

**Foraging ecology and sampling of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) and its host *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) in wild host trees**

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**Abstract**

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive agricultural and nuisance pest in the United States and an increasing number of countries abroad. In the absence of other long-term solutions, biological control presents one of the most promising *H. halys* management options. An adventive population of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), one of the most effective egg parasitoids of *H. halys* in their shared native range in Asia, was discovered in the Maryland in 2014 and in Frederick County, VA in 2015. Adventive populations of *T. japonicus* have also been detected recently in several other states and show strong indications of ongoing range expansion. Despite their importance, the foraging ecology of *H. halys* and especially of *T. japonicus* in the USA need further study. Small pyramid traps baited with a low-dose *H. halys* aggregation pheromone lure and deployed as vertical transects in the canopy of female tree of heaven (*Ailanthus altissima* (Mill.) Swingle), male tree of heaven, or hackberry (*Celtis occidentalis* L.), captured significantly more *H. halys* adults and nymphs near the top of the canopy than at mid- or lower canopy, regardless of host plant. The majority of *H. halys* egg masses collected via destructive sampling of felled tree of heaven were from mid-canopy, and the majority of those parasitized by *T. japonicus* were also from mid-canopy, with no *T. japonicus* emergence from egg masses collected from the lower canopy. A comparison of sentinel *H. halys* egg masses and yellow sticky traps deployed as vertical transects in tree of heaven revealed that the majority of *T. japonicus* detections occurred at mid-canopy and that while both methods proved to be equally effective, yellow sticky traps were more efficient for this purpose. This led to the development of a novel *T. japonicus* sampling method in which a yellow sticky trap is deployed atop a bamboo pole in the mid-canopy of wild host trees. This method was employed to compare the effect of habitat type on *T. japonicus* captures in female tree of heaven growing in spatially isolated patches, thin windbreaks, and at the edge of contiguous woodlots. Consistent

habitat effects on *T. japonicus* detections were not found between sampling years, but provided the first documentation of the seasonal activity of *T. japonicus* in the USA. A study of host plant effects on *T. japonicus* detection in mid-canopy yellow sticky traps did not reveal consistent host plant effects on captures. These data provide important insights into the foraging ecology of *H. halys* and *T. japonicus* in the USA, as well as efficient sampling methods for future studies of *T. japonicus*.

**Foraging ecology and sampling of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) and its host *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) in wild host trees**

**Nicole F. Quinn**

**General Audience Abstract**

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive agricultural and nuisance pest from Asia that is widely distributed in the United States and is present in an increasing number of countries abroad. In the absence of other long-term management solutions, biological control via the actions of natural enemies presents a promising brown marmorated stink bug management option.

*Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), also known as the samurai wasp, is one of the most effective egg parasitoids of brown marmorated stink bug in Asia. Samurai wasp arrived in the USA without deliberate introduction and was first detected in Maryland in 2014 and subsequently in Frederick County, VA in 2015. It has continued to expand its range in the USA and was recently detected in several eastern and western states. Despite their importance, little is known of where brown marmorated stink bug eggs, nymphs, and adults are most likely to be found in wild trees. Even less is known about where samurai wasp is likely to occur within its new range. To address these issues, traps containing a lure to attract brown marmorated stink bug were placed in the upper, middle, and lower canopy of female tree of heaven (*Ailanthus altissima* (Mill.) Swingle), male tree of heaven, and hackberry (*Celtis occidentalis* L.). Significantly more brown marmorated stink bug adults and nymphs were caught in traps near the top of trees than in those at other canopy locations, with no effect of host plant species observed. The majority of brown marmorated stink bug egg masses collected from felled tree of heaven were from the middle of the canopy. The majority of samurai wasps emerged from egg masses collected from the mid-canopy, while none emerged from those collected from the lower canopy. In a comparison of sentinel brown marmorated stink bug egg masses and yellow sticky traps placed in the upper, middle, and lower canopy of tree of heaven, the majority of samurai wasp detections occurred at mid-canopy. Both methods were found to be effective, but yellow sticky traps required less time and effort to use. This led to the development of a new sampling method for samurai wasp, in which a yellow sticky

trap was deployed atop a bamboo pole in the mid-canopy of wild host trees. Using this technique, the effect of habitat type on samurai wasp captures was examined, revealing no consistent effects on detections. However, this study provided the first documentation of when samurai wasps are present and most abundant during the spring and summer. A study of host plant effects on samurai wasp captures, also using yellow sticky traps placed in the mid-canopy of trees, did not reveal consistent host plant effects on captures. These data provide important insights into the interactions of brown marmorated stink bug, samurai wasp, and their environment in the Mid-Atlantic region of the USA, as well as an efficient tool with which to study the samurai wasp.

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Published manuscripts presented as Chapters 2 and 3 herein were each coauthored by Nicole Quinn, Elijah Talamas, Tracy Leskey, and Chris Bergh.

## **Chapter 1**

### **Introduction and Literature Review**

#### **1.1 Invasive species**

Invasive species are defined as, “species that [are]: 1) non-native (or alien) to the ecosystem under consideration; and 2) whose introduction causes or is likely to cause economic or environmental harm or harm to human health” (USDA/NISIC 2019). In the United States, there are approximately 50,000 invasive species, 4,500 of which are arthropods (Pimentel et al. 2005). Invasive species pose one of the greatest threats to agriculture (Paini et al. 2016). They are responsible for at least \$70 billion per year in economic costs globally (Bradshaw et al. 2016) and up to \$13.5 billion each year in agricultural losses and management costs in the USA (Pimentel et al. 2005). Many conditions have led to the increase in establishment of invasive species, including an increase in the speed and volume of global trade, which has afforded greater opportunity for potential invasive species to expand their range into new areas, despite improvements in monitoring and detection (Hulme 2009). The enemy release hypothesis (David et al. 2017) purports that invasive species can dominate the ecosystems in which they arrive by outcompeting native species for resources and experiencing reduced mortality in the absence of their coevolved natural enemies. Release from biological control allows invasive species to experience disproportionately higher survivorship, aiding in their rapid establishment and spread (Liu and Stiling 2006). Invasive species can reduce biodiversity by outcompeting native species and can become the dominant species relatively quickly (Sanders et al. 2003). Climate change may also increase the negative effects of invasive species by allowing them to expand their range and fill the ecological void left by native species that cannot tolerate changes to their environment (Hellman et al. 2008). Eradication of invasive species is labor-intensive and expensive compared with preventing their establishment (Leung et al. 2002), although preventing their establishment can be difficult (Simberloff et al. 2013).

#### **1.2 Brown marmorated stink bug, *Halyomorpha halys***

One of the most important recent herbivorous invasive species is *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), the brown marmorated stink bug (BMSB) (Leskey and Nielsen

2018). *Halyomorpha halys* has undergone several name revisions, including *Pentatoma halys* Stål, *Poecilomes mistus* Uhler, *Dalpada brevis* Walker, and *Dalpada remota* Walker (Hoebeke and Carter 2003). These species are considered synonymous (Josifov and Kerzhner 1978). In 2002 the species was revised again to *H. halys* (Rider et al. 2002).

The first *H. halys* specimens in the USA were purportedly sighted in 1996 in Allentown, PA, although were not identified until 2001 (Hoebeke and Carter 2003). Genetic analyses have determined that *H. halys* populations in the USA originated from Beijing, China (Xu et al. 2014). *Halyomorpha halys* likely arrived in the USA via goods imported from Asia, but the exact route or timing of introduction is unknown (Hoebeke and Carter 2003). Detections of *H. halys* were first reported from the Mid-Atlantic region in the early and mid-2000s (Hamilton 2009, Nielsen et al. 2013, Wallner et al. 2014). In 2010, an outbreak of *H. halys* resulted in severe losses in Mid-Atlantic tree fruit production (Leskey et al. 2012a). *Halyomorpha halys* quickly increased its range to include several states in the Midwest and New England, where it has continued to injure crops (Leskey and Nielsen 2018). A separate introduction of *H. halys* on the west coast has resulted in its establishment in Oregon, Washington State, and California as well (Xu et al. 2014, Leskey and Nielsen 2018). *Halyomorpha halys* has now been detected or established in more than 42 USA states, (Hahn et al. 2016) and several countries abroad, including Canada (Fogain and Graff 2011, Abram et al. 2017a), Chile (Faúndez and Rider 2017), Switzerland (Haye et al. 2015a), France, (Callot and Brua 2013), Germany (Heckmann 2012), Great Britain (Malumphy 2014), Greece (Milonas and Partsinevelos 2014), Hungary (Vetek et al. 2014), Italy (Maistrello and Dioli 2014), Lichtenstein (Arnold 2009), Romania (Macavei et al. 2015), Georgia (Gapon 2016), and Sicily (Carapezza and Lo Verde 2017). Based on climate-matching studies, *H. halys* will likely continue to spread (Kriticos et al. 2017) and has been intercepted at ports of entry in New Zealand (Harris 2010) and Australia (Walker 2009, Horwood et al. 2019), but has not yet established there. The spread of *H. halys* in the USA has been associated with railways (Wallner et al. 2014), suggesting that human activity is a key component of its range expansion.

### **1.3 Life history**

In Asia, *H. halys* adults emerge from overwintering in early spring (Lee et al. 2013). In the eastern USA, Bergh et al. (2017) showed that adults emerged from experimental overwintering shelters between April and June, with peak emergence in May. Females typically oviposit on the underside of host plant leaves, laying a median of 28 eggs in each clutch (Nielsen et al. 2008). Females are synovigenic and can mate and lay eggs throughout their lifetime, producing an average of 9.3 egg masses (Nielsen and Hamilton 2009a). Eggs are light green in color, barrel-shaped, and affixed to the substrate with an adhesive (Hoebeke and Carter 2003, Lee et al. 2013, Tognon et al. 2017). At 25°C, nymphs emerge within 5-6 days (Lee et al. 2013) or 6.1 degree days (DD) above 14.17° C (Nielsen et al. 2008). First instars remain with the egg mass, acquiring gut symbionts from the egg chorion that are essential to their development (Taylor et al. 2014). Nymphs then disperse and begin feeding on plants, with adults feeding on the same hosts (Leskey and Nielsen 2018). After five nymphal instars, *H. halys* molt to the adult stage (Nielsen et al. 2008). A total of 685 DD above the minimum developmental threshold of 14.17° C are needed for development from egg hatch to reproductive maturity (Nielsen et al. 2008, 2009a). *Halyomorpha halys* has been reported to be univoltine or bivoltine depending upon environmental conditions and geographic location (Nielsen and Hamilton 2009a, Lee et al. 2013). In the fall, adults seek sheltered locations in which to overwinter, often in large aggregations, including underneath tree bark, in cracks and crevices in rocky outcrops, and in human-made structures (Kobayashi and Kimura 1969, Qin 1990, Lee et al. 2014a, Bergh and Quinn 2018). A citizen scientist study conducted by Hancock et al. (2019) found that dispersing *H. halys* alighted in greatest numbers on homes in rural areas that had exteriors made of natural materials such as wood, cement, or stone exteriors and that were brown, tan, or grey in color.

#### **1.4 Pest Status**

Due to its overwintering behavior, *H. halys* can be a significant nuisance pest to homeowners in areas with high populations. Some homes are heavily invaded in the autumn during the period of adult dispersal to overwintering sites; for example, over 26,000 overwintering *H. halys* were collected in a single home in Maryland (Inkley 2012). Adults enter buildings through crevices as small as 4 mm (Chambers et al. 2019), where they remain for the duration of diapause (Bergh et al. 2017). However, *H. halys* is primarily considered an agricultural pest and is known to feed on

over 170 host plants, including many of economic importance (Leskey and Nielsen 2018) and many wild species (Bakken et al. 2015, Acebes-Doria et al. 2016a). Soybeans are one of the most economically important crops in the USA and are susceptible to injury from *H. halys* feeding (Owens et al. 2013), as are small fruits (Wiman et al. 2015), sweet corn (Cissel et al. 2015) and vegetables (Zobel et al. 2016). Tree fruit have been among the crops most affected by *H. halys*. The outbreak of *H. halys* in the Mid-Atlantic region in 2010 resulted in losses of over \$37 million to the apple crop (Herrick 2011) and severe impacts to many peach orchards (Leskey et al. 2012a). Fruit injury from *H. halys* feeding on apples and peaches is expressed as external depressions and discolorations and necrotic tissue internally (Joseph et al. 2015). Unlike the native stink bug pests of tree fruit, feeding by both adult and late-instar nymphal *H. halys* can injure apples and peaches, especially if feeding occurs late in the season (Acebes-Doria et al. 2016b). Fruit that are fed upon in the mid- and late-season show the most injury compared with fruit injured earlier in the season, but early-season feeding can also reduce fruit marketability, especially in peaches (Nielsen and Hamilton 2009b, Acebes-Doria et al. 2016b).

*Halyomorpha halys* is a highly dispersive pest that moves among crops and wild hosts throughout the season (Bakken et al. 2015, Blaauw et al. 2016, Leskey and Nielsen 2018). In a 22-hour period, adults on a flight mill flew up to 117 km (Lee and Leskey 2015) and 4<sup>th</sup> and 5<sup>th</sup> instars were capable of walking 20 m in <5 hours (Lee et al. 2014b). Fruit on trees in orchard borders adjacent to woodlots often had greater levels of injury than those in the orchard interior (Joseph et al. 2014) and *H. halys* abundance is often greatest at the edge of cropped areas (Venugopal et al. 2015). In soybeans, increased feeding at field edges results in “stay green” syndrome, in which the plants fail to senesce (Hill et al. 2006, Venugopal et al. 2015).

### **1.5 Monitoring and management of *H. halys***

The invasion of a new pest requires the development of effective monitoring and management tools. Initially, Nielsen et al. (2013) used black light traps to sample *H. halys*. Subsequently, it was shown that *Halyomorpha halys* is cross-attracted to the aggregation pheromone of *Plautia stali* Scott (Hemiptera: Pentatomidae), 2,4,6-E,E,Z methyl-decatrienoate (MDT) (Khrimian et al. 2008). Leskey et al. (2012c) found that captures of *H. halys* were greater in black pyramid traps

baited with MDT compared with other trap colors and designs. In 2014, a male-produced *H. halys* aggregation pheromone, (3S,6S,7R,10S)-10,11- epoxy-1-bisabolen-3-ol and (3R,6S,7R,10S)-10,11-epoxy-1- bisabolen-3-ol, of *H. halys*, was identified (Khrimian et al. 2014). Combining MDT lures and lures containing the male aggregation pheromone significantly increased (synergized) captures of *H. halys* (Weber et al. 2014). Initially, black pyramid traps baited with lures containing the aggregation pheromone and pheromone synergist became the standard method for *H. halys* sampling. Subsequently, dual pheromone-MDT lures deployed with clear sticky panels mounted atop wooden stakes in the ground have proven effective. Although captures in baited pyramid traps typically exceeded those in sticky traps, captures in sticky traps and pyramid traps were significantly correlated, regardless of whether population density was low, medium, or high (Acebes-Doria et al. 2018).

Although alternative tactics for managing *H. halys* and its injury are being investigated (Blaauw et al. 2014, Morrison et al. 2016a, Short et al. 2017), current management practices in tree fruit orchards continue to rely primarily on air-blast applications of broad-spectrum insecticides (Leskey and Nielsen 2018). The insecticides used in fruit orchards differ widely in their efficacy against *H. halys* (Leskey et al. 2012b, Kuhar and Kamminga 2017) and most of the effective products have relatively brief residual activity (Leskey et al. 2014). Moreover, orchards can be invaded continuously throughout the growing season by adults moving from the many wild tree species bordering most orchards (Nielsen and Hamilton 2009a), resulting in the need for multiple insecticide applications (Leskey and Nielsen 2018). The use of broad-spectrum insecticides against *H. halys* during the post-bloom period in apple orchards has contributed to the outbreak of secondary pests, such as woolly apple aphid (*Eriosoma lanigerum*), San Jose scale (*Quadraspidiotus perniciosus*), and European red mite (*Panonychus ulmi*) (Leskey et al 2012a). Other management options such as pheromone-based attract-and-kill (Morrison et al. 2016a), pheromone trap-based thresholds to inform spray timing (Short et al. 2017), and border sprays (Blaauw et al. 2014) have shown considerable potential to reduce insecticide inputs against *H. halys* in orchards, but are not considered long-term solutions to the *H. halys* problem (Leskey and Nielsen 2018). One of the most promising long-term management strategies for suppressing *H. halys* populations in the landscape is biological control, or the use of natural enemies to regulate *H. halys* populations (Leskey and Nielsen 2018).

