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Sampling Methods for Adventive *Trissolcus japonicus* (Hymenoptera: Scelionidae) in a Wild Tree Host of *Halyomorpha halys* (Hemiptera: Pentatomidae)

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Abstract

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an invasive pest that has established in much of the United States. Adventive populations of an effective Asian egg parasitoid of *H. halys*, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), have been detected in several states, including Virginia, and its geographic range is expanding. Documenting changes in its distribution and abundance have thus become key research priorities. For these specific purposes, surveillance of *T. japonicus* over large geographic areas using sentinel *H. halys* egg masses may not be optimally efficient, and examination of alternative sampling tactics is warranted. In 2016, sentinel *H. halys* egg masses were deployed as vertical transects in the canopy of female *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae) in Virginia. A brief follow-up study in 2016 using yellow sticky traps deployed in the same trees yielded captures of *T. japonicus*, leading to a comparison of vertical transects of sentinel eggs and yellow sticky traps in 2017. Both methods yielded *T. japonicus* detections only in the middle and upper tree canopies, whereas other known *H. halys* parasitoids were detected in the lower, middle, or upper canopies. Based on this information, a method for deploying yellow sticky traps in the middle canopy of *H. halys* host trees was assessed in 2017, yielding *T. japonicus* captures. A comparison of estimated time inputs revealed that traps were more efficient than sentinel eggs in this regard. Results are discussed in relation to the utility of each sampling method to address specific questions about the range expansion and ecology of *T. japonicus*.

Key words: biological control, parasitoids, invasive species, monitoring

Brown marmorated stink bug, *Halyomoprha halys* (Stål) (Hemiptera: Pentatomidae), is a polyphagous agricultural and nuisance pest from Asia that continues to cause significant crop protection issues in the United States (www.stopbmsb.org, Leskey and Nielsen 2018). Its management has relied heavily on broad spectrum insecticides, which can mitigate crop injury but have incited secondary pest outbreaks (Rice et al. 2014) and do not adequately reduce its populations in the surrounding landscape. Consequently, there is much interest in biological control to regulate *H. halys* populations in crop and noncrop habitats. Some indigenous natural enemies attack *H. halys* but have not regulated its populations sufficiently (Abram et al. 2017). In Asia, the egg parasitoid, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), is a key natural enemy of *H. halys* (Yang et al. 2009, Zhang et al. 2017) and has been evaluated in quarantine in the United States since 2007 (Talamas et al. 2015). An adventive population of *T. japonicus* was detected in Maryland in 2014 (Talamas et al. 2015); subsequent detections have been reported from Virginia, West Virginia, Maryland, Delaware, New York, New Jersey, Pennsylvania, Ohio, Michigan, Oregon, Washington, California, and Washington, DC. Consequently, documenting the spread of *T. japonicus* has become a major research objective.

To date, most field data on *H. halys* natural enemies have been derived from assessments of naturally laid egg masses or from sentinel egg masses deployed on host plants (reviewed in Abram et al. 2017). While these sampling methods can document egg predation and parasitism, they pose some challenges for determining the distribution and spread of *T. japonicus* across large geographic areas. Collection of naturally laid egg masses is time-consuming (Bakken et al. 2015) and the use of sentinel eggs requires maintaining a colony of *H. halys*. The acceptability of *H. halys* eggs to *T. japonicus* declines within a few days of oviposition (Qiu et al. 2007, Yang et al. 2018), and sentinel eggs are typically retrieved after 72 h. Moreover, sentinel eggs are vulnerable to predation during deployment (reviewed in Abram et al. 2017), and may underestimate parasitism (Jones et al. 2014). Scouting for naturally laid eggs and deployment of sentinels has usually involved sampling from the ground (Abram et al. 2017). However, Quinn et al. (2019) found that most *H. halys* egg masses were in the middle and upper tree canopies of tree of heaven, *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae), and that those parasitized by *T. japonicus* were exclusively from the middle and upper tree canopies, suggesting that sampling from the ground may reduce the likelihood of *T. japonicus* detection.

Adventive populations of *T. japonicus* in the United States are anticipated to impact *H. halys* populations. Given certain limitations of current methods for tracking its establishment and spread, alternative tools and standardized protocols for more extensive and intensive surveillance of *T. japonicus* are needed. Yellow sticky traps have been used to sample other parasitoids, such as braconids (Vargas et al. 1991) and trichogrammatids (Romeis et al. 1998). Other scelionids, such as *Trissolcus basalis* (Wollaston), have demonstrated an innate preference for the color yellow (Ferreira Santos de Aquino et al. 2012), suggesting that yellow sticky traps may be effective for sampling *T. japonicus*. Here, we report studies that evaluated sentinel eggs and yellow sticky traps for detecting *T. japonicus* in a common *H. halys* tree host.

Materials and Methods

Five female tree of heaven growing at the woods edge next to fruit orchards in Frederick County, VA, were used for sampling in 2016 and 2017. As described previously (Quinn et al. 2019), an eyebolt was inserted near the top and bottom of the trunk of each tree, and a pulley system created by running a rope through these and attaching the ends enabled sampling devices to be attached to the rope and spaced evenly along the trunk at lower, middle, and upper canopy locations.

