stink bug egg masses at the same sites to evaluate potential non-target effects of *T. japonicus* in the field, which were shown to occur in laboratory studies. We found *T. japonicus* in a single *H. halys* egg mass, which constitutes the first record of this Asian parasitoid in Michigan. Native predators and parasitoids caused minimal mortality of *H. halys* eggs and we did not find evidence of non-target effects of *T. japonicus* on native stink bug species. These findings open the door to initiation of a classical biological control program using an efficient, coevolved parasitoid from the native range of *H. halys*.

Keywords: Samurai wasp, brown marmorated stink bug, BMSB, biological control, sentinel egg masses, *Halyomorpha halys*

Invasive insects can cause significant economic damage to crops, especially in large monocultures (Bradshaw et al. 2016), potentially because they exist in their invaded ranges without their coevolved natural enemies (Roy et al. 2011). The brown marmorated stink bug, *Halyomorpha halys* (Stål), is an invasive pentatomid pest that was first detected in the United States in 1996 (Hoebeke and Carter 2003). It is capable of feeding on over 200 host plants, including many species of agricultural importance and has caused significant economic damage in the mid-Atlantic region (Leskey et al. 2012, Leskey and Nielsen 2018). Control of *H. halys* currently relies on pesticide applications, largely due to the absence of alternative control strategies like biological control agents (Rice et al. 2014). Native natural enemies have very limited impact on *H. halys* populations, with egg parasitoids attacking usually < 5% of egg masses (Abram et al. 2017, Dieckhoff et al. 2017). Thus, repeated applications of broad-spectrum insecticides over the grow-

ing season are necessary to control this pest in cropping areas, but given the vast host range of *H. halys*, populations can always persist in natural areas and recolonize crops. Biological control can suppress *H. halys* numbers across the landscape but to date no effective natural enemies have been found in Michigan.

Two parasitoid species that attack *H. halys* in its native range have been under consideration for release as classical biological control agents since 2007, with one, *Trissolcus japonicus* (Ashmead), undergoing host range testing. *Trissolcus japonicus* was found to develop on at least seven native stink bug species in Oregon (Hedstrom et al. 2017) and 15 native species in Michigan (Botch and Delfosse 2018), which would likely prevent its approval for field release. Nevertheless, *T. japonicus* found its own way into the United States, most likely from parasitized *H. halys* egg masses (Talamas et al. 2015b). Adventive populations were initially detected in Maryland (Talamas et

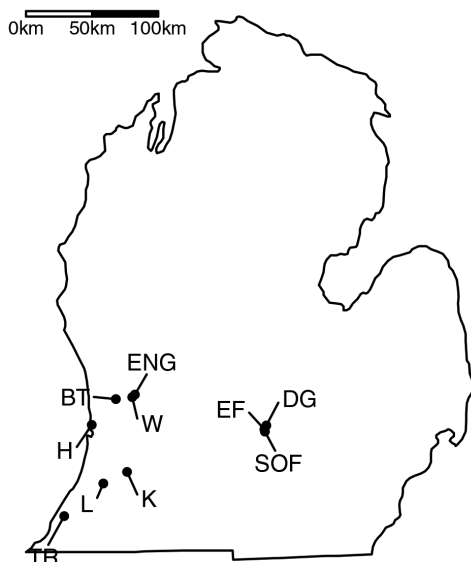


Figure 1: Map of sites where sentinel egg masses were deployed. Site codes are listed in Table 1. *Trissolcus japonicus* was captured at the MSU Student Organic Farm (SOF) in August 2018. *Trissolcus euschisti* was found at the orchard site ENG, and *T. brochymenae* was found at the mixed site W.

al. 2015b) and soon thereafter in neighboring states (Buffington et al. 2018). Based on the pattern of discovery, *T. japonicus* populations are gradually moving west and north, having been found in Pennsylvania and Ohio in 2017. Adventive populations have also been found in western Canada and Oregon, although these are presumably from a separate introduction (Milnes et al. 2016; Hedstrom et al. 2017).

Given that *T. japonicus* is an oligophagous foreign species for which field release has not been permitted, its intentional movement across state lines is not allowed. Thus, biological control programs aiming to use this species can only be initiated once it is detected in the field in a given state. We set out to assess the presence of *T. japonicus* and current levels of parasitism and predation by native species by deploying sentinel egg masses of *H. halys* at a network of sites across southern Michigan. In addition, we deployed sentinel eggs of native stink bug species that were shown to be attacked in the laboratory by *T. japonicus* to assess non-target effects in field settings. We report the first record of *T. japonicus* in Michigan and find no evidence of non-target parasitism. The detection and capture of live individuals

now allows for a biological control program to be mounted against *H. halys* in Michigan.

Materials and Methods

We deployed sentinel egg masses of *H. halys* between May and October 2018 (Fig. 1). Egg masses were collected from laboratory colonies maintained at Michigan State University (initial propagule provided by the New Jersey Department of Agriculture: Beneficial Insects Facility). To test for non-target parasitism in the field we also deployed egg masses of two species of native stink bugs, *Podisus maculiventris* (Say) and *Thyanta custator* (Fab.), which were shown to be successfully parasitized by *T. japonicus* in laboratory studies.