## 1.6 Biological control

Biological control is one of the safest and most effective mechanisms by which pest populations and crop injury can be reduced (Bianchi et al. 2006, van Lenteren et al. 2006, 2018). Aside from biological control that occurs naturally and without human intervention in some systems, there are essentially three ways by which biological control can be manipulated or promoted: conservation, augmentation, and introduction (classical). Conservation biological control is the provisioning of appropriate habitat resources, such as food, alternative hosts, nesting areas, overwintering habitat, and shelter from climatic changes, with the intent to improve the abundance and efficacy of native natural enemies (Landis et al. 2000). Augmentative biological control is the release of natural enemies in a given area to temporarily supplement local biological control, as with control of *Bemisia argentifolii* (Gennadius) (Homoptera: Aleyrodidae) using the parasitoid *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) (Hoddle et al. 1997, van Lenteren 2012). Classical biological control involves the importation, rearing, and release of a pest's natural enemy in a new location to reduce pest populations. Some of the most successful examples of biological control of insects utilized classical biological control, such as releasing the vedalia beetle, *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae) against cottony cushion scale (*Icerya purchasi*) (Maskell) (Hemiptera: Monophlebidae) (Caltagirone 1981, Caltagirone and Doult 1989). The overall establishment rate of introduced natural enemies via classical biocontrol is relatively low, at only 10-34% (Gurr and Wratten 2012), but they are more likely to establish if their coevolved hosts are present in sufficient numbers and the landscape contains relatively stable habitats, such as forests (Hall and Ehler 1979).

The implementation of modern classical biological control requires thorough testing of potential exotic natural enemies prior to release to determine key biological information, such as physiological and behavioral host range, and dispersal capacity. Centrifugal testing of these parameters has resulted in relatively few occurrences of non-target effects after release (van Lenteren et al. 2006). Even when deliberately introduced after careful consideration, classical biological control efforts can fail, meaning that the introduced natural enemy does not establish or does not sufficiently reduce pest populations, due to climatic incompatibility, predation or

parasitism, or lack of alternative hosts, among other causes (Hall and Ehler 1979, Stiling 1993, Gurr and Wratten 2012, Goldson et al. 2014). Occasionally, adventive populations of exotic natural enemies have established in new areas. Adventive natural enemies are those that arrive in a new locality by any means rather than via deliberate release efforts (Pemberton 1964). An adventive population of *Stethoconus japonicus* (Schumacher) (Hemiptera: Miridae), a predator of azalea lace bug (*Stephanitis pyrioides*) (Scott) (Hemiptera: Tingidae), was discovered incidentally during a voltinism study by Neal et al. (1991). The arundo wasp (*Tetramesa romana*) (Walker) (Hymenoptera: Eurytomidae) was to be released to control the giant reed (*Arundo donax*) (L.) (Poales: *Poaceae*) in the southwestern USA, but prior to its release several adventive populations of *T. romana* were detected in southern Texas (Racelis et al. 2009). Endemic to Europe, adventive populations of the predatory 14-spotted ladybird beetle (*Propylea quatuordecimpunctata*) (L.) (Coleoptera: Coccinellidae) were detected in the USA and Canada (Hoebeke and Wheeler 1996, Pervez and Omkar 2011). Populations of *Trichopoda pennipes* Fabr. (Diptera: Tachinidae), a North American parasitoid of pentatomids, including *Nezara viridula* (L.) (Hemiptera: Pentatomidae), were discovered in Italy (Colazza et al. 1996). Greater risk of non-target effects can be associated with adventive biological control agents, especially when they are redistributed. For example, the European thistle weevil (*Larinus planus*) (Fabr.) (Coleoptera: Curculionidae), a natural enemy of Canada thistle (*Cirsium arvense*) (L.) (Asterales: Asteraceae), was released in Colorado after the discovery of an adventive population in the northeastern USA and subsequently attacked native thistles in its introduced range (Louda et al. 2003). Overall, the establishment of adventive biological control agents occurs infrequently and has not been well-studied.

### **1.7 Biological control of *H. halys* by native natural enemies in the USA**

In its native Asian range, *H. halys* does not have the same pest status as it does in the United States. This is due in part to release from natural enemy control, when invasive species flourish in the absence of their coevolved natural enemies (Vilà et al. 2005, Liu and Stiling 2006). Generalist arthropod natural enemies, which tend to be more abundant than specialists in agricultural landscapes (Landis et al. 2000), may have the potential to regulate *H. halys* populations but as yet have not suppressed it adequately in the USA, typically causing less than

25% of observed egg mortality (Abram et al. 2017b). A two-year study in organic orchards in the USA showed that only 19-20% of *H. halys* eggs were attacked by natural enemies, with the majority of biological control provided by generalist chewing predators (Ogburn et al. 2016). Katydid (Orthoptera: Tettigoniidae) and ground beetles (Coleoptera: Carabidae) are among the most effective predators of *H. halys* eggs, with earwigs (Dermaptera), crickets (Orthoptera: Gryllidae), and jumping spiders (Araneae: Salticidae) playing a lesser role (Morrison et al. 2016b). *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) and *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae) showed higher predation on eggs of the native, spined soldier bug, *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) than on those of *H. halys* (Abram et al. 2014b). The microsporidian, *Nosema maddoxi* sp. nov. (Microsporidia: Nosematidae), originally isolated from green stink bug (*Chinavia hilaris*) (Say) (Hemiptera: Pentatomidae), has been detected in wild *H. halys* populations and is suspected to contribute to decreased survival and fecundity (Hajek et al. 2018).

Several tachinid parasitoids (Diptera: Tachinidae) will oviposit on *H. halys* adults and nymphs, although this is uncommon (Abram et al. 2017b). Parasitoids in the families Scelionidae, Platygasteridae, Eupelmidae, and Encyrtidae will parasitize *H. halys* and other pentatomid eggs (Abram et al. 2017b). In a long-term study of the egg parasitoids of *H. halys* in unmanaged habitats in Delaware, Dieckhoff et al. (2017) reported very low levels of parasitization by the native parasitoid complex, suggesting that they did not contribute significantly to *H. halys* biological control. Cornelius et al. (2016) observed no differences in parasitization of sentinel *H. halys* egg masses deployed in exotic or native host plants, but observed greater parasitization of eggs deployed at the edge of unmanaged woods. The extent to which the native parasitoid taxa attack *H. halys* is variable and may be habitat-dependent. For example, Herlihy et al. (2016) reported significantly greater *H. halys* sentinel egg parasitization by *T. japonicus* and native *Trissolcus* in wooded habitat compared to soy or apple plantings. Abram et al. (2017b) summarized the results of *H. halys* biocontrol research in North America, reporting that, generally, *Telenomus* species (Hymenoptera: Scelionidae) were detected most frequently in orchard and field/vegetable crop habitats, *Anastatus* spp. (Hymenoptera: Eupelmidae) in forests, orchards, and ornamentals, while *Trissolcus* spp. were associated with semi-natural and forest habitats. Parasitism of wild and sentinel *H. halys* eggs can vary from 0-59% (Abram et al.

2017b). Native parasitoids, such as *Trissolcus euschisti* (Ashmead) (Hymenoptera: Scelionidae), will attack *H. halys* eggs, but often cannot complete development due to the host's immune response (Konopka et al. 2018, 2019). This non-reproductive host mortality may be a source of *H. halys* mortality in the field, but is difficult to quantify in wild *H. halys* populations (Kaser et al. 2018a). The addition of methyl salicylate to the *H. halys* aggregation pheromone in association with sentinel egg masses deployed on a variety of plant species did not appear to enhance predation or parasitization (Morrison et al. 2018). In contrast to the findings reported by Abram et al. (2017b) showing that *Telenomus podisi* was a common parasitoid of *H. halys* eggs in orchard and field/vegetable crops, Tognon et al. (2017) observed that kairomones from *H. halys* eggs inhibit parasitization by *Telenomus podisi* Ashmead and *Trissolcus erugatus* Johnson (Hymenoptera: Scelionidae). In a follow-up study however, they demonstrated that the same parasitoids learned to recognize these cues as being indicative of a suitable host (Tognon et al. 2019), meaning that they could potentially attack *H. halys* eggs with greater frequency over time.

### **1.8 Biological control of *H. halys* by *Trissolcus japonicus***

The parasitoid of *H. halys* most frequently collected in Asia is *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) (Zhang et al. 2017), a solitary endoparasitoid of pentatomid eggs (Yang et al. 2009) that was recently given the ESA-approved common name, samurai wasp. Other *Trissolcus* spp., *Ooencytus* spp. (Hymenoptera: Encyrtidae), and *Anastatus* spp. have been observed less frequently (Arakawa and Namura 2002, Lee et al. 2013, Zhang et al. 2017). Talamas et al. (2015a) developed a key to identify and differentiate *T. japonicus* from other Nearctic *Trissolcus* species. Female *T. japonicus* parasitize an average of 42 eggs and prefer eggs that are less than 72 hours old (Qiu et al. 2007). Yang et al. (2009) reported that up to 80% of wild *H. halys* egg masses were parasitized by *T. japonicus*, and in the laboratory, 70.6% of *H. halys* eggs parasitized by *T. japonicus* produced females (Zhang et al. 2017). Unfertilized *H. halys* eggs produced a greater proportion of female *T. japonicus* than fertilized eggs (Yang et al. 2018). In the laboratory, *T. japonicus* demonstrated a strong preference for *H. halys* eggs in dual choice experiments, although this was slightly reduced when *T. japonicus* emerged from the eggs of other pentatomid species (Botch and Delfosse 2018). In China, non-target pentatomids were attacked by *T. japonicus* under laboratory and field conditions, but in the field, it emerged most

frequently from *H. halys* eggs (Zhang et al. 2017). Other species of parasitoids exhibit much lower levels of successful parasitization of *H. halys* eggs compared with *T. japonicus*, possibly due to differences in developmental compatibility or the efficiency of the venom injected (Haye et al. 2015b), but the exact mechanisms which impede development remain unknown (Konopka et al. 2019). The innate immune response of hosts can reduce successful parasitoid development in other host-parasitoid relationships (Abdel-Latif and Hilker 2008). When parasitoids attack an unsuitable host, the nontarget host species can act as an “evolutionary trap,” reducing the reproductive success of the parasitoid, potentially affecting population dynamics of both host and parasitoid (Abram et al. 2014a, Konopka et al. 2018). Under laboratory conditions, *T. japonicus* will attack the eggs of some stink bugs native to the USA (e.g. the beneficial *Podisus maculiventris* (Say)) (Abram et al. 2014a), and New Zealand (e.g. *Cermatulus nasalis nasalis* (Westwood)) (Charles et al. 2019).

The efficacy of *T. japonicus* against *H. halys* in its native range made it an appealing candidate for classical biological control of *H. halys* in the USA. As such, starting in 2007, *T. japonicus* from Beijing, China were tested in quarantine at the USDA/ARS Beneficial Insect Introduction Research Unit in Delaware and other laboratories in the USA. In 2014, an adventive population of *T. japonicus* was detected from six sentinel egg masses at the edge of wooded habitat in Beltsville, MD (Talamas et al. 2015b). Microsatellite analysis of specimens from this detection revealed that they were genetically distinct from those in quarantine culture in the USA, ruling out their escape from quarantine (Bon et al. 2017). Sampling and identification efforts in the Mid-Atlantic were subsequently intensified, and in 2015 *T. japonicus* was detected in Winchester, VA (J.C. Bergh, unpublished data). Subsequently, it has been detected in 12 states and the District of Columbia in the USA (Herlihy et al. 2016, Milnes et al. 2016, Hedstrom et al. 2017, Morrison et al. 2018), and in Canada, Italy, and Switzerland (Costi et al. 2019, Peverieri et al. 2018, Stahl et al. 2019, Abram et al. 2019). The spread of *T. japonicus* in the USA and other parts of the world where *H. halys* is present is expected to continue, depending upon climate and host availability (Nystrom Santacruz et al. 2017, Avila and Charles 2018).

The ecological impact of *T. japonicus* on native pentatomids and their parasitoids needs further study. There is some concern about the risk of nontarget effects by *T. japonicus* (Zhang et al.

2017, Avila and Charles 2018). However, Hedstrom et al. (2017) reported that adventive *T. japonicus* attacked a higher proportion of *H. halys* than native pentatomid eggs in paired choice tests, suggesting limited non-target impact. In laboratory studies, *T. japonicus* responded more strongly to leaf surfaces contaminated by *H. halys* compared with those contaminated by *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), further suggesting *H. halys* is its preferred host (Boyle et al. 2017). *Trissolcus japonicus* were significantly attracted to volatiles from plants on which *H. halys* had oviposited and fed on and did not respond to clean plants or those on which *P. maculiventris* had oviposited (Bertoldi et al. 2019). In a field study in Washington state, adventive *T. japonicus* attacked native pentatomids at significantly lower rates than *H. halys* eggs (Milnes and Beers 2019). Konopka et al. (2017) concluded that *T. japonicus* has the potential to add to total biological control of *H. halys* rather than outcompeting native parasitoids. This is due to counterbalance competition in which *T. japonicus* is a superior extrinsic competitor via guarding egg masses while *Anastatus bifasciatus* is a superior intrinsic competitor given its ability to develop in hosts of varying ages (Konopka et al. 2017).

*Trissolcus japonicus* does not appear to respond to the *H. halys* aggregation pheromone (Weber et al. 2017, Morrison et al. 2018), but is attracted to n-tridecane, a defensive compound produced by *H. halys* (Zhong et al. 2017). *Trissolcus japonicus* host searching intensity, as measured by its speed of movement and turning frequency, increased in the presence of the footprints of gravid *H. halys*, even when egg masses or adults were not physically present (Boyle 2017). Bertoldi et al. (2019) found that *T. japonicus* responded to adult male and female *H. halys* kairomones but did not respond to *H. halys* egg kairomones. Other *Trissolcus* species respond to plant volatiles (Colazza et al. 2004, Islamoğlu and Koçak 2014), contact cues from egg adhesives (Conti et al. 2003, 2004), and the color yellow (Ferreira Santos de Aquino et al. 2012). The majority of *Trissolcus* studied thus far seem to rely most heavily on kairomones from adult pentatomids rather than the eggs themselves (Bruni et al. 2000, Conti et al. 2003, 2004, Salerno et al. 2006, Laumann et al. 2009, Tognon et al. 2016, Boyle 2017, Rondoni et al. 2017, Bertoldi et al. 2019). This is known as the “infochemical detour strategy” (Vet and Dicke 1992) for solving the host cue reliability-detectability problem, in which the more conspicuous but less reliable cues of non-target stages of the host are used to locate the target life stage. However, the cues utilized by *T. japonicus* to locate egg masses in the field are not well understood.

Little is known of the habitat preferences of *T. japonicus*. In Asia, it was detected most frequently in areas of high *H. halys* density but was not associated with a particular habitat or host plant (Zhang et al. 2017). *Trissolcus semistriatus* (Nees) (Hymenoptera: Scelionidae) and *Trissolcus grandis* (Thomson) (Hymenoptera: Scelionidae), parasitoids of *Eurygaster integriceps* (Puton) (Hemiptera: Scutelleridae) eggs in Iran, were associated with semiarid climates with mild winters and summers (Yasemi et al. 2015, 2016). In the USA, Tillman (2016) found more pentatomid egg masses parasitized by *Trissolcus* species in forest habitats compared with cultivated areas. In a study of the effect of habitat partitioning by host plant of *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) egg parasitization in the field, *T. euschisti* only attacked egg masses on hackberry trees (*Celtis occidentalis*) (L.) (Rosales: Cannabaceae), but attacked none of the eggs deployed on alfalfa (Okuda and Yeargan 1988). Other *Trissolcus* species require nectar or honey sources to improve their survival and fecundity (Rahat et al. 2005, Foti et al. 2017), and in the laboratory, the provisioning of honey improves *T. japonicus* survival (C. Dieckhoff unpublished data). Presumably, *T. japonicus* in the field also require floral resources in addition to pentatomid eggs to reproduce, but their foraging ecology in the field remains to be described. The first detection of *T. japonicus* in Washington State occurred via a wild-collected *H. halys* egg mass collected from a lightly wooded habitat in a suburban area (Milnes et al. 2016). In New Jersey, *T. japonicus* emerged from sentinel egg masses that had been deployed in a commercial peach orchard (Kaser et al. 2018b), but thus far *T. japonicus* has not been reported from wild egg masses from within an orchard or other cropped area. As one of the most important natural enemies of *H. halys* in its native range, it is essential to improve our understanding of the biology and ecology of adventive populations of *T. japonicus* in the USA, which will rely heavily on sampling and monitoring.