Halyomorpha halys egg masses that were ≤24 h old and comprised of >20 eggs were obtained from a colony of field-collected adults and used as sentinels. Each mass was removed from the substrate and affixed to double-sided tape (Scotch, The 3M Company, Maplewood, MN) on a 3 × 5 cm piece of white cardstock. Any remaining sticky surface was covered with fine white sand. Cards with eggs were held at −80°C for 1–21 d before deployment. Abram et al. (2017) reported that attacks of frozen and fresh *H. halys* egg masses by native parasitoids were equivalent. Clothespins were used to affix the cards to the underside of leaves on excised shoots of female tree of heaven in 500 ml plastic bottles with water, which were attached to the rope with cable ties (Fig. 1). In 2016, egg masses were deployed for 72 h each week from 24 May through 12 August, during periods when the probability of rain or windy conditions was low. Upon retrieval, eggs were held at 25°C and a 16:8 (L:D) h photoperiod, and parasitoid emergence was assessed daily for 6 wk. Parasitoids that emerged were identified to species or genus.

At the conclusion of this study, an exploratory evaluation of yellow sticky traps (one-sided, 23 × 14 cm, Alpha Scents, West Linn, OR) was conducted in the same five trees in which eggs had been deployed. Traps were attached to the rope and positioned at the lower, middle, and upper canopy locations for three, 7-d intervals between mid-August and early September. In 2017, sticky traps or sentinel eggs were deployed on alternating weeks from 31 May through 24 August in the same trees used in 2016 and at the same canopy positions described previously. Captured specimens suspected of being a potential *H. halys* parasitoid were removed in situ on a piece of the trap and identified to species or genus.

Also in 2017, one backfolding yellow sticky trap (46 × 28 cm) (Alpha Scents) was deployed in the middle canopy of 10 female tree of heaven growing near apple orchards by attaching it to the top of a 4.8 m bamboo pole that was suspended from a branch via a

Fig. 1. *Halyomorpha halys* sentinel egg mass attached to foliage of an excised tree of heaven shoot and deployed in a vertical transect along the trunk of mature female tree of heaven.

wire hook below the trap (Fig. 2). Traps were replaced at 7-d intervals from 30 June through 21 July, and parasitoids of interest were removed and identified as described above. All data are presented using descriptive statistics.

Results

In 2016, 135 sentinel egg masses were deployed. Six (4.4%) yielded adult parasitoids, including *T. japonicus* (n = 3), *Trissolcus euschisti* (Ashmead) (Hymenoptera: Scelionidae) (n = 2), and *Telenomus podisi* Ashmead (Hymenoptera: Platygastridae) (Scelionidae) (n = 1). All detections of *T. japonicus* were from the middle (n = 2) or upper canopy (n = 1) and occurred between 13 June and 22 July. Based on the number of adults that emerged, the percentage of eggs parasitized per mass ranged from 7 to 96 (mean = 53.2%) for *T. japonicus*, 29 to 96 (mean = 62.5%) for *T. euschisti*, and 18 for *T. podisi*.

Across three 7-d sampling intervals in late summer 2016, two *T. japonicus* were captured in middle canopy sticky traps and one in an upper canopy trap. Other *H. halys* parasitoids captured included *T. euschisti* (upper trap, n = 1), *Trissolcus brochymenae* (Ashmead) (Hymenoptera: Scelionidae) (middle trap, n = 1), *Ooencyrtus johnsoni* (Howard) (Hymenoptera: Encyrtidae) (Encyrtidae) (lower trap, n = 2; upper trap, n = 1), and *Telonomus* spp. (Hymenoptera: Platygastridae) (lower trap, n = 1).

In 2017, one sentinel egg mass in the middle canopy was parasitized by *T. japonicus* in late June (100% eggs parasitized). Three

Fig. 2. Yellow sticky trap atop a 4.8 m bamboo pole (A) in close-up and (B) as deployed in the middle canopy of mature female tree of heaven.

middle canopy traps and one upper canopy trap each captured one *T. japonicus* between June and September. Two egg masses from the middle canopy yielded *Anastatus* spp. (Hymenoptera: Eupelmidae) (56–64% of eggs parasitized) and one mass from the upper canopy produced *T. euschisti* (89% parasitized). Other parasitoids in traps included one *T. thyantae* Ashmead (lower canopy), seven *Anastatus* spp. (three lower, one middle, three upper canopy), three *Telenomus* spp. (two lower, one middle canopy), and one Encyrtidae (lower canopy).

Backfolding sticky traps deployed in the middle canopy of 10 trees over 3 wk in 2017 yielded 12 *T. japonicus* (0.4 ± 0.36 SE per trap), 11 of which were on one trap.