Egg masses were deployed in ten locations (Fig. 1) across central and western Michigan in the primary fruit and vegetable growing region of Michigan where *H. halys* populations have been most prevalent since its arrival in the state. The sites consisted of a diversity of cropping systems, including apples, blueberries, and farms with mixed crops. All sites had large non-agricultural areas nearby in the form of adjacent wood lots or fallow fields. A description of the sites and the number of sentinel egg masses of each species at each site is listed in Table 1. Native stink bug and *H. halys* egg masses were deployed following the protocol of previous sentinel egg mass programs in the eastern United States (Ogburn et al. 2016). Due to fluctuations in egg mass availability, the monitoring period of each site differed (Table 1). Either fresh egg masses laid within a 24-hour period or frozen (at -80°C for three minutes) eggs were deployed. Eggs were left in the field for 2–3 days and then brought back to the laboratory and kept at 20°C until nymphs or parasitoids had emerged. Any parasitoids that emerged from the sentinel egg masses were identified using the identification tools of Talamas et al. (2015a).

Eggs deployed early in the season ($N = 142$) at 3 sites (ENG, W, TR) from May through July were assessed for signs of natural enemy attack (both parasitism and predation). Eggs deployed during this earlier period were left in the field for 48 hrs, after which they were collected and assessed for signs of natural enemy attack using a compound microscope. This was conducted using the protocols of Ogurn et al. (2016) with the exception that egg masses were not dissected to check for partially developed parasitoids or other signs of unsuccessful parasitism. Six weeks after nymph emergence, egg masses were reassessed to determine hatch rate, and to check for emerged parasitoids. During this period, many egg masses became too moldy to assess from accumulated moisture whilst

Table 1: Sites where sentinel egg masses were deployed. Details of the main crop and the number of egg masses from each stink bug species are listed, as well as the first and last date on which egg masses were deployed. Egg mass numbers in parentheses are the number of frozen egg masses deployed.

Site	Crop	<i>H. halys</i>	<i>P. maculiventris</i>	<i>T. custator</i>	Start	End
SOF	Mixed	78 (10)	23 (2)	2 (0)	08/14/18	09/28/18
EF	Mixed	20 (9)	7 (1)	4 (2)	08/14/18	09/25/18
DG	Mixed	38 (0)	0	0	09/13/18	10/05/18
BT	Blueberry	1 (1)	3 (3)	1 (1)	09/14/18	09/14/18
ENG	Apple	25 (7)	0	0	05/23/18	08/15/18
W	Mixed	8 (0)	0	0	06/13/18	06/15/18
TR	Mixed	14 (7)	0	0	05/22/18	06/14/18
DG	Mixed	38 (0)	0	0	09/13/18	10/05/18
L	Blueberry	2 (2)	4 (4)	0	09/04/18	09/17/18
K	Blueberry	2 (1)	2 (2)	1 (1)	09/04/18	09/17/18
H	Blueberry	1 (1)	3 (3)	1 (1)	09/14/18	09/14/18

Table 2: All parasitoid emergences from sentinel egg masses. All sentinel egg masses that were parasitized by native parasitoids were frozen.

Site	Sentinel egg mass species	Date deployed	Date retrieved	Parasitoid species	Number of individuals
ENG	<i>H. halys</i>	05/23/18	05/25/18	<i>Trissolcus euschisti</i>	2 (2 males)
W	<i>H. halys</i>	06/13/18	06/15/18	<i>Trissolcus brochymenae</i>	5 (5 males)
ENG	<i>H. halys</i>	07/09/18	07/11/18	<i>Trissolcus euschisti</i>	9 (2 males)
SOF	<i>H. halys</i>	08/14/18	08/16/18	<i>Trissolcus japonicus</i>	5 (2 males)

deploying egg masses during rainy weather. Any egg masses that were too moldy to assess were discarded and are not included in the data presented here. Later in the season from August through October most sentinel egg placements focused around the area where *T. japonicus* had been captured and only rates of parasitism were assessed due to time constraints (Table 1).

Results

Trissolcus japonicus emerged from a single *H. halys* egg mass that was deployed on 14 August 2018 at the Michigan State University Student Organic Farm (site code: SOF) three miles south of the East Lansing campus. A fresh egg mass was attached to a paw-paw tree (Magnoliales: Annonaceae, *Asimina triloba*, Dunal) located in an organic garden that included a diversity of native and imported tree species, many weeds, and ornamental and agricultural crop species including peaches, grapes, and raspberries. Three male and two female *T. japonicus* individuals emerged 23 days later on 6 September 2018.