### **1.9 Monitoring parasitoids of *H. halys***

Monitoring and studying adventive populations of *T. japonicus* necessitates the use of effective sampling tools. There is a wide range of methods available for sampling parasitoids in general, including sweep-nets, malaise traps, pan traps, flight intercept traps, canopy fogging, sticky traps, and sentinel hosts (Chambers et al. 1986, Weseloh 1986, Noyes 1989, Fraser et al. 2008,

Krewenka et al. 2011, Bennett and Gratton 2012, Letourneau et al. 2012, Veijalainen et al. 2012). All of these techniques offer tradeoffs in performance and sampling efficiency, depending upon the environment in which they are deployed and the parasitoid species targeted. For example, Noyes (1989), found yellow pan traps to be among the most effective sampling methods for scelionids, but their efficacy was reduced in areas of low visibility (e.g. thick vegetation). Most studies of *H. halys* parasitoids, many of which are scelionids, have used sentinel egg masses (Abram et al. 2017b). As discussed in Chapter 3, sentinel egg masses are essential to addressing key biological questions, such as 1) the incidence of egg predation and parasitization, 2) which parasitoids attack *H. halys* eggs in the field, and 3) whether these species can develop successfully in *H. halys* eggs. However, for questions about monitoring the presence, establishment, spread, and relative abundance of parasitoids, the use of sentinel eggs may not be optimal. Despite all of the resources required to deploy sentinel egg masses, it is not unusual for fewer than 2.5% of sentinel eggs deployed to be parasitized (Abram et al. 2017b), a relatively low return on sampling effort. Recently, Garipey et al. (2018) developed a method for identifying parasitoids within sentinel egg masses via DNA barcoding, but this method is not widely or readily accessible. Given the anticipated spread of *T. japonicus* and its impact on *H. halys* populations, there is a need to develop a simple, optimized, and standardized sampling protocol that is based on the biology of the host and the parasitoid and that can be used widely to improve our understanding of the distribution, biology, and ecology of the adventive population.

### **1.10 Justification for Research**

*Trissolcus japonicus* is anticipated to significantly impact *H. halys* populations in the USA, as it does in Asia. Since 2014, the number and rate of detections of adventive *T. japonicus* populations in the USA has increased steadily, although little is currently known about key aspects of its foraging ecology and its geographic distribution. This is compounded by the inefficiency of current monitoring techniques for addressing certain research questions. There is a need to develop efficient monitoring tools for *T. japonicus* to understand when and where to sample for it most effectively, improving the ability to track its establishment, spread, and population growth, and ultimately evaluate its impact on *H. halys* populations.

This dissertation aims to describe and quantify aspects of the foraging ecology of *H. halys* and its natural enemies, with a focus on *T. japonicus*, via various sampling methods. In Chapter 2, the vertical distribution of *H. halys* and its natural enemies in wild host trees is described. Chapter 3 compares sentinel egg masses and yellow sticky traps for sampling *T. japonicus* and other *H. halys* parasitoids. In Chapter 4, the effects of habitat type on captures of *T. japonicus* and other *H. halys* parasitoids and the seasonal phenology of *T. japonicus* captures is presented. Lastly, Chapter 5 examines the effects host plant species on *T. japonicus* and other *H. halys* parasitoid captures.

### **1.11 Research objectives**

**Objective 1:** Determine the vertical distribution of *H. halys* life stages and its egg parasitoids in the canopy of tree hosts.

**Objective 2:** Compare sampling methods for monitoring *T. japonicus* and other *H. halys* parasitoids.

**Objective 3:** Determine the effect of habitat type on the frequency of *T. japonicus* captures and document the seasonality of captures.

**Objective 4:** Determine the effect of *H. halys* host plant species on captures of *T. japonicus* and other *H. halys* parasitoids.

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## Chapter 2

### Vertical sampling in tree canopies for *Halyomorpha halys* (Hemiptera: Pentatomidae) life stages and its egg parasitoid, *Trissolcus japonicus* (Hymenoptera: Scelionidae)

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#### 2.1 Abstract

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive agricultural and nuisance pest that has established across much of the USA and caused significant crop losses in the Mid-Atlantic region. While it has been monitored extensively using ground-deployed pheromone traps, the vertical distribution of its life stages in the canopy of wild tree hosts has not been examined. In Virginia, small pyramid traps baited with “low-dose” *H. halys* pheromone lures were deployed via a pulley system at the lower, mid-, and upper canopy of female tree of heaven (*Ailanthus altissima* (Mill.) Swingle) in 2016 and 2017 and male *A. altissima* and hackberry (*Celtis occidentalis* L.) in 2017. Weekly captures of adults and nymphs were recorded throughout each season. Each year, additional female *A. altissima* trees were felled during the two main periods of *H. halys* oviposition. The number and relative locations of all pentatomid egg masses found on foliage were recorded and any parasitoids that emerged from them were identified. *Halyomorpha halys* adults and nymphs were captured in greatest numbers in upper canopy traps and in lowest numbers in traps near the tree base. More *H. halys* egg masses were collected from mid-canopy than from the lower or upper canopy. The adventive egg parasitoid, *Trissolcus japonicus* (Ashmead), emerged most frequently from egg masses found at

mid-canopy and was not recovered from those in the lower canopy. Results are discussed in relation to the foraging ecology of *H. halys* and its natural enemies, including *T. japonicus*.

## 2.2 Introduction

*Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is an invasive pest from Asia (Hoebeke and Carter 2003) that feeds or reproduces on many cultivated and wild plants ([www.stopbmsb.org](http://www.stopbmsb.org)). A widespread outbreak of *H. halys* in the Mid-Atlantic region of the USA in 2010 resulted in losses of over \$37 million to the apple crop and severe impacts to many peach orchards (Leskey et al. 2012). Given its broad host range, high mobility (Lee et al. 2014, Wiman et al. 2015, Lee and Leskey 2015), and propensity to “hitchhike” in human conveyances, *H. halys* has now been detected or established in 44 states, four Canadian provinces ([www.stopbmsb.org](http://www.stopbmsb.org)), and several countries abroad (Leskey and Nielsen 2018).

*Halyomorpha halys* is not known to reside permanently in any crop, but moves into crops from its many wild hosts (Bakken et al. 2015). It is considered a perimeter-driven pest, and injury from its feeding is often greatest at crop borders next to wooded areas (Leskey et al. 2012, Joseph et al. 2014, Venugopal et al. 2015, Bergmann et al. 2016). Given its wide distribution and mobility in the landscape, insecticide applications can reduce *H. halys* injury to crops but likely do not have a substantial effect on its pest pressure overall. Most of the effective insecticides for managing *H. halys* injury to crops are toxic to natural enemies, resulting in disruption of integrated pest management programs and frequent outbreaks of secondary pests (Rice et al. 2014, Leskey and Nielsen 2018). Thus, effective biological control of *H. halys* in non-crop habitats is considered a key element of its sustainable management.

In the USA, numerous native parasitoids and predators that attack *H. halys* have been identified, but have not regulated its populations adequately (Abram et al. 2017). During the summer of 2014, an adventive population of the Asian parasitoid of *H. halys* eggs, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), was detected from samples collected in Beltsville, MD (Talamas et al. 2015a). Subsequent detections have occurred in the Mid-Atlantic and Pacific Northwest (reviewed in Leskey and Nielsen 2018). In Frederick County, VA, *T. japonicus* has

been detected annually since 2015 (Quinn, unpublished data). Given the important role of *T. japonicus* in regulating *H. halys* in its native Asian range (Yang et al. 2009, Zhang et al. 2017), this significant development may result in improved biocontrol services in the USA (Abram et al. 2017).

Typically, pheromone-based monitoring of *H. halys* has used ground-deployed traps (Leskey et al. 2015, Morrison et al. 2015). However, anecdotal and experimental observations suggest that *H. halys* activity may be most abundant in the upper canopy of host trees. B.D. Short (USDA ARS, personal communication) observed high numbers of *H. halys* and appreciable feeding injury from it at the top of tree of heaven, *Ailanthus altissima* (Mill.) Swingle, and Joseph et al. (2014) recorded greatest fruit injury from *H. halys* at the top of apple trees in commercial orchards. If *H. halys* density is stratified vertically in the host tree canopy, the distribution and density of its egg masses, and thus of its egg parasitoids, may also be similarly stratified. Understanding these distributions would greatly enhance our capacity to monitor the presence, abundance, and impact of its egg parasitoids, including *T. japonicus*.

This study presents data from trapping and destructive sampling studies that examined: 1) the vertical distribution of *H. halys* captures in pheromone traps, 2) the vertical distribution of its egg masses, and 3) the predation and parasitization of these egg masses and the parasitoid species recovered from them.

## **2.3 Materials and Methods**

### *Trapping Sites and Tree Hosts*

All studies were conducted in Frederick County, VA. The host trees used for trapping were located at Virginia Tech's Alson H. Smith, Jr. Agricultural Research and Extension Center (AREC) near Winchester, VA (39.112867, -78.284029) and at three commercial fruit orchards within 20 km of the AREC. Given that tree of heaven was the most common deciduous tree species in the local landscape (Acebes-Doria et al. 2017) and that it can harbor large populations of *H. halys* in the eastern USA (Bakken et al. 2015) (Fig. 2.1), female tree of heaven (n = 5) were

selected for sampling in 2016 and 2017, using the same trees each year. In 2017, male tree of heaven ( $n = 5$ ) and hackberry, *Celtis occidentalis* L. ( $n = 5$ ) were also sampled. Hackberry is a host of *H. halys* ([www.stopbmsb.org](http://www.stopbmsb.org)) and was the second most common deciduous tree species recorded at the woodland edge in this region (Acebes-Doria et al. 2017). All trees were located at the woods edge adjacent to experimental or commercial apple and peach orchards. Each sample tree was  $\geq 20$  m from other trees of the same sex or species and  $\geq 101$  m from other trees having traps. Trees of similar height ( $9.2 \text{ m} \pm 0.29 \text{ SE}$ ), DBH ( $24.1 \text{ cm} \pm 1.3 \text{ SEM}$ ), and architecture (i.e. mostly upright, with branching along most of the trunk), were selected. All trees bore fruit during the season, with the exception of male tree of heaven.

### *Pheromone Trap Transects*

Professional arborists inserted an eye bolt near the top and bottom of the trunk of each tree. A pulley system was created by running a rope through these and attaching the ends, creating a loop that enabled raising and lowering the traps easily for sampling along the length of the trunk. The rope was attached to the bottom eye bolt using a carabiner, and the vertical distance between the top and bottom bolt was measured, enabling even spacing of traps. Three 61 cm tall pyramid traps (Dead-Inn, AgBio, Westminster, CO) were deployed in each tree (Fig. 2.2A). These were baited with a low-dose residential *H. halys* lure containing its aggregation pheromone, (3*S*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol and (3*R*,6*S*,7*R*,10*S*)-10,11-epoxy-1-bisabolen-3-ol (Khrimian et al. 2014) and its pheromone synergist, methyl (2*E*,4*E*,6*Z*)-decatrienoate (MDT) (Weber et al 2014), as well as the main component of the aggregation pheromone of *Euschistus* spp., methyl decadienoate, at loadings of 17 mg, 17 mg, and 7.5 mg, respectively (AgBio, Westminster, CO). Along with lures, half of a dichlorvos-impregnated strip (Vaportape, Hercon, York, PA) hung within the collection jar atop the trap served as the killing agent. Lures and dichlorvos strips were replaced at 14 day intervals, according to manufacturer specifications and field data (Joseph et al. 2013). The bottom trap was attached to the tree trunk at  $1.04 \pm 0.08 \text{ SE m}$  above the ground, and the two other traps were attached to the rope and positioned, respectively, at the approximate midpoint between the upper and lower bolts and just below the upper bolt (Fig. 2.2 A–B). Traps at mid- and upper-canopy, respectively, were  $4.30 \pm 0.39 \text{ SE m}$  and  $8.60 \pm$

0.79 SE m above the bottom traps. The number of *H. halys* adults and nymphs captured in each trap was recorded weekly from mid-April until mid-October.

### Destructive Sampling

Mature female tree of heaven growing at the edge of woods next to three commercial fruit orchards within 12 km of the AREC were chosen, using the same selection criteria described above. Trees selected for destructive sampling  $\geq 230$  m away from trees with pheromone traps. In 2016, trees were felled between 21–23 June ( $n = 5$ ) and between 2–5 August ( $n = 5$ ), and in 2017, trees were felled between 20–21 June ( $n = 5$ ) and 8–9 August ( $n = 5$ ). The June and August samples occurred within the predicted periods of oviposition by the overwintering and summer generations, respectively, of adult *H. halys* (Nielsen et al. 2016). After felling, total tree height, height of each branch at its growth point from the trunk, and stump circumference ( $\sim 1$  m above the ground) were measured, and all branches were flagged and numbered. The mean ( $\pm$  SE) height of the felled trees in 2016 and 2017, respectively, was  $9.9 \pm 0.47$  m and  $10.16 \pm 3.22$  m. The number of fruiting structures (samaras) was also recorded. All leaves were inspected, and all pentatomid egg masses found were collected and identified using visual appearance and comparisons with the field guide of Herbert et al. (2014). For each egg mass found, the length of the branch on which it occurred, its linear distance from the trunk (based on branch length), and its approximate height were recorded. The foliage from each branch was placed in paper landscape bags and air-dried in a greenhouse for determination of foliar biomass.

Egg masses were held individually in small Petri dishes in a controlled-environment chamber at 25°C and a 16:8 L:D photoperiod. The eggs in each mass were counted and categorized as to whether they were intact, had yielded nymphs previously (based on the presence of an egg burster), had yielded parasitoids previously (based on the presence of circular parasitoid emergence holes), or had been preyed upon, as per Morrison et al. (2016). Masses with intact eggs were monitored daily for emergence of *H. halys* nymphs or parasitoids. Specimens of all parasitoids that emerged were identified using the keys of Johnson (1984), Talamas et al. (2015b), and Burks (1967).

### Statistical Analysis

Data analyses used SAS 9.4 (SAS Institute, Cary, NC) and  $\alpha = 0.05$ . For each year, captures of *H. halys* from vertical trap transects were log transformed and compared among trap locations (upper, mid, or lower canopy) and tree type (female tree of heaven, male tree of heaven, or hackberry) using generalized linear mixed models (GLMMs) with Laplace approximation and log normal distribution, with trap location as the main effect. The interaction of trap location and sampling date were nested within sample tree as random effects. From the destructive sampling, Kendall's Tau-b correlation coefficients were used to examine the relationships between the number of pentatomid egg masses collected, *T. japonicus* detection, tree height, branch height, branch length, tree circumferences, linear distance from trunk, dry foliar biomass, and number of samaras.

## 2.4 Results

### *Pheromone Trap Transects*

In 2016, there was a significant effect of trap location on mean weekly captures of *H. halys* adults ( $F_{2,121} = 39.87$ ,  $P < 0.01$ ) and nymphs ( $F_{2,59} = 35.61$ ,  $P < 0.01$ ) in female tree of heaven. Adult captures differed significantly among the three canopy locations, with highest and lowest numbers in the upper and lower traps, respectively (Fig. 2.3). Captures of nymphs were significantly greater in the upper canopy than in mid- or lower canopy, which did not differ significantly from each other (Fig. 2.3).

In 2017, no significant effect of tree species on mean weekly captures of adults ( $F_{2,296} = 0.085$ ,  $P = 0.43$ ) or nymphs ( $F_{2,141} = 0.33$ ,  $P > 0.05$ ) (Fig. 2.4A); therefore, host species was excluded from the model and captures were compared by canopy location. Captures of adults in the upper canopy were significantly greater than in the mid- and lower canopy, which did not differ significantly from each other ( $F_{2,296} = 55.81$ ,  $P < 0.05$ ) (Fig. 2.4B). Similarly, nymph captures were significantly greater in the upper canopy than in the mid- and lower canopy, which did not differ ( $F_{2,141} = 2.40$ ,  $P > 0.05$ ) (Fig. 2.4B).