Discussion

Quinn et al. (2019) reported that naturally laid *H. halys* egg masses parasitized by *T. japonicus* were found only in the middle and upper thirds of the canopy of felled female tree of heaven. Although relatively few *T. japonicus* were detected during the present studies, which was not unexpected given that it is likely in early stages of establishment, the distribution of its detections in the tree canopy via vertical transects of sentinel *H. halys* egg masses and yellow sticky traps paralleled those from our previous research. *Trissolcus japonicus* has been recovered from *H. halys* egg masses found or deployed in the lower tree canopy (Cornelius et al. 2016, Herlihy et al. 2016), but these data suggest that the likelihood of detection may be enhanced by sampling higher in the tree.

Yellow sticky traps appear to be an effective sampling tool for addressing some, but not all questions about the field ecology of *H. halys* parasitoids, including *T. japonicus*. However, these traps and sentinel eggs each have attendant strengths and weaknesses. One major difference between them is the time and labor inputs required, summarized in Fig. 3. Aside from the inputs required to maintain an *H. halys* colony and to search daily for newly laid egg masses, preparing eggs for use as sentinels and tracking their fate upon retrieval from the field is comparatively time-consuming. In contrast, sticky traps can be processed immediately upon retrieval and captured specimens on small pieces of trap can be quickly identified in situ (E.J.T. and N.F.Q., personal observation). Captures of nontarget insects in sticky traps can range from few to many per trap, depending on the site and sampling period during the spring and summer. Foliage or other organisms or their remnants (e.g., feathers, hair) may occasionally occlude portions of the trapping surface. Traps enable longer sampling intervals than sentinel eggs, given the rapidly diminishing acceptability of ≥3-d-old eggs to *T. japonicus* (Qiu et al. 2007, Yang et al 2018). Predation of sentinel eggs is relatively common (Morrison et al. 2016, Abram et al. 2017), adversely affecting results if parasitoid detection is intended. While other parasitoid species known to attack *H. halys* eggs (Abram et al. 2017) were captured in traps, determination of which taxa attack the eggs of *H. halys* and native pentatomids and their rates of attack and parasitization can be addressed only via sentinel or naturally laid egg masses.

In summary, yellow sticky traps deployed in the middle canopy of an *H. halys* host tree have proven effective for sampling *T. japonicus*. The efficiency and effectiveness of efforts to track changes in its distribution and relative abundance on a large geographic scale is expected to benefit from use of this trap; other *H. halys* researchers have recently reported *T. japonicus* captures using them (N.F.Q., personal observation). As well as providing basic surveillance data on the presence or absence of *T. japonicus* captures, the traps have also shown utility for addressing certain ecological questions. For example, N. F. Quinn (unpublished data) captured >200 *T. japonicus* between May and September 2018, revealing new information about its seasonal phenology and the effects of habitat type on detection frequency. Our future research will continue to examine the seasonal phenology of *T. japonicus*, patch size and tree species effects on its presence and abundance, and annual changes in the relative proportions of *T. japonicus* and other *H. halys* parasitoids captured.

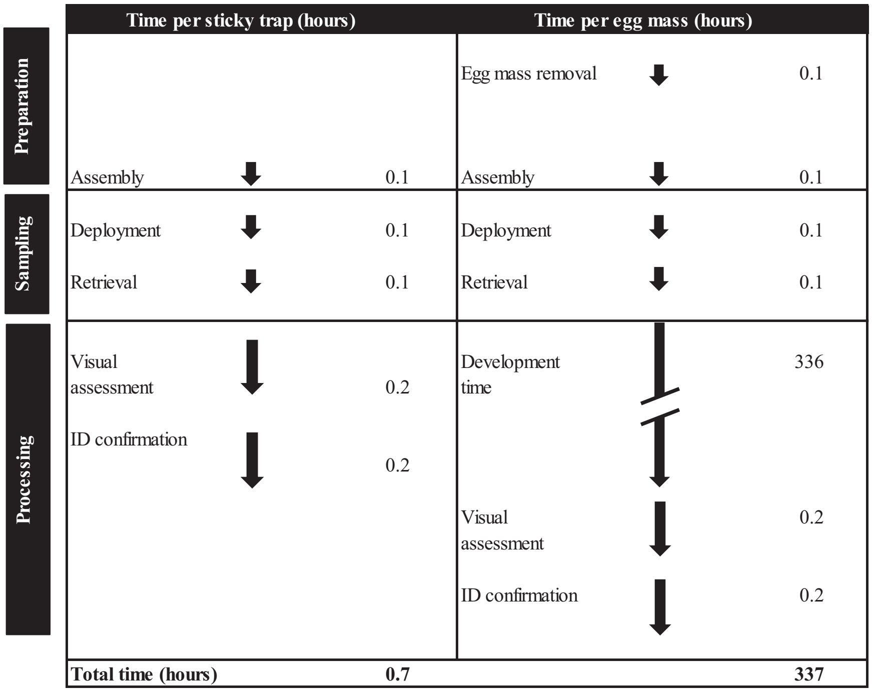

Fig. 3. Estimated time required to monitor parasitoids of *H. halys* using sentinel egg masses or yellow sticky traps. Time requirements for colony maintenance will vary depending upon size and productivity of colony and are therefore excluded here. Travel time to and from field sites was held constant between methods.

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