Of the 142 egg masses deployed to measure natural enemy attack rate, eight were lost during deployment and 14 became too moldy to assess, leaving a total of 120 egg

masses containing 3239 individual *H. halys* eggs. These egg masses contained an average of 27.0 eggs and of these an average of 9.06 eggs successfully emerged as nymphs (33.5% hatch rate). Chewing predation occurred on three egg masses, affecting ten eggs in total (2.5% of egg masses, 0.3% of individual eggs). Incomplete chewing predation (like that associated with spider feeding, Morrison et al. 2016) occurred on 12 egg masses, affecting 34 eggs (10.0% of egg masses, 1.05% of individual eggs). Sucking predation associated with hemipteran predators occurred on seven egg masses affecting 13 individual eggs (5.22% of egg masses, 0.4% of individual eggs). Parasitism occurred on three egg masses from two different species with a total of 12 adult parasitoids that successfully emerged from parasitized eggs (2.1% of egg masses, 0.4% of individual eggs). Including all 189 *H. halys* egg masses and both native and non-native parasitoids, the overall parasitism rate was 2.1%. Only native parasitoids emerged from these eggs, *Trissolcus brochymenae* (Ashmead) and *Trissolcus euschisti* (Ashmead). All emerging parasitoids from sentinel eggs are detailed in Table 2, with the site and dates the sentinel egg masses were deployed, and the number and species of emerging parasitoids.

Discussion

The discovery of *T. japonicus* in Michigan will have a major impact on the way *H. halys* is managed in the state. Currently, pesticides are the primary tactic used to combat the pest; however, now a classical biological control program can be initiated as in New York and Oregon where mass rearing and release of *T. japonicus* are already underway (BMBS SCRI Annual Report 2017). We captured *T. japonicus* at an organic farm, despite deployment of eggs at the edges of numerous conventionally managed orchards and mixed farms, which suggests that pesticide applications might negatively affect this parasitoid and limit its potential as a biological control agent in some cropping areas (Wilkinson et al. 1975, Croft 1990, Ndakidemi et al. 2016), although Kaser et al. (2018) recorded *T. japonicus* in a managed peach orchard. Further research is therefore required in order to determine how current chemical control regimes could be amended to form an integrated pest management strategy for *H. halys* that complements classical biological control by *T. japonicus* (Roubos et al. 2014).

Our discovery of *T. japonicus* indicates continued westward range expansion from the east coast. This is also one of the northernmost records of *T. japonicus* east of the Rocky Mountains. Despite its cold winter weather, Michigan falls into the predicted range suitable for *T. japonicus* (Avila and Charles 2018) and it is therefore likely that *T. japonicus* populations will continue to persist, at least in the southern half of the state.

Currently, *T. japonicus* has not been approved for release in the United States or permitted for interstate redistribution largely due to its potential to attack native stink bug species, such as *P. maculiventris*, which is a predatory stink bug and an important biological control agent in its own right (Botch and Delfosse 2018). In the laboratory, *T. japonicus* shows strong preference for *H. halys* eggs and often rejects non-target species for oviposition but only when it is reared on its primary host (Botch and Delfosse 2018). Attack on non-target species was shown to increase with prior exposure to native stink bugs but also resulted in decreased brood and adult sizes of *T. japonicus*. We deployed sentinel egg masses from native stink bugs throughout the state including the place where *T. japonicus* was detected but did not find any signs of non-target attacks. These results might suggest that *T. japonicus* prefers *H. halys* over native stink bugs in the field. However, the number of sentinel egg masses was relatively low and additional replication of both *H. halys* and native stink bug egg masses is required to better under-

stand the distribution and population sizes of *T. japonicus* in Michigan and its realized host range in the field.

We found two native congeners, *T. brochymenae* and *T. euschisti* emerging from frozen egg masses and overall very low natural enemy utilization of *H. halys* eggs by native species. This is congruent with previous studies reporting parasitism rates of less than 5% and predation rates between 4.4–12.7% (Ogburn et al. 2016, Cornelius et al. 2016, Abram et al. 2017). Frozen eggs are thought to be more susceptible to parasitism from native parasitoids (Herlihy et al. 2016) because the eggs cannot mount an immune response and defend themselves once the hosts have ceased development (Haye et al. 2015). Despite our small sample size, we found that native parasitoids only emerged from frozen *H. halys* egg masses. The two species we caught, *T. euschisti* and *T. brochymenae*, are both common parasitoids of sentinel *H. halys* egg masses across the United States, but frequently fail to complete development on live *H. halys* eggs (Abram et al. 2017). We did not dissect egg masses to assess for parasitism but only measured parasitoid emergence, which likely underestimated the rate of parasitism and the non-reproductive effects native parasitoids have on *H. halys* populations (Abram et al. 2018). The fact that native parasitoids attack *H. halys* egg masses, and that a small proportion do emerge as adult parasitoids, suggests native parasitoids have the potential to exploit *H. halys* as hosts but require additional adaptations. Thus, *H. halys* populations could grow largely unchecked in North America and *T. japonicus* may represent the only effective natural enemy to be used for biological control of this pest. Further work should focus on exploring the continued range expansion of *T. japonicus* and measuring its impact on *H. halys* populations both in managed and natural areas.

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