### *Destructive Sampling: Egg Masses*

In total, 38 and 20 pentatomid egg masses were collected in 2016 and 2017, respectively. All egg masses were recovered from the underside of leaves. Fifty-one (87.9%) of these were identified as *H. halys*, with the remainder identified as *Chinavia hilaris* (Say) (n = 2), *Nezara viridula* (L.) (n = 2), *Euschistus* spp. (n = 2), and *Podisus maculiventris* Say (n = 1). *Halymorpha halys* egg masses were found in eight of the ten trees in 2016 (range = 1 to 8 per tree) and in six of the ten trees (range = 1 to 6 per tree) in 2017. The majority (58.8%) of the 51 *H. halys* egg masses were from the middle third of the canopy (n = 30), with essentially equal numbers from the upper (n = 11) and lower (n = 10) thirds. Of the seven variables measured from the felled trees (Table 2.1), the number of *H. halys* egg masses found in 2016 was significantly correlated with branch length (r = 0.20, n = 157,  $P < 0.001$ ), linear distance from the trunk (r = 0.24, n = 157,  $P = 0.045$ ) and foliar biomass (r = 0.29, n = 157,  $P < 0.0001$ ), and with branch length (r = 0.20, n = 122,  $P = 0.007$ ) in 2017. A marginally significant correlation was found between egg masses and branch height in 2017 (r = 0.14, n = 126,  $P = 0.05$ ).

### *Destructive Sampling: Parasitization and Predation*

All 51 *H. halys* egg masses collected in 2016 and 2017 produced nymphs or parasitoids before and/or after collection, or showed signs of predation. Twenty-four (47.1%) produced only nymphs, eleven (21.6%) yielded only parasitoids, and nine (17.6%) showed indications of predation only. The remainder produced both nymphs and parasitoids (n = 3; 5.9%), yielded nymphs and showed some degree of predation (n = 3; 5.9%), or showed both predation and produced parasitoids (n = 1; 2.0%). Of the 14 egg masses from which adult parasitoids emerged in the laboratory, eight yielded *T. japonicus* adults ( $99.1 \pm 0.9$  SE % eggs parasitized). These were distributed among six trees (range = 1 to 3 per tree), with six egg masses from the middle third of the canopy and the remaining two from the upper third. Detections of *T. japonicus* were positively correlated with tree height (r = 0.15, n = 157,  $P = 0.03$ ) and dry foliar biomass (r = 0.26, n = 117,  $P = 0.008$ ) in 2016, and with branch height (r = 0.15, n = 126,  $P = 0.04$ ) in 2017 (Table 2.1). Six *H. halys* egg masses produced adults of native parasitoid species, including *Telenomus podisi* Ashmead (n = 1; 88.5% eggs parasitized), Pteromalidae (n = 1; 32.1% eggs

parasitized), *Trissolcus brochymenae* Ashmead (n = 1; 85.7% eggs parasitized), and *Anastatus* spp. Motuchulsky (n = 3;  $97.4 \pm 1.8$  SE % eggs parasitized). Two of the *H. halys* egg masses that yielded *Anastatus* spp. were collected from the lower third of the canopy, otherwise all other masses yielding native parasitoids were found in mid-canopy. Of the native pentatomid egg masses collected, the *Podisus maculiventris* egg mass produced *Anastatus* spp., one *Euschistus servus* (Say) egg mass yielded *T. podisi*, one *C. hilaris* egg mass produced *Anastatus redivii* (Howard), and neither *Nezara viridula* egg mass was parasitized. Egg masses showing evidence of complete or partial predation ( $\geq 1$  predated egg; n = 13) had  $81.4 \pm 7.9$  SE % eggs preyed upon.

## 2.5 Discussion

Detection of an adventive population of *T. japonicus* in Maryland in 2014 (Talamas et al. 2015a, Herlihy et al. 2016), and subsequent detections elsewhere in the USA (Leskey and Nielsen 2018), highlighted the need to develop efficient and effective sampling protocols to track its geographic distribution, spread, and population growth. Thus, information about the foraging ecology and distribution of both *H. halys* and *T. japonicus* in the canopy of *H. halys* host trees are of critical importance. Previously, the only information about the distribution of *H. halys* adults and nymphs in the canopy was from anecdotal observations suggesting a higher density in the upper portion of wild hosts. Furthermore, there was no information on the distribution of *H. halys* egg masses, the resource for *T. japonicus*, in the tree. I hypothesized that the distribution of *H. halys* would reflect the distribution of its egg masses and therefore the distribution of *T. japonicus* detections from eggs. Using captures of *H. halys* adults and nymphs in pheromone traps as a proxy for its vertical distribution in known tree hosts, our results indicated that these life stages were most abundant in the mid- and upper canopy. This finding was consistent between two tree species, the sexes of tree of heaven, and between sampling years, despite differences in the number of *H. halys* captured in female tree of heaven in 2016 and 2017. Conforming to these findings, destructive sampling from felled female tree of heaven revealed that 80% of the *H. halys* egg masses occurred in the mid- and upper canopy. Moreover, detections of *T. japonicus* occurred only from *H. halys* egg masses collected from the mid- and upper canopy.

In the absence of information about the distance over which *H. halys* responds to a point source of pheromone, I attempted to reduce the zone of attraction by using a commercially available “residential” lure marketed for homeowner use that contained a lower loading of pheromone than lures used to monitor *H. halys* in agricultural systems. Admittedly, sampling *H. halys* adults and nymphs in the canopy using vertical transects of pheromone traps raises questions about the behavioral response of the insect to the lures. However, the spatial coincidence between captures in traps and the location of egg masses in trees without traps concurs with anecdotal observations of high density in the upper canopy (B.D. Short, personal communication) and experimental data showing greatest fruit injury in the upper canopy of apple trees (Joseph et al. 2014). Moreover, laboratory and field experiments by Acebes-Doria et al. (2016, 2017) showed that *H. halys* nymphs were negatively gravitactic and positively phototactic, and were captured in greater numbers walking up versus down tree trunks. *Euschistus servus*, a common native stink bug pest of many crops, was also captured in greater numbers in pheromone traps in the upper canopy of pecan trees than in other tree locations (Cottrell et al. 2000). Owens et al. (2013) reported that approximately 80% of the population of *C. hilaris* and *E. servus* were located in the upper canopy of mature soybean plants, independent of environmental conditions. Martinson et al. (2015) demonstrated that seasonal differences in *H. halys* abundance across a range of ornamental host trees were associated with host phenology; *H. halys* moved among the hosts in concert with the presence of fruiting structures during the season and were less abundant in experimentally de-fruited trees than in trees with fruit. Interestingly, despite differences in the presence of fruiting structures among the hosts used in the present study in 2017, no significant effect of plant species or sex on the number of *H. halys* adults or nymphs captured was observed.

Pentatomids exhibit differing preferences for oviposition sites. For example, *E. servus* oviposits more commonly on smaller cotton bolls, whereas *N. viridula* prefers larger bolls (Huang and Toews 2012). In general, *H. halys* is considered an arboreal species (Rice et al 2014), although it also feeds and oviposits on a range of plants and shrubs ([www.stopbmsb.org](http://www.stopbmsb.org)) and typically deposits its eggs on the underside of host plant leaves (Nielsen and Hamilton 2009). Female tree of heaven was selected as a model host for most of this work, given that its native range in Asia broadly overlaps with that of *H. halys* and that it very common host of *H. halys* in the eastern

USA (Acebes-Doria et al. 2017, Bakken et al. 2015) that can support large populations of nymphs and adults (Bergh pers. observation; Fig. 2.1). Bakken et al. (2015) used visual inspection of foliage from the ground to document the presence and seasonal abundance of *H. halys* life stages, including egg masses, across a broad range of native and non-native hosts in the eastern USA, and showed that tree of heaven was among the hosts that supported the highest *H. halys* populations. In the present study, destructive sampling of felled female tree of heaven revealed that the preponderance of *H. halys* egg masses were recovered from the mid- and upper canopy, suggesting that ground-based sampling may underestimate egg mass density.

Of the variables examined in relation to the specific location of the *H. halys* egg masses collected, only branch length showed a significant positive correlation with egg mass location in both years. The architecture of tree of heaven is quite different from many of the other common tree hosts of *H. halys*, producing foliage primarily near the end of branches, with long sections of branch that are sparsely foliated (Kowarik and Säumel 2007). Consequently, the outcomes of these analyses must be interpreted in context of the host trees in which sampling occurred. Furthermore, variation among felled trees in the numbers of egg masses collected and a low sample size overall (58 masses from 20 trees) likely affected the outcome of correlation analyses of the other variables.

As for *H. halys*, previous surveillance efforts for *T. japonicus* have also occurred primarily at ground level. In the USA, numerous researchers have detected it using sentinel or naturally laid *H. halys* egg masses in the lower canopy of host trees, and captured it in yellow sticky traps deployed in the lower canopy (Bergh, personal observation). In Asia, ground-based inspection of foliage for *H. halys* egg masses and deployment of sentinel eggs (Zhang et al. 2017) has also yielded *T. japonicus*. Hoelmer (personal communication) stated that, "... in my Asia surveys, a large majority (2/3 to 3/4) of all *H. halys* egg masses discovered during ground-based searches were consistently 100% parasitized by *T. japonicus*. Clearly, *T. japonicus* can locate egg masses within lower strata of host plants, even if the majority of eggs are deposited higher in trees." Unlike in its native Asian range, *T. japonicus* appears to be in early stages of establishment and spread in many parts of the USA, and new state records have occurred each year since its initial detection in Maryland. However, it has not yet been reported from all states where *H. halys* is

established or from all regions within states where detections have occurred. Given that it likely remains a relatively rare species in many locations, our results suggest that sampling for *T. japonicus* in the lower tree canopy may affect the likelihood and frequency of its detection, particularly where its populations are small.

Although few parasitized *H. halys* egg masses were collected from felled trees, this is the first report of *H. halys* biological control in the USA in which *T. japonicus* was the predominant species, based on the percentage of egg masses attacked, and it appears to be establishing well in Frederick County, VA. For example, a female tree of heaven that was growing too close to one of the trees used in the trapping study was felled on 25 June, 2017, and inspection of its foliage revealed seven *H. halys* egg masses, all of which subsequently yielded adults of *T. japonicus*. The even smaller number of *H. halys* egg masses from which native parasitoids emerged permits only species-level comparisons with previous research. Detection of *Anastatus*, *T. podisi*, and *T. brochymenae* concurred with the findings from naturally laid *H. halys* egg masses collected from forest and other habitats (Abram et al. 2017, Dieckhoff et al. 2017, Jones et al. 2017), although other parasitoids were reported in those studies. Across these and the present study, *Anastatus* was the predominant native parasitoid. Dieckhoff et al. (2017) found that the same parasitoid complex attacked both naturally laid and sentinel *H. halys* egg masses. Using sentinel egg masses, Herlihy et al. (2016) and Cornelius et al. (2016) also recovered, among other species, *Anastatus*, *T. podisi*, and *T. brochymenae*, with *Anastatus redivii* being predominant in the former study.

More research on the biotic and abiotic factors that influence the foraging ecology of *T. japonicus* will be required to optimize the effectiveness and efficiency of sampling tools and protocols. Many parasitoids have demonstrated niche partitioning. In a case of spatial niche partitioning, different species of parasitoids of Mexican *Anastrepha* Shiner (Diptera: Tephritidae) differed in their vertical and horizontal distributions in the canopy of fruit trees (Sivinski et al. 1997). Other parasitoids attack hosts on different plant species at different rates. For example, Okuda and Yeargan (1988) observed higher levels of pentatomid egg parasitization by *T. podisi* on alfalfa than hackberry, and the reverse for *Trissolcus euschisti* (Ashmead). It is unknown whether kairomonal cues associated with *H. halys* egg masses attract *T. japonicus* from

some distance, but Tognon et al. (2016) showed that two closely related species, *T. podisi* and *Trissolcus erugatus* Johnson, were attracted to the egg adhesive of *Euchistus conspersus* Uhler. Recently, Boyle (2017) demonstrated that female *T. japonicus* responded to semiochemical cues left by the tarsi of gravid female *H. halys*, perhaps suggesting that the density of *H. halys* in the tree canopy may influence where *T. japonicus* forages most frequently. Similarly, *Trissolcus basalis* (Wollaston) exhibited increased movement speed and turning when exposed to substrate-borne kairomones from *Nezara viridula* (Colazza et al. 2014). Species of *Trissolcus* are also able to discriminate between cues left by walking male and female pentatomids, spending significantly more time on substrates on which females had walked (Peri et al 2013). Furthermore, feeding (Conti and Colazza 2012) and oviposition (Colazza et al. 2014) by pentatomids can induce plants to emit volatiles that attract egg parasitoids. Abiotic factors such as light intensity, temperature, and relative humidity may be directly affected by canopy density (Parker 1995) and may also influence *T. japonicus* foraging behavior.

Our findings add new insights into the foraging ecology of *H. halys* and *T. japonicus* in arboreal habitats. While the sampling methods used (i.e. deploying a pulley system and felling trees) may not be ideal for general adoption, they yielded important information that may guide the development of new and efficient methods to monitor *T. japonicus* in the canopy of *H. halys* host trees, toward documenting its habitat preferences and changes in its geographic distribution and relative abundance. As one example, Quinn (unpublished data) used yellow sticky traps atop bamboo poles that enabled trap deployment in the mid-canopy of host trees to show that the frequency of *T. japonicus* detections in Frederick County, VA, has increased markedly since the present studies were completed, boding well for its potential to impact *H. halys* populations.

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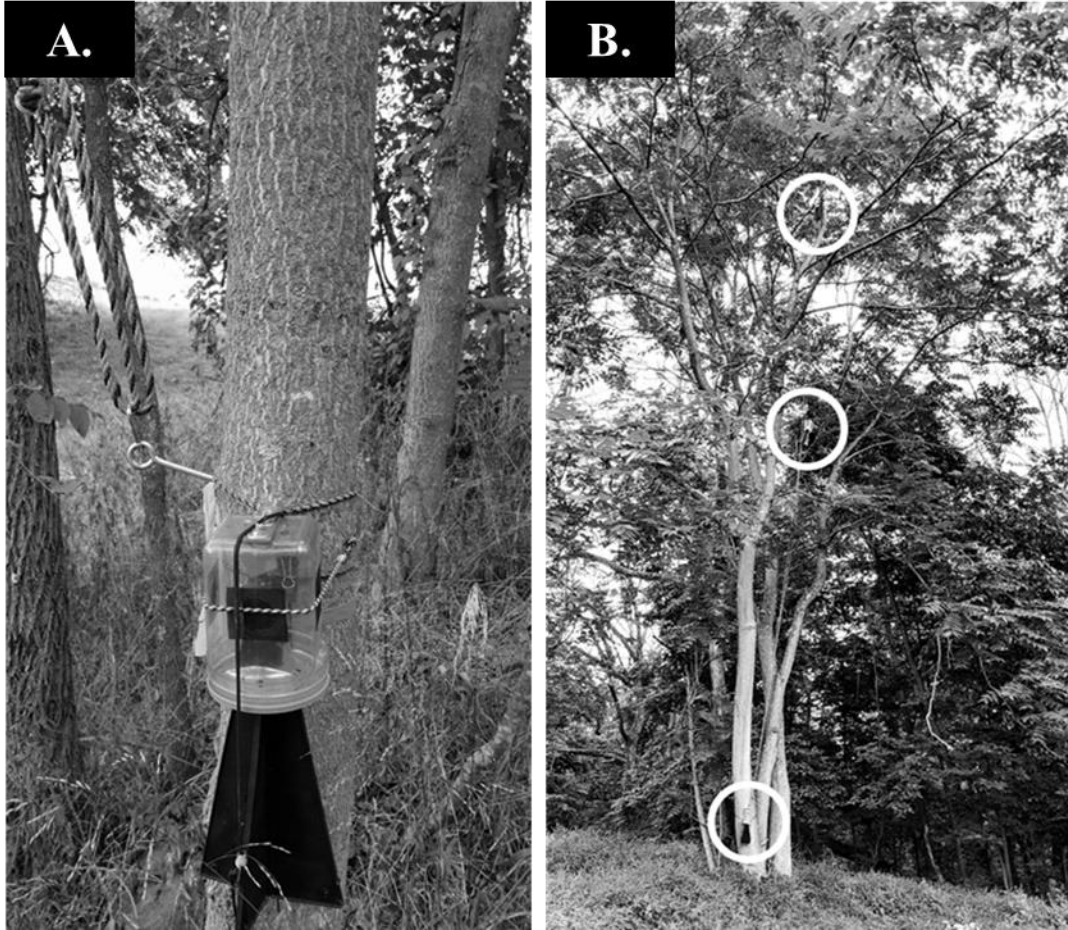
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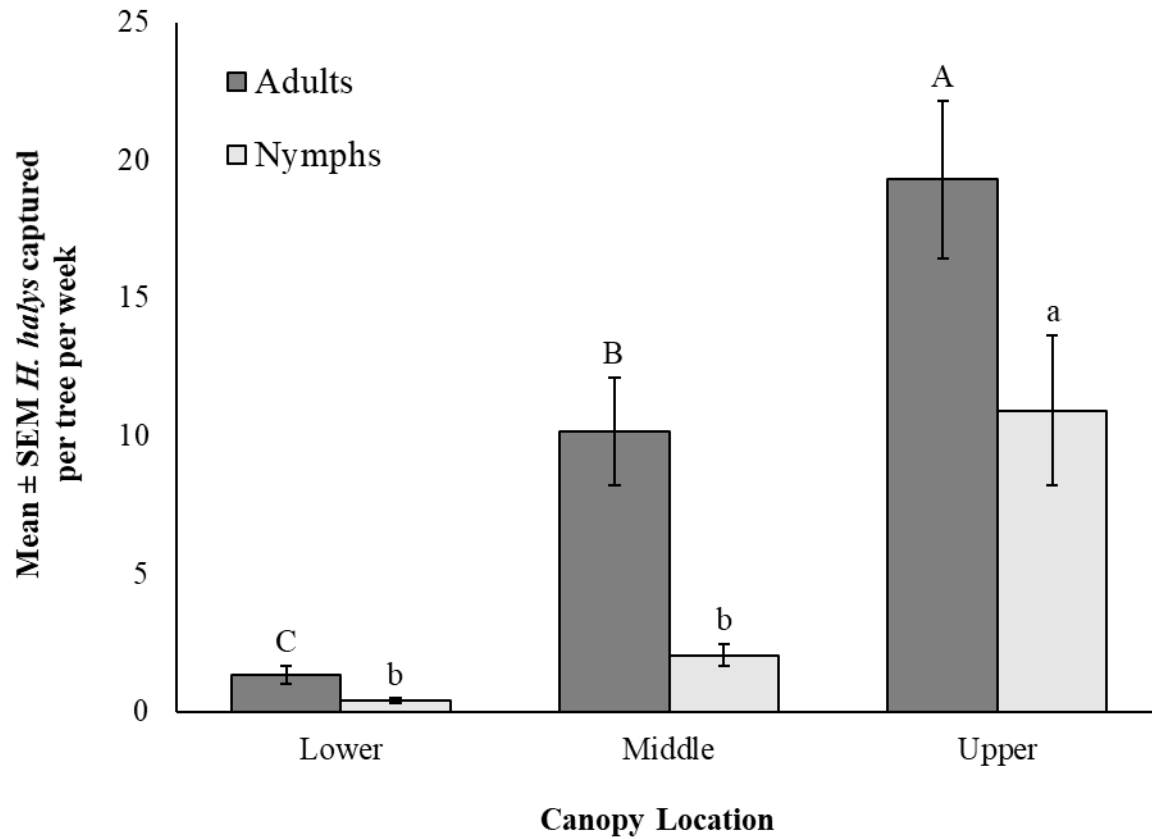
## 2.7 Figures and tables



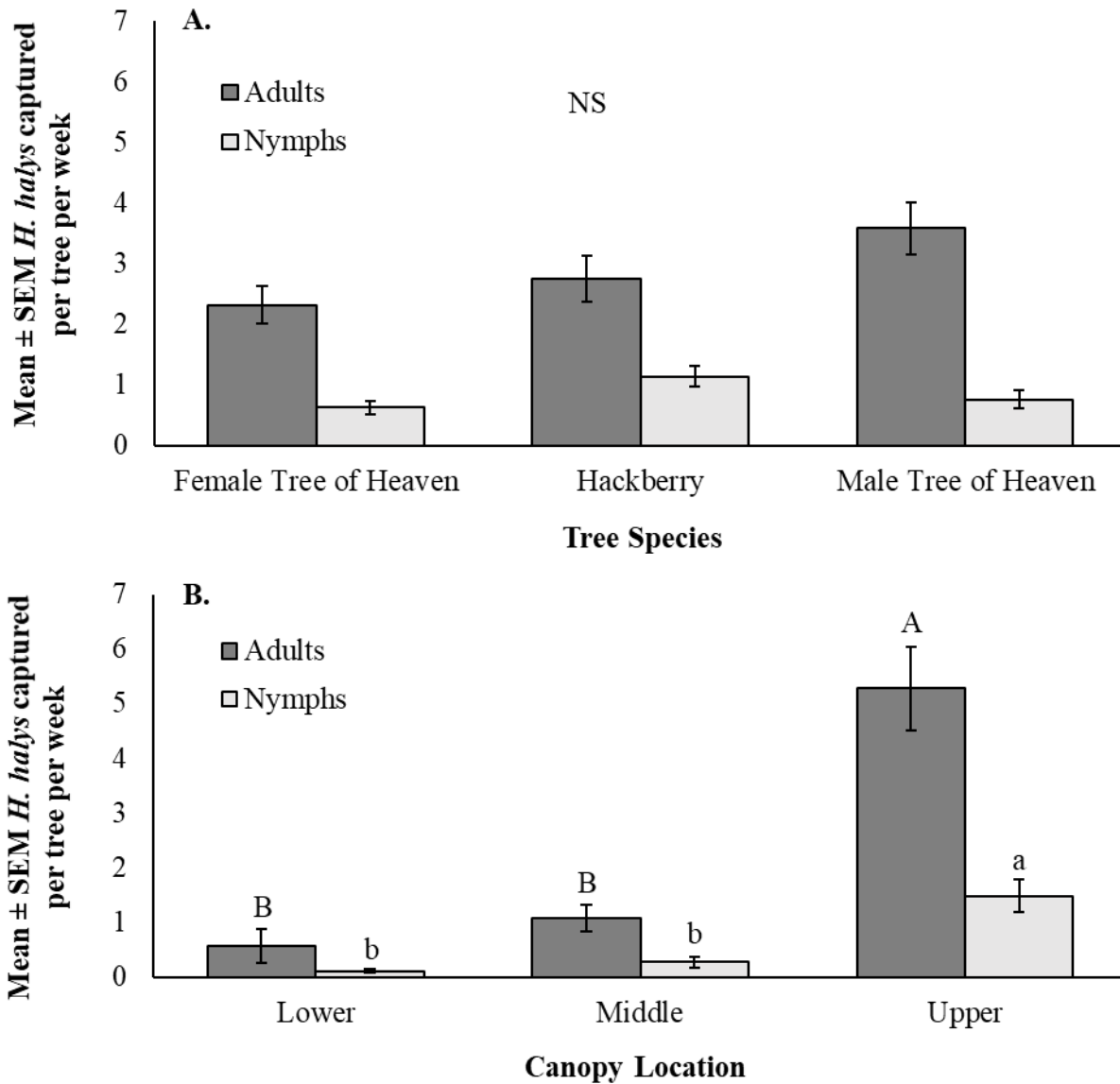
**Figure 2.1.** Nymphal *H. halys* on the samaras of a female tree of heaven (*Ailanthus altissima*) at Virginia Tech's research station near Winchester, VA. Photo credit: J.C. Bergh



**Figure 2.2.** A) Pheromone-baited pyramid trap deployed in tree of heaven (*Ailanthus altissima*), B) pheromone-baited pyramid traps (indicated by circles) at the upper, middle, and lower canopy of female tree of heaven. (*Ailanthus altissima*) near Winchester, VA.



**Figure 2.3.** Mean ( $\pm$  SE) weekly captures of *H. halys* adults and nymphs in pheromone-baited pyramid traps at lower, middle, and upper canopy locations in female *Ailanthus altissima* in 2016. Bars with the same upper or lower case letter are not significantly different.



**Figure 2.4.** Mean ( $\pm$  SE) weekly captures of *H. halys* adults and nymphs in pheromone-baited pyramid traps at lower, middle, and upper canopy locations in 2017 by (A) tree species and (B) canopy location. Bars with the same upper or lower case letter are not significantly different.

**Table 2.1.** Kendall tau-b correlation coefficient values for the relationship between measurements from felled female tree of heaven, *H. halys* egg masses, and *T. japonicus* detections in 2016 and 2017.

Measurement	2016		2017	
	Number of egg masses	<i>T. japonicus</i> detections	Number of egg masses	<i>T. japonicus</i> detections
Tree height	0.07	0.15**	0.11	0.08
Branch height	0.01	0.12	0.14*	0.15**
Branch length	0.20*	0.12	0.20**	0.04
Tree circumference	0.09	0.01	0.002	0.07
Linear distance of egg mass from trunk	0.24**	0.07	0.38	0.14
Foliar biomass by branch	0.29*****	0.26***	0.0004	0.09
Number of samaras	0.11	0.13	0.11	0.03

\*  $P = 0.05$

\*\*  $P < 0.05$

\*\*\*  $P < 0.01$

\*\*\*\*  $P < 0.001$

\*\*\*\*\*  $P < 0.0001$

## Chapter 3

### **Sampling methods for adventive *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) in a wild tree host of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae)**

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**Quinn, N. F., E. J. Talamas, T. C. Leskey, and J. C. Bergh. 2019.** Sampling methods for adventive *Trissolcus japonicus* (Hymenoptera: Scelionidae) in a wild tree host of *Halyomorpha halys* (Hemiptera: Pentatomidae). *J. Econ. Entomol.* 112: 1997–2000.

#### **3.1 Abstract**

*Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) is an invasive pest that has established in much of the US. 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 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*.

### 3.2 Introduction

Brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), is a polyphagous agricultural and nuisance pest from Asia that continues to cause significant crop protection issues in the USA ([www.stopbmsb.org](http://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 non-crop 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 USA 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*, 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 USA 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, I report studies that evaluated sentinel eggs and yellow sticky traps for detecting *T. japonicus* in a common *H. halys* tree host.

### **3.3 Sampling 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 eye-bolt 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  $\leq 24$  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 x 5 cm piece of white cardstock. Any remaining sticky surface was covered with fine white sand. Cards with eggs were held at  $-80^{\circ}$  C for 1 to 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. 3.1). 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 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 back-folding yellow sticky trap (46 × 28 cm) (Alpha Scents, West Linn, OR) 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 wire hook below the trap (Fig. 3.2). 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.

### 3.4 Results

In 2016, 135 sentinel egg masses were deployed. Six (4.4%) yielded adult parasitoids, including *T. japonicus* (n = 3), *Trissolcus euschisti* (Ashmead) (n = 2), and *Telonomus podisi* Ashmead (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) (middle trap, n =

1), *Ooencyrtus johnsoni* (Howard) (Encyrtidae) (lower trap, n = 2, upper trap, n = 1), and *Telenomus* spp. (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 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. (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. (3 lower, 1 middle, 3 upper canopy), three *Telenomus* spp. (2 lower, 1 middle canopy), and one Encyrtidae (lower canopy).

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

### 3.5 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 (Herlihy et al. 2016, Cornelius 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. 3.3. 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. Talamas, N.F. Quinn, personal observation). Captures of non-target 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  $\geq 3$  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 (Quinn, 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, 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.

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### 3.8 Figures



**Figure 3.1.** *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.



**Figure 3.2.** 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.

	Time per sticky trap (hours)		Time per egg mass (hours)	
Preparation			Egg mass removal ↓	0.1
	Assembly ↓	0.1	Assembly ↓	0.1
Sampling	Deployment ↓	0.1	Deployment ↓	0.1
	Retrieval ↓	0.1	Retrieval ↓	0.1
Processing	Visual assessment ↓	0.2	Development time ↓ // ↓	336
	ID confirmation ↓	0.2		Visual assessment ↓
			ID confirmation ↓	0.2
	<b>Total time (hours)</b>	<b>0.7</b>		<b>337</b>

**Figure 3.3.** 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.

## Chapter 4

### Seasonal activity of *Trissolcus japonicus* and the effect of habitat type on detection frequency

#### 4.1 Abstract

In Asia, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) is among the most effective egg parasitoids of brown marmorated stink bug, *Halyomorpha halys*. Adventive populations of *T. japonicus* have been detected in the USA since 2014, including annual detections in Frederick County, VA since 2015. Currently, there is no information about the seasonal period of activity of *T. japonicus* in the USA or whether habitat characteristics affect its presence and/or relative abundance. Both of these elements relate directly to the development of optimally efficient surveillance and monitoring protocols to document the presence, spread, and changes in abundance of *T. japonicus*. To evaluate seasonal trends in *T. japonicus* captures and the effect of habitat type on its detection, sampling via yellow sticky traps deployed in the mid-canopy of female tree of heaven was conducted in three habitat types in 2018 and 2019: 1) discrete, spatially isolated patches, 2) thin tree lines and windbreaks, and 3) at the edge of contiguous woodlots. Despite very different climatic conditions during the spring and summer of 2018 and 2019, seasonal trends in *T. japonicus* detections were very similar. Pooled captures across habitat types each year revealed first captures in about mid-May with subsequent captures on most weeks through mid- to late August. In both years, captures peaked in mid-July and early August. Significant effects of habitat type on *T. japonicus* captures were found in 2018, but not 2019. In 2018, captures of *T. japonicus* were significantly greater in windbreaks than at the edge of woodlots. Based on the reported sex ratio of adult *T. japonicus* emerging from *H. halys* egg masses, the sex ratio of *T. japonicus* captured in traps differed significantly from expected values in both years. Ultimately, this improves our capacity to detect and study *T. japonicus*, allowing for more efficient evaluation of its distribution and impact on *H. halys* populations.

## 4.2 Introduction

*Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive, herbivorous stink bug from Asia that has been a severe agricultural pest in portions of the USA since the late 2000's (Leskey and Nielsen 2018). It is also a significant nuisance pest, invading and entering private residences and other buildings during its search for overwintering locations in the fall, and then moving into living spaces during the following winter and spring. For example, Inkley (2012) collected >26,000 individuals from the interior of his Maryland home in 2011. *Halyomorpha halys* is highly polyphagous, feeding on many wild plants and economically important crops (Leskey and Nielsen 2018). Tree fruit production has been impacted significantly, with Mid-Atlantic apple growers collectively experiencing crop value loss of over \$37 million in 2010 (Herrick 2011). *Halyomorpha halys* feeding on apples causes discolored external depressions and internal necrosis (Nielsen and Hamilton 2009, Acebes-Doria et al. 2016), which reduce their value and marketability. Conventional tree fruit producers primarily use broad-spectrum insecticides to prevent *H. halys* injury (Leskey and Nielsen 2018), but this is not viewed as a sustainable, long-term solution.

Biological control is considered an important aspect of long-term, sustainable *H. halys* management (Leskey and Nielsen 2018) and therefore warrants greater study. Native parasitoids collected from *H. halys* sentinel egg masses often include: *Anastatus* spp. (Hymenoptera: Eupelmidae), *Telenomus* spp. (Hymenoptera: Scelionidae), *Gryon* spp. (Hymenoptera: Scelionidae), and *Trissolcus* spp. (Hymenoptera: Scelionidae) (Abram et al. 2017). Endemic natural enemies in the USA do not attack *H. halys* at high enough rates to regulate *H. halys* populations sufficiently (Abram et al. 2017). Dieckhoff et al. (2017) reported very low levels of parasitization by native parasitoids in unmanaged habitats, suggesting that they did not contribute significantly to *H. halys* biological control. Similarly, a study in Delaware found no differences in parasitization of sentinel *H. halys* egg masses deployed in exotic or native host plants, but reported greater parasitization of eggs deployed at the edge of unmanaged woods (Cornelius et al. 2016). However, parasitoid detections are somewhat habitat dependent, with *Anastatus* species being among the more commonly detected parasitoids in ornamental systems

(Jones et al. 2017) and *Trissolcus* species tending to dominate wooded habitats (Herlihy et al. 2016).

*Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), is one of the most important natural enemies of *H. halys* in Asia. In their shared native range, *T. japonicus* attacks up to 80% of wild *H. halys* egg masses (Yang et al. 2009, Zhang et al. 2017). Based on its effectiveness against *H. halys* and in anticipation of *H. halys* outbreaks in the USA, a quarantine program to evaluate the host range and non-target effects of *T. japonicus* began in 2007 at the USDA/ARS Beneficial Insect Introduction Research Unit in Delaware and subsequently at several other quarantine facilities in the USA. *Trissolcus japonicus* demonstrated an ability to attack the eggs of native pentatomid species in no-choice assays, but attacked *H. halys* at relatively greater rates in dual-choice assays in which *H. halys* eggs were included in the laboratory (Hedstrom et al. 2017, K. Hoelmer pers. comm.) and the field (Milnes and Beers 2019).

An adventive population of *T. japonicus* was detected in Beltsville, MD in 2014 (Talamas et al. 2015a) and has since been detected in 12 states and the District of Columbia (Herlihy et al. 2016, Milnes et al. 2016, Hedstrom et al. 2017, Morrison et al. 2018), and in Canada, Italy, and Switzerland (Costi et al. 2019, Peverieri et al. 2018, Stahl et al. 2018, Abram et al. 2019). In Virginia, *T. japonicus* has been present in Frederick county since at least 2015 (Bergh unpublished), where it has been detected annually ever since (Chapters 2 and 3). *Trissolcus japonicus* is expected to have major impacts on *H. halys* population dynamics in the relatively near term, requiring ongoing, intensified monitoring of *T. japonicus* spread and activity. Most monitoring efforts for natural enemies of *H. halys* have utilized sentinel egg masses (Abram et al. 2017). However, Quinn et al. (Chapter 3) determined that sticky traps deployed in the mid-canopy of wild trees were as effective as sentinel egg masses deployed at the same height for detecting *T. japonicus*. This sampling tool and protocol reduced resource inputs and increased sampling efficiency, enabling more efficient surveillance of *T. japonicus* and investigation of relevant aspects of its ecology.

Little is known about the distribution of adventive *T. japonicus* in the landscape or its habitat requirements. Habitat provides resources such as food and shelter that are essential to

maintaining natural enemy activity (Landis et al. 2000). Landscape simplification as a result of agricultural intensification can reduce biological control efficacy by removing habitat resources (Rusch et al. 2012) and increasing natural enemy exposure to pesticides (Geiger et al. 2010). Herlihy et al. (2016) reported that *T. japonicus* emerged from sentinel egg masses deployed at the edge of a woodland strip in Beltsville, MD, but not from those in an abandoned apple orchard or a soybean planting nearby. *Trissolcus japonicus* was first detected in Washington State from a wild *H. halys* egg mass collected from a lightly wooded habitat in a residential area in Vancouver, WA (Milnes et al. 2016). However, the effect of habitat characteristics on detection of *T. japonicus* in the USA has not been formally studied.

While there is limited information on the effects of patch size or habitat fragmentation on the presence/distribution of *T. japonicus*. Generally, parasitoids are negatively impacted by simplified landscapes with low habitat connectivity (Bianchi et al. 2006). For example, the egg parasitoid *Foersterella reptans* Nees (Hymenoptera: Tetracampidae) is most abundant in larger habitats with high host plant density and connectivity (Heisswolf et al. 2008). There are exceptions however based upon the parasitoid's life history. Elzinga et al. (2006) observed that the presence of *Microplitis tristis* (Nees) (Hymenoptera: Braconidae) was not affected by patch isolation, while *Eurylabus tristis* (Gravenhorst) (Hymenoptera: Ichneumonidae) was negatively impacted, possibly due in part to differing dispersal abilities (Elzinga et al. 2006). Abram et al. (2017) reviewed the North American surveys of attacks of *H. halys* egg masses by native parasitoids in field/vegetable crops, orchards, ornamental trees, forests, and semi-natural/urban habitats. Although species from most genera were recovered from most habitats, species of *Telenomus* were most prevalent from field/vegetable crops and orchards, *Anastatus* species were prevalent in all but field/vegetable crops, and *Trissolcus* species tended to be more prevalent in ornamental, semi-natural/urban, and forest habitats than in field/vegetable crops, and were not reported from orchards. Herlihy et al. (2016) documented significantly greater levels of parasitization by native *Trissolcus* species and *T. japonicus* when sentinel *H. halys* eggs were deployed in wooded habitats compared to soy or apple plantings, whereas *Telenomus podisi* was only recovered from eggs placed in soybean fields.

In order to more effectively monitor *T. japonicus* establishment and spread in the USA, there is a need for a greater understanding of the habitats used by *T. japonicus*. Furthermore, understanding the seasonal activity of natural enemies in the field is essential to optimize surveillance and to enhance monitoring of their performance in biological control programs. In Asia, detections of *T. japonicus* from sentinel and field-collected egg masses begin in May, peak in July and August, and end in early September (Zhang et al. 2017). While the phenology of *T. japonicus* in the USA is suspected to be similar to that in its native range, this has not been documented. This chapter presents results from studies examining the effects of habitat on adventive *T. japonicus* captures and the seasonality of *T. japonicus* activity in the field.

### 4.3 Materials and Methods

#### *Field sites*

All study sites were adjacent to commercial and experimental orchards within 10 km of Virginia Tech's Alson H. Smith Jr. Agricultural Research and Extension Center (AREC) in Winchester, VA (39.112867, -78.284029) (Fig. 4.1). In the USA, tree of heaven (*Ailanthus altissima*), also an invasive Asian species (Kowarik and Säumel 2007), often grows prolifically in disturbed or semi-disturbed locations (Call and Nilsen 2003). Three common habitat types in which this tree grows locally are; 1) spatially isolated, often circular patches associated with rock breaks in otherwise cultivated fields, 2) windbreaks or hedgerows, and 3) the edge of wooded areas (Fig. 4.2). Acebes-Doria et al. (2017) reported that tree of heaven was the most abundant species at the forest edge (about 25% of all trees) but much less common in the forest interior (about 4% of all trees). All of these habitat types are commonly found near or adjacent to commercial tree fruit orchards and thus were selected for sampling (n = 5 sites per type). Measurements from each site yielded the following; 1) isolated patches: area =  $278.6 \pm 88.1$  SE m<sup>2</sup>, width at widest point =  $17.3 \pm 3.6$  m; 2) windbreaks:  $2,629.2 \pm 827.5$  SE m<sup>2</sup>, width at widest point =  $10.7 \pm 1.7$  m; 3) woods edge:  $649,866.4 \pm 254,141.6$  SE m<sup>2</sup>, width at widest point =  $977.8 \pm 130.0$  m.

### *Trapping for Trissolcus japonicus*

The same sites were used for trapping in 2018 and 2019. At each site, one backfolding yellow sticky trap (23 x 28 cm, Alpha Scents, West Linn, OR) was deployed in the mid-canopy of female tree of heaven atop a 4.8 m bamboo pole, as described in Chapter 3, at the edge of the habitat. Traps were deployed on 3 May, 2018 and replaced at  $7 \pm 2$ -day intervals through 21 September. Data presented for 2019 reflect trapping from 20 April until 30 September.

All parasitoids of interest captured (i.e. those considered to be potential *H. halys* parasitoids) were tentatively identified *in situ* in the laboratory, following Talamas et al. (2015b) and sent to Dr. E.J. Talamas (Florida Department of Agriculture and Consumer Services, Division of Plant Industry) for species confirmation with the exception of *Anastatus* spp. Male and female *T. japonicus* were differentiated based on their antennal morphology (Yang et al. 2016); female antennae have 11 antennomeres while male antennae have 12 and a more elongated and cylindrical antennal flagellum. Due to time and schedule constraints associated with species confirmation by Dr. Talamas and dissertation preparation, specimens from the last two weeks of sampling have not had their identifications confirmed. The complete data set for 2019 will be included in the future publication.

### *Data analysis*

Captures of *T. japonicus* each year were pooled across dates and compared among habitat types using the Kruskal-Wallis test followed by the Bonferroni corrected Dunn's test (SAS Institute, Cary, NC; SAS Institute Inc. 2018). For each year, total captures of male and female *T. japonicus* pooled across all habitat types by week are presented. Comparison of observed versus expected sex ratios each year used a  $\chi^2$  analysis, based on the findings of Yang et al. (2018), who reported that female *T. japonicus* accounted for 72.3% of the adults that emerged from fertilized *H. halys* eggs.

## 4.4 Results

### *Effect of habitat type*

In 2018 and 2019, respectively, 101 and 104 specimens of *T. japonicus* were captured on yellow sticky traps across all habitats sampled. In 2018, 83.2% of which were female, and the observed female:male ratio differed significantly from expected ( $\chi^2 = 9.7$ ,  $df = 1$ ,  $P < 0.01$ ). There was a significant effect of habitat type on *T. japonicus* captures ( $\chi^2 = 8.31$ ,  $df = 2$ ,  $P < 0.05$ ); significantly more were captured in windbreaks than at the woods edge ( $z_{\text{windbreak}} = 7.9$ ,  $z_{\text{woods edge}} = 6.9$ ,  $P < 0.05$ ) (Fig. 4.3A). In 2019, 95.2 % of *T. japonicus* captured were female. As in 2018, the female:male sex ratio differed significantly from expected ( $\chi^2 = 27.2$ ,  $df = 1$ ,  $P < 0.01$ ). In 2019 however, habitat type did not significantly affect captures ( $\chi^2 = 0.25$ ,  $df = 2$ ,  $P > 0.05$ ) (Fig. 4.3B).

### *Seasonal captures of Trissolcus japonicus*

In 2018, the first capture of *T. japonicus* was recorded on 18 May (Fi. 4.4A). Captures were recorded on most weeks through 14 September, with peak captures on 13 July and 10 August. The last detection of *T. japonicus* was recorded on 14 September. Interestingly, males were captured only between mid-June and late August. In 2019, the date of first *T. japonicus* capture (13 May, Fig. 4.4B) was similar to that of 2018. Again, *T. japonicus* was captured on most weeks between mid-May and early September. Peak captures occurred on 22 July and between 5 and 11 August and the last capture was recorded from a trap retrieved on 2 September. Female captures predominated in 2019, with males recorded only on 11 August.

### *Captures of native parasitoids of H. halys*

In 2018 and 2019, respectively, 61 and 159 specimens of native parasitoids of *H. halys* were captured on yellow sticky traps across all habitats sampled. All native species listed are known to develop successfully in *H. halys* sentinel eggs to varying degrees (Abram et al. 2017). In 2018, these included *Trissolcus brochymenae* (Ashmead) (Hymenoptera: Scelionidae), *Trissolcus*

*euschisti* (Ashmead) (Hymenoptera: Scelionidae), *Trissolcus thyantae* Ashmead (Hymenoptera: Scelionidae), *Trissolcus edessae* (Ashmead) (Hymenoptera: Scelionidae), *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae), *Telenomus* spp. (Hymenoptera: Scelionidae), *Anastatus* spp., and Encyrtidae (Table 4.1). Captures in 2019 included *T. brochymenae*, *T. thyantae*, *T. euschisti*, *T. podisi*, *Telenomus persimilis* Ashmead (Hymenoptera: Scelionidae), *Telenomus* spp., *Gryon* spp. (Hymenoptera: Scelionidae), *Anastatus* spp., and Encyrtidae (Table 4.2).

#### 4.5 Discussion

The spatial ecology of parasitoids and their hosts has been notoriously difficult to quantify (Cronin and Reeve 2005). Typically, it is thought that decreased habitat size and increased fragmentation reduce parasitization rates (Valladares et al. 2006) and parasitoid abundance (Elzinga et al. 2007), and is thus why captures of *T. japonicus* might be expected to be greatest in our largest habitat type: woods edge. However, the effect of habitat size on parasitoid abundance is dependent upon the parasitoid in question. Some parasitoids, such as *Hyposoter horticola* (Gravenhorst) (Hymenoptera: Ichneuemonidae), have a dispersal capability sufficient to colonize patchily distributed hosts across greater distances more easily than others (Van Nouhuys and Hanski 2002). In a field study of *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae), 369 individuals were released in a 50 x 100 m area tomato planting, resulting in the parasitization of about 50% of sentinel *N. viridula* egg masses deployed (Justo et al. 1997). Wright and Diez (2011) found that released *T. basalis* dispersed up to 75 m in the field in a two-week period and parasitized more eggs in weedy pastures than in macadamia orchards. The distribution of parasitoids is typically a reflection of the distribution of their hosts within the landscape, which is often patchy (Hassel and May 1973, Hassel and May 1974). For example, the parasitoid *Foersterella reptans* Nees (Hymenoptera: Tetracampidae), exhibits a strong density-dependent response to host density, regardless of habitat size or quality (Heisswolf et al. 2008). In 2018, the fewest *T. japonicus* were captured at the edge of woodlots and most were captured in windbreaks, with all habitats being equal in captures in 2019. In their review of native natural enemies of *H. halys*, Abram et al. (2017) noted that across currently available studies *Trissolcus* species were associated primarily with wooded habitats (Abram et al. 2017). However, the wooded habitats listed in this review varied in size and other characteristics, with *Trissolcus* species prevalence relatively even among ornamental, semi-natural/urban habitats,

and forests, all of which are comprised of varying sizes of patches of predominately trees. This suggests that the impact of habitat size on *T. japonicus* abundance is variable. However, while it is interesting to note that the first detection of *T. japonicus* in the USA occurred in habitat similar to the windbreak habitat sampled in for this work (Talamas et al. 2015a), this may be coincidental. Positive density-dependent responses of parasitoids to hosts have been documented in other *Trissolcus* species (Meats and Castillo Pando 2000, Amir-Maafi and Parker 2002). Therefore, *T. japonicus* abundance may be most influenced by *H. halys* density.

The sex ratio of *T. japonicus* captures was more female-biased in both years than expected from laboratory studies (Yang et al. 2018), in which 72.3% of adults from *H. halys* were females. As solitary parasitoids, scelionids tend to have heavily female-biased sex ratios (Godfray 1994). Egg masses are likely to only be parasitized by a single female *T. japonicus* due to its tendency to vigorously defend the egg mass from other parasitoids (Konopka et al. 2017). *Trissolcus japonicus* is protandrous, with males emerging 12-24 hours before females in the laboratory (N. F. Quinn pers. obs.). In other *Trissolcus* species that also exhibit protandry, the first male to emerge typically mates with the majority of the females (Loch and Walter 2002), reducing local mate competition (Waage 1982). The more heavily female-biased sex ratio found in our traps may be due to: 1) differential longevity, 2) early colonization, 3) differences between laboratory and field studies, or 4) sampling constraints. In laboratory studies of other *Trissolcus* species, males live 6.5-12.3 days less than females (Powell and Shepard 1982), and therefore may be potentially less likely than females to be captured over time. Other parasitoids can adjust the sex ratio based on their environment. For example, when female *Telenomus heliothidis* Ashmead (Hymenoptera: Scelionidae) encountered more female conspecifics, fewer female wasps emerged from egg masses that they attacked subsequently (Strand 1988). Based on the findings of Strand (1988), the observed female-skewed sex ratio may reflect the fact that *T. japonicus* is still actively colonizing the area in which trapping was conducted, and individual female *T. japonicus* may be less likely to encounter other female wasps compared to their native range. Additionally, the studies of *T. japonicus* reproduction by Yang et al. (2018) were conducted in the laboratory, and therefore may not reflect the ecology of *T. japonicus* under field conditions. A more precise estimate of the in-field sex ratio of *T. japonicus* might be reached through

intensification of future sampling efforts in order to increase the total *T. japonicus* captured (Dixon et al. 2005).

Synchrony of parasitoids and their hosts influences parasitoid population size and rate of colonization (Van Nouhuys and Lei 2004). Phenological mismatch caused by unusually warm springs or other conditions can reduce observed parasitism (Evans et al. 2013). In China, Zhang et al. (2017) reported that the phenology of *T. japonicus* activity, as measured by emergence from sentinel egg masses, is synchronous with the periods of *H. halys* egg laying, with the greatest number of sentinel eggs attacked in July and August. In this study, the phenology of *T. japonicus* appeared to be relatively synchronous with that of *H. halys*, based on known periods of *H. halys* oviposition; two peaks of *T. japonicus* captures reported herein occurred shortly after predicted periods of peak *H. halys* oviposition (Nielsen et al. 2016). This synchrony increases the likelihood of the persistence of adventive *T. japonicus* (Godfray et al. 1994). While the effect of habitat type on *T. japonicus* captures varied between years, captures in both years indicated that, 1) *T. japonicus* was first detected in mid-May, 2) captures peaked in mid-July and early August, and 3) detections declined markedly after mid-August, with final detections occurring in early September. Similar seasonal activity trends in 2018 and 2019 were particularly notable given the environmental differences between years, with 2019 being drier than 2018. In 2018, the mean maximum air temperature from May through August at the AHS-AREC was  $28.1\text{ }^{\circ}\text{C} \pm 0.35$  and total precipitation was 77.8 cm, while in 2019 the mean air temperature was  $28.1\text{ }^{\circ}\text{C} \pm 0.42$  and the total precipitation was 36.5 cm. This suggested that *T. japonicus* seasonal activity remained stable despite yearly climate variation, which bodes well for its persistence and for sustained biological control of *H. halys* over time. Another interesting aspect of the temporal distribution of male and female captures in 2018 is the potential implications on the overwintering biology of *T. japonicus*. Given that female *T. japonicus* are mated at eclosion, those that enter overwintering sites are likely mated. The lack of captures of males early and late in the season may suggest that only female *T. japonicus* overwinter and the first males captured in a given year are from eggs parasitized within that year, but this remains to be verified.

While several other parasitoids were detected, including native several that are known to attack *H. halys* eggs, captures of *T. japonicus* were much higher than those of any other species.

Interestingly, while in 2018 only 40.2% of the parasitoids captured were native species (i.e. not *T. japonicus*), in 2019, 62.5% were native species, a 154.4% increase. The community of native parasitoids described here was similar in composition to that reported by Tillman (2016) in an environment without *T. japonicus*. This suggests that, as Konopka et al. (2017) concluded, *T. japonicus* may be able to successfully coexist with native species in the biological control of *H. halys*. Interestingly, while Herlihy et al. (2016) detected *T. podisi* only in soybean fields, it was captured in all habitat types in both years in this study. This suggests either that *T. podisi* is more widely distributed in the environment than previously reported or that the visual stimulus provided by yellow sticky traps can attract parasitoids from greater distances than sentinel egg masses.

This study indicated that yellow sticky traps were effective for detecting *T. japonicus* in habitats of different sizes, allowing greater sampling flexibility and ease of adoption. It also provided further validation of the efficacy of these traps deployed in the mid-canopy of tree hosts for monitoring *T. japonicus*. Additionally, documentation of the seasonal activity of adventive *T. japonicus* in the Mid-Atlantic USA can be used to target the timing of *T. japonicus* monitoring efforts. Ultimately this can improve our ability to study the ecology and track the spread of this important *H. halys* biological control agent, even in areas of relatively low *T. japonicus* density.

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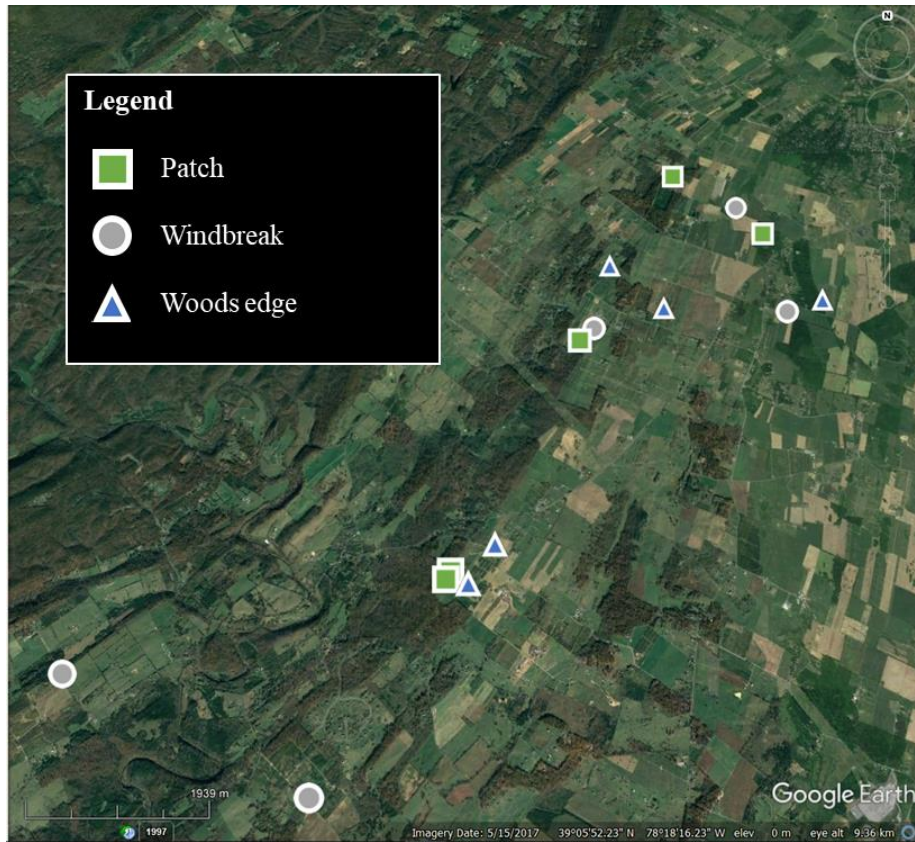
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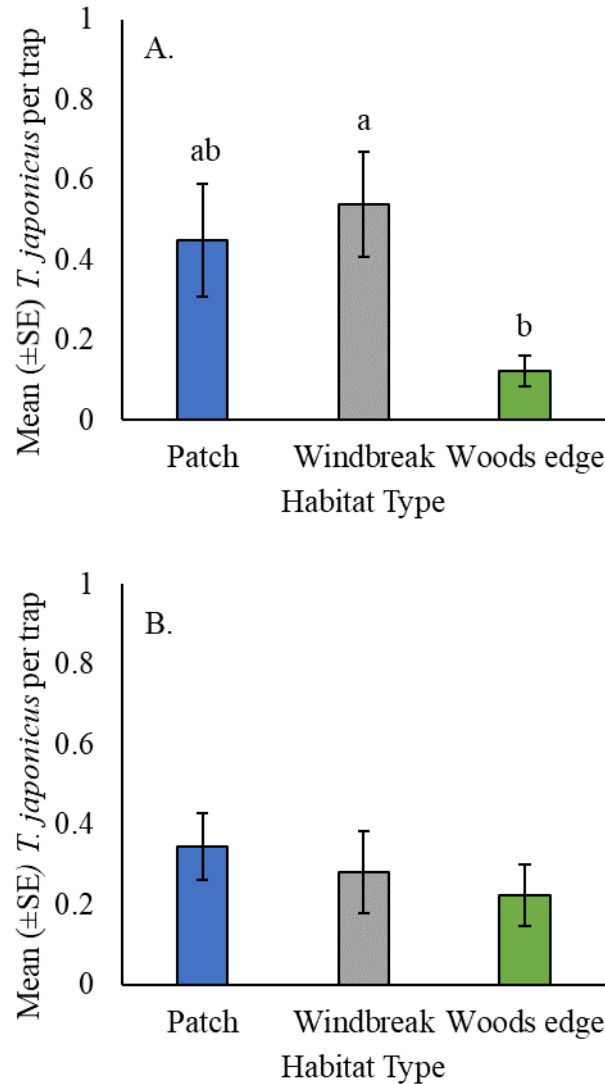
## 4.6 Figures and tables



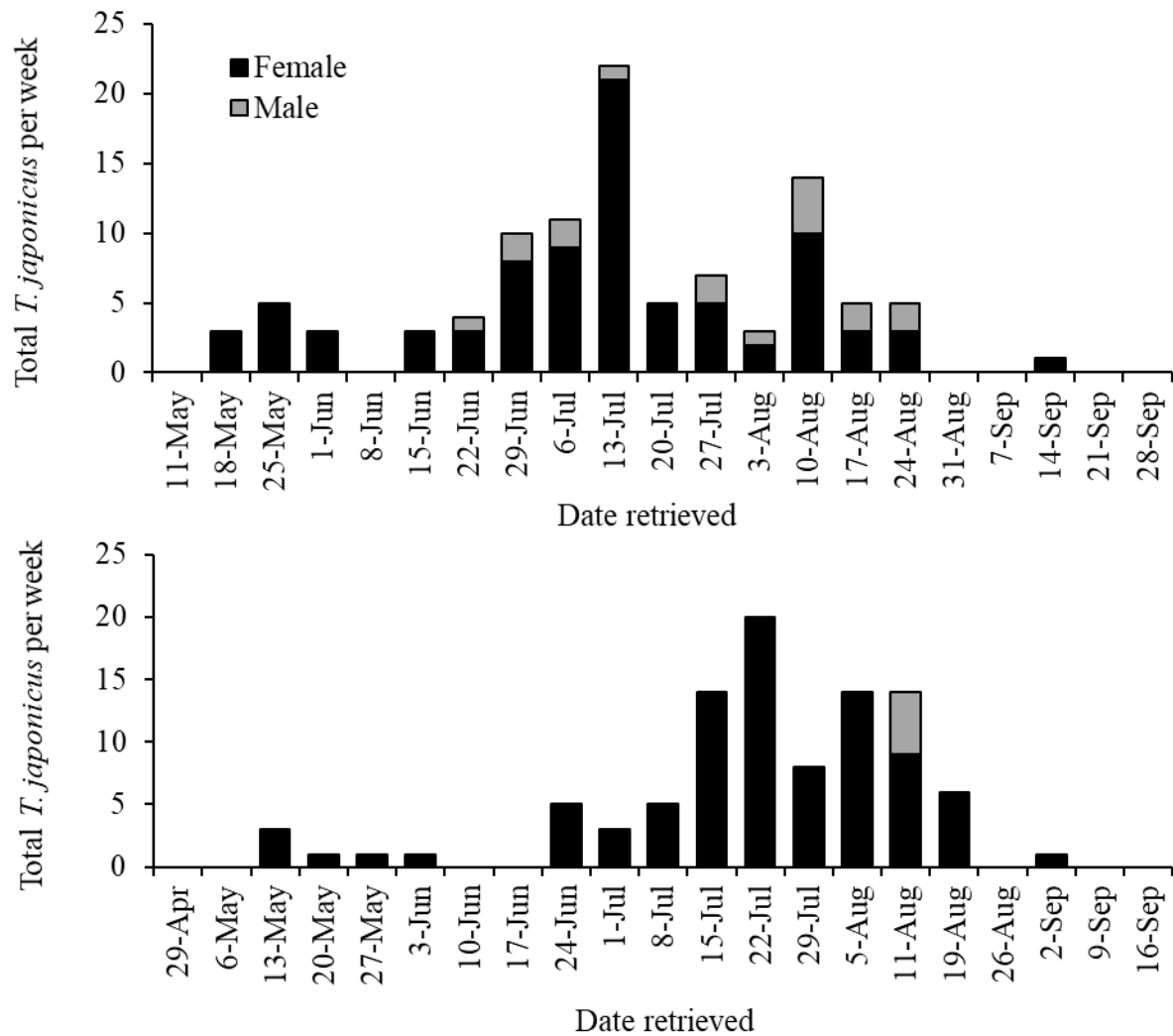
**Figure 4.1.** Distribution of sites where yellow sticky traps for sampling *Trissolcus japonicus* were deployed in the mid-canopy of mature female tree of heaven growing in three habitat types in Frederick County, VA, 2018 – 2019.



**Figure 4.2.** Representative habitat types in which *Trissolcus japonicus* was sampled using yellow sticky traps deployed in the mid-canopy of mature female tree of heaven in Frederick County, VA in 2018 and 2019; A) Patch: Isolated patches of predominantly tree of heaven, B) Windbreak: long narrow patch of mixed wild trees and shrubs, C) Woods edge: larger contiguous woodlot of wild trees and shrubs.



**Figure 4.3.** Mean number of *T. japonicus* captured in yellow sticky traps deployed in mid-canopy of mature female tree of heaven in Frederick Co., VA by habitat type in: A) 2018 and B) 2019. Traps were deployed on 3 May, 2018 and replaced at  $7 \pm 2$ -day intervals through 21 September. Data presented for 2019 reflect trapping from 20 April until 30 September. Note that specimen identifications from traps collected on September 23 and September 30 2019 have not been verified at the time of this writing. Bars with the same letters within the same figure are not significantly different from one another. (Kruskal-Wallis test and Bonferroni corrected Dunn's test,  $P > 0.05$ ).



**Figure 4.4.** Seasonal weekly captures of *T. japonicus* in yellow sticky traps deployed in mid-canopy of mature female tree of heaven in Frederick Co., VA in A) 2018 and B) 2019. Traps were deployed on 3 May, 2018 and replaced at  $7 \pm 2$ -day intervals through 21 September. Note that specimen identifications from traps collected on September 23 and September 30 2019 have not been verified at the time of this writing.

**Table 4.1.** Parasitoids of *H. halys* captured in yellow sticky traps deployed in mid-canopy of mature female tree of heaven in Frederick Co., VA, 2018. All parasitoids listed are known to emerge from *H. halys* sentinel egg masses (Abram et al. 2017).

Habitat	<i>Trissolcus</i>					<i>Telenomus</i>		<i>Anastatus</i>	Encyrtidae
	<i>japonicus</i>	<i>brochymenae</i>	<i>thyantae</i>	spp.	<i>edessae</i>	<i>podisi</i>	spp.	spp.	
Patch	40	2	0	4	0	2	2	2	0
Windbreak	50	6	0	14	1	3	2	1	0
Woods edge	11	2	1	11	0	4	5	4	2
<i>Grand Total</i>	<i>101</i>	<i>10</i>	<i>1</i>	<i>29</i>	<i>1</i>	<i>9</i>	<i>9</i>	<i>7</i>	<i>2</i>

**Table 4.2.** Parasitoids of *H. halys* captured in yellow sticky traps deployed in mid-canopy of mature female tree of heaven in Frederick Co., VA, 2019. All parasitoids listed are known to emerge from *H. halys* sentinel egg masses (Abram et al. 2017). Note that specimen identifications from traps collected on September 23 and September 30, 2019 have not been verified at the time of this writing.

Habitat	<i>Trissolcus</i>				<i>Telenomus</i>			<i>Gryon</i>	<i>Anastatus</i>	Encyrtidae
	<i>japonicus</i>	<i>brochymenae</i>	<i>thyantae</i>	<i>euschisti</i>	<i>podisi</i>	<i>persimilis</i>	spp.	spp.	spp.	
Patch	43	2	5	18	20	0	9	1	5	2
Windbreak	35	4	7	25	17	1	2	1	5	0
Woods edge	26	4	7	15	6	2	10	0	4	1
<i>Grand Total</i>	<i>104</i>	<i>10</i>	<i>19</i>	<i>58</i>	<i>43</i>	<i>3</i>	<i>21</i>	<i>2</i>	<i>14</i>	<i>3</i>

## Chapter 5

### Host plant effects on *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) detections

#### 5.1 Abstract

*Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) is considered a highly effective Asian egg parasitoid of brown marmorated stink bug (*Halyomorpha halys*) (Stål) (Hemiptera: Pentatomidae). Since 2014, several adventive populations of *T. japonicus* have been detected in the USA and are showing indications of geographic range expansion, prompting research on surveillance tactics that may enable efficient and optimized monitoring and a deeper understanding of relevant aspects of its foraging ecology. Yellow sticky traps have proven effective for monitoring *T. japonicus*, but a better understanding of when and where it is present, including host plant effects on its detection frequency, may further enhance sampling effectiveness. In 2017, yellow sticky traps deployed in the mid-canopy of mature female tree of heaven (Mill.) Swingle (Sapindales: Simaroubaceae) growing at the edge of woodlands adjacent to apple orchards in Frederick Co., VA were paired with a nearby trap in; 1) black walnut (*Juglans nigra*) L. (Fagales: Juglandaceae), 2) black locust (*Robinia pseudoacacia*) L. (Fabales: Fabaceae), or 3) hackberry (*Celtis occidentalis*) L. (Rosales: Cannabaceae), also growing at the woods edge. In 2018, pairs of tree of heaven and black cherry (*Prunus serotina*) Ehrh. (Rosales: Rosaceae) were added. In 2017 and 2018, respectively, traps deployed throughout August and from mid-June through mid-August were collected and replaced at 7-day intervals. In 2019, following information on the effects of habitat type on *T. japonicus* detections (see Chapter 4), host plant effects were examined using three yellow sticky traps per site, deployed in tree of heaven, black walnut, and black locust growing in the same windbreaks near commercial fruit orchards. Traps were replaced at 7-day intervals between mid-June and mid-August. Consistent host plant effects on captures of *T. japonicus* and other parasitoids were not observed among years. Results are discussed in relation to the optimization of *T. japonicus* sampling efficiency and aspects of its foraging ecology.

## 5.2 Introduction

Brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a polyphagous, invasive agricultural and nuisance pest from Asia (Hoebeke and Carter 2003) that feeds on over 170 wild and cultivated host plants (Leskey and Nielsen 2018). In 2010, an outbreak of *H. halys* resulted in over \$37 million in losses to Mid-Atlantic apple growers (Herrick 2011). Given its broad host range, high mobility, (Lee et al. 2014, 2015, Lee and Leskey 2015), and ability to “hitchhike” in human conveyances (Kriticos et al. 2017), *H. halys* has been detected or established throughout much of North America and in several countries abroad (Leskey and Nielsen 2018).

*Halyomorpha halys* invades croplands from wild hosts adjacent to cultivated areas (Bakken et al. 2015). Apples harvested from orchard borders next to wooded areas often exhibit the greatest levels of injury from *H. halys* (Joseph et al. 2014), where the abundance of *H. halys* is also greatest (Venugopal et al. 2015). While chemical control strategies can reduce *H. halys* injury to crops in the short-term, they likely do not affect *H. halys* population dynamics on a larger spatial scale. Additionally, treatment of orchards with broad-spectrum insecticides that are needed against *H. halys* have disrupted integrated pest management programs by increasing the frequency of secondary pest outbreaks (Rice et al. 2014, Leskey and Nielsen 2018). Biological control of *H. halys* in non-crop habitats is considered a more sustainable, long-term alternative to chemical control.

Native predators and parasitoids in the USA have not adequately regulated *H. halys* populations (Abram et al. 2017). In the native Asian range of *H. halys*, the egg parasitoid, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), is among the most effective natural enemies of *H. halys*, attacking up to 80% of wild *H. halys* egg masses, often parasitizing all of the eggs within the mass (Qiu 2007, Yang et al. 2009, Zhang et al. 2017). In 2014, an adventive population of *T. japonicus* was discovered serendipitously in Beltsville, MD (Talamas et al. 2015a), prompting more intensive and extensive surveillance that has resulted in subsequent detections in 12 US states and 2 Canadian provinces (Leskey and Nielsen 2018, stopbmsb.org). In Frederick County, VA, *T. japonicus* has been detected annually since 2015 (Bergh

unpublished, Chapters 2, 3) In Oregon, adventive *T. japonicus* parasitized 67% of sentinel *H. halys* eggs, and in Washington State, it attacked and parasitized significantly fewer sentinel egg masses of native pentatomids than *H. halys* (Milnes and Beers 2019). Adventive *T. japonicus* are associated with arboreal habitats (Talamas et al. 2015a, Herlihy et al. 2016, Milnes and Beers 2019, Chapters 2, 3), but the role of host plants in its foraging ecology is unknown.

Parasitoids often utilize semiochemical cues to locate their hosts (Lewis and Martin 1990). *Trissolcus japonicus* is attracted to n-tridecane, a component of *H. halys* defensive compounds (Zhong et al. 2017), but does not respond to the *H. halys* aggregation pheromone (Weber et al. 2017, Morrison et al. 2018). In Petri dish assays, *T. japonicus* walking speed and turning frequency increased in response to footprints of gravid *H. halys* on leaf tissue, regardless of the plant species (Boyle 2017). *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) was significantly attracted to volatiles induced by feeding and oviposition of *Nezara viridula* (L.) (Hemiptera: Pentatomidae) on French bean plants (*Phaseolus vulgaris* L.) (Colazza et al. 2004). *Trissolcus japonicus* were significantly attracted to volatiles from plants on which *H. halys* had oviposited and fed compared to control plants (Bertoldi et al. 2019). Other *Trissolcus* species respond to plant volatiles (Colazza et al. 2004, Islamoğlu and Koçak 2014), but the role of host plant species in long-range host finding by *T. japonicus* is unknown. In Virginia, wild *H. halys* egg masses collected from the mid-canopy of tree of heaven *Ailanthus altissima* (Mill.) and Swingle (Sapindales: Simaroubaceae) exhibited relatively higher levels of parasitization, including by *T. japonicus*, than those from other portions of the canopy (Chapter 2).

Yellow sticky traps are used commonly to sample and monitor certain species of pest and beneficial insects (Heathcote 1957, Gerling and Horowitz 1984, Heinz et al. 1992, Atakan and Canhilal 2004, Quinn et al. 2017), including *T. japonicus* and other parasitic Hymenoptera (Hoelmer and Simmons 2008, Tognon et al. 2016, Chapter 3). While yellow sticky traps are gaining acceptance and adoption by researchers to document the establishment and spread of *T. japonicus* in the USA, optimally efficient sampling protocols for this purpose have not been well defined. Toward this end, the questions of when and where to conduct surveillance for *T. japonicus* are particularly important. Given that the effect of host plant on its detection has not

been studied but could have important consequences, this study explores the effects of wild host tree species on detections of *T. japonicus* and other parasitoids of *H. halys*.

### 5.3 Materials and Methods

#### *Sites, sampling devices, and sampling trees*

Trees were sampled at the edge of woodlands (2017 and 2018) and in windbreaks (2019) that were near or adjacent to commercial and experimental orchards within 10 km of Virginia Tech's Alson H. Smith Jr. Agricultural Research and Extension Center (AREC) in Winchester, VA (39.112867, -78.284029). Backfolding yellow sticky traps (23 x 28 cm, Alpha Scents, West Linn, OR) were affixed to and deployed atop 4.8 m bamboo poles suspended from a mid-canopy branch via a hook, as described in Chapters 3 and 4.

Selection of the tree species used for trapping was based on; 1) census data, 2) known hosts of *H. halys*, and 3) leaf architecture. The height and trunk diameter at breast height (DBH), were recorded for every tree, using a Nikon Forestry Pro Hypsometer (Nikon Corporation, Tokyo, Japan) and meter tape, respectively. Acebes-Doria et al. (2017) reported that tree of heaven was the most abundant species at the edge of woodlands bordering tree fruit orchards in this region, and, as in our previous studies (Chapters 2-4), was the standard host used (height = 10.5 m  $\pm$  2.3 SE, DBH = 0.2 m  $\pm$  0.02 SE). The other species selected, black walnut, *Juglans nigra*, L. (Fagales: Juglandaceae) (height = 11.3 m  $\pm$  4.0 S, DBH = 0.8 m  $\pm$  0.4 SE), black locust, *Robinia pseudoacacia* L. (Fabales: Fabaceae) (height = 7.7 m  $\pm$  2.6 SE, DBH = 0.1 m  $\pm$  0.02 SE), hackberry, *Celtis occidentalis* L. (Rosales: Cannabaceae) (height = 16.1 m  $\pm$  8.1 SE, DBH = 0.3 m  $\pm$  0.05 SE), and black cherry, *Prunus serotina* Ehrh. (Rosales: Rosaceae) (height = 8.2 m  $\pm$  3.7 SE, DBH = 0.2 m  $\pm$  0.01 SE), were also among the most common species recorded by Acebes-Doria et al. (2017) and all are known hosts of *H. halys* (Bakken et al. 2015). In addition to representing a diversity of plant families, these trees differ in leaf structure. Tree of heaven, black walnut, and black locust have compound leaves, while hackberry and black cherry have simple leaves.

### *Simultaneous trapping in paired tree species at the forest edge*

In 2017, traps were deployed in mature tree of heaven growing at the forest edge and paired with a trap in black walnut, black locust, or hackberry ( $n = 5$  per species pairing), with  $\geq 10$  m and  $\leq 25$  m between trees in each pair. Traps were deployed from 31 July until 29 August, 2017 and replaced at  $7 \pm 2$ -day intervals. In 2018, the same trees used in 2017 were included in the study, as well as 5 pairs of tree of heaven and black cherry. Traps were deployed from 13 June until 20 September, 2018, and replaced at  $7 \pm 2$ -day intervals.

### *Simultaneous trapping in three tree species in windbreaks*

In 2018, trapping for *T. japonicus* showed that significantly more *T. japonicus* were captured in female tree of heaven growing in windbreaks and spatially isolated patches than along the edge of woods (Chapter 4). Thus, in 2019 windbreaks ( $n = 5$ ) containing tree of heaven, black walnut, and black locust were identified for sampling. Adjacent trap trees in each windbreak were  $23.7 \pm 8.6$  m apart and the distance between the traps at the ends of the sampling area was  $47.4 \pm 15.9$  m. Traps were deployed from 17 June until 11 August, 2019, and replaced at  $7 \pm 1$ -day intervals.

For all studies, all parasitoids of interest on the traps (i.e. Scelionidae considered potential *H. halys* parasitoids, per Abram et al. 2017) were tentatively identified *in situ* under a microscope in the laboratory, using the key from Talamas et al. (2015b), and subsequently sent to Dr. E.J. Talamas (Florida Department of Agriculture and Consumer Services, Division of Plant Industry).

### *Analysis*

In 2017 and 2018, captures of *T. japonicus* were compared by tree species pair using a Wilcoxon signed-rank test, while in 2019 captures were compared among three host species using the Kruskal-Wallis test followed by the Bonferroni corrected Dunn's test. All statistical comparisons used SAS (SAS Institute, Cary, NC; SAS Institute Inc. 2018) and were considered significant at  $P < 0.05$ .

## 5.4 Results

### *Simultaneous trapping in paired tree species at the forest edge: Trissolcus japonicus*

In total, 24 *T. japonicus* were captured during the four weeks of sampling in 2017 (Table 5.1). All specimens of *T. japonicus* were captured in tree of heaven, but tree species was not a significant effect; tree of heaven vs black locust,  $S = -3$ ,  $P > 0.05$ , tree of heaven vs black walnut,  $S = -5$ ,  $P > 0.5$ , tree of heaven vs hackberry,  $S = -3$ ,  $P > 0.05$  (Fig. 5.1).

In total, 42 *T. japonicus* were captured during 12 weeks of sampling in 2018 (Table 5.2). Of all *T. japonicus* captured, 66.7% were from tree of heaven, but again tree species did not significantly affect captures for any host pairing; tree of heaven vs black locust,  $S = -1.5$ ,  $P > 0.05$ , tree of heaven vs black walnut,  $S = -10$ ,  $P > 0.05$ , tree of heaven vs hackberry  $S = -11$ ,  $P > 0.05$ , tree of heaven vs black cherry,  $S = -1$ ,  $P > 0.05$  (Fig. 5.2).

### *Simultaneous trapping in three tree species in windbreaks: Trissolcus japonicus*

During the 8-week sampling period in 2019, 13 *T. japonicus* were captured (Table 5.3), 61.5%, 38.5%, and 0.0% of which were captured in black locust, black walnut, and tree of heaven, respectively. The host plant in which traps were deployed significantly affected *T. japonicus* captures ( $\chi^2 = 6.32$ ,  $df = 2$ ,  $P < 0.05$ ). Significantly more *T. japonicus* were captured in traps in black locust trees versus tree of heaven ( $z_{\text{black locust}} = 8.4$ ,  $z_{\text{tree of heaven}} = 7.3$ ,  $P < 0.05$ ) (Fig. 5.3), while captures in black walnut were not significantly different from those in black locust or tree of heaven ( $z_{\text{black walnut}} = 7.8$ ,  $z_{\text{tree of heaven}} = 7.3$ ,  $P > 0.05$ ) (Fig. 5.3).

### *Captures of native Scelionidae*

In 2017 and 2018, respectively, 12 and 52 specimens of native Scelionidae were captured on yellow sticky traps in paired host trees at the forest edge. In 2017, these included *Trissolcus brochymenae* (Ashmead) (Hymenoptera: Scelionidae), *Trissolcus euschisti* (Ashmead) (Hymenoptera: Scelionidae), *Telenomus* spp. (Hymenoptera: Scelionidae), and *Gryon* spp.

(Hymenoptera: Scelionidae) (Table 5.1). Captures in 2018 included *Trissolcus thyantae* Ashmead (Hymenoptera: Scelionidae), *T. euschisti*, *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae), *Telenomus* spp., and *Gryon* spp. (Table 5.2). In 2019, 48 specimens of native scelionids were captured on traps in three host tree species in windbreaks, including *T. brochymenae*, *T. thyantae*, *T. euschisti*, *Trissolcus edessae* Fouts (Hymenoptera: Scelionidae), *T. podisi*, and *Telenomus* spp. (Table 5.3). Of the native species captured, all are known to develop successfully in *H. halys* sentinel eggs, albeit to varying degrees (Abram et al. 2017).

In 2017 and 2018, *T. japonicus* was the predominant species captured, representing 66.7% and 44.7% of all parasitoids in the respective years (Tables 5.1-5.2). In 2019, *T. japonicus* represented only 21.3% of total captures (Table 5.3). Of the native species, only *T. euschisti* was captured every year, and was the most abundant native species detected each year and in total (54.5% of the native captures) (Tables 5.1-5.3). *Telenomus podisi* was captured in two years and was the second most abundant native species, representing 25% of total native detections. With the exception of *T. edessae*, which was captured in only one year, all other native species were captured in two of the three years, but were somewhat or very rare, representing between 0.9 and 8.9% of native species.

## 5.5 Discussion

While yellow sticky traps have yielded novel information that can guide surveillance and monitoring efforts for *T. japonicus* (Chapters 3, 4), examination of ecological factors associated with its distribution in the landscape may lead to refined sampling protocols and improved detection efficiency. Given the ongoing spread of this important adventive parasitoid in the USA, questions of when and where to sample for *T. japonicus* have become especially relevant. The relatively robust *T. japonicus* population in Frederick Co., VA afforded the unique opportunity to address some of these questions. The data presented here did not indicate a consistent effect of host tree species on the frequency of *T. japonicus* captures. *Trissolcus japonicus* was not detected in any tree sampled except tree of heaven in 2017, was detected equally among all hosts in 2018, then not detected in tree of heaven in 2019. One important consideration is that in 2017 the sample size was much lower than in subsequent years, meaning that differences among hosts,

if present, were unable to be captured. Parasitoid distributions tend to be stochastic and aggregative, with parasitoids spending more time in areas of greater host density, thereby aggregating in those areas over time (Morrison 1986). The lack of *T. japonicus* captures on sticky traps deployed in native hosts in 2017 and tree of heaven in 2019 may well be a result of this stochastic process, especially given that the mean number of wasps per trap was close to zero in each year. Future efforts with more intensive sampling may circumvent this issue by providing sufficient statistical power.

Tree of heaven has been used as a standard or model host for this study and others (Chapters 2, 3, 4) due to its relative abundance (Acebes-Doria et al. 2017) and late-season suitability as a host for *H. halys* (Acebes-Doria et al. 2016). It is important to bear in mind that *H. halys*, *T. japonicus*, and tree of heaven are all endemic to the same regions in Asia (Kowarik, and Säumel 2007, Yang et al. 2009, Lee et al. 2013), leading one to reasonably speculate that *T. japonicus* might preferentially forage there due to their shared evolutionary history. However, despite their common origins, there did not appear to be a strong association between captures of *T. japonicus* and tree of heaven. In his review of novel associations between insects, including parasitoids, and plants, Agosta (2006) suggested that such associations are more flexible than previously thought, with ecological fitting occurring relatively easily so long as the novel plant shares similarities with the ancestral host. In fact, when an herbivore shifts hosts, its natural enemies may often follow in quick succession. This was demonstrated by the parasitoids of goldenrod (*Solidago* spp.) herbivores, which followed their hosts from *Solidago altissima* to *Solidago gigantea*, with no consistent differences in parasitization by host plant, and variations in attack rates attributed to site and seasonal variation (Heard et al. 2006). In scouting efforts in Asia, K. Hoelmer (pers. comm.) has observed lower levels of *T. japonicus* parasitization of egg masses on tree of heaven compared to other hosts sampled. Zhang et al. (2017) observed numerically greater parasitization of wild *H. halys* egg masses collected from black locust trees, but did not examine parasitization by host plant experimentally or statistically. Given the broad host range of *H. halys* (Bakken et al. 2015) and the diversity of feeding and/or reproductive hosts available to it in this region (Acebes-Doria et al. 2017), *T. japonicus* foraging should theoretically not be limited to a single habitat (Chapter 4) or host plant. An important consideration is our poor understanding of *H. halys* ovipositional hosts in its invaded range. Although Bakken et al. (2015)

observed few *H. halys* egg masses on wild trees, those found were on plants in 17 different families, suggesting relatively indiscriminate oviposition site selection given the range of relatedness and diversity of characteristics of these host plants. In counts of wild *H. halys* egg masses Formella et al. (2019) did not observe a significant difference among hosts in the number of egg masses present. *Trissolcus japonicus* may be following the kairomone trail left by gravid *H. halys* rather than a plant-specific cue as suggested by the findings of Boyle (2017), which showed that gravid *H. halys* footprints elicited strong host-seeking responses from *T. japonicus* regardless of the species of plant tissue on which they occurred. Captures of parasitoids on sticky traps are greater in hosts and habitats in which their hosts are present (Weseloh 1986). Yellow sticky traps attract insects by providing a “supernormal” foliage-type stimulus that elicits food or host seeking behavior (Prokopy 1972). It appears that in the absence of kairomones deposited by the tarsi of *H. halys*, the supernormal visual cue provided by the trap, rather the host plant itself, may be a strong attractant for *T. japonicus*.

Captures of native *H. halys* parasitoids varied among years but did not seem to be affected by plant species. This suggests that, similar to our findings with *T. japonicus*, host plant did not play a major role in captures of other *H. halys* parasitoids on yellow sticky traps. Milnes and Beers (2019) observed significantly lower levels of *T. japonicus* parasitization of sentinel eggs of native pentatomids than *H. halys* in the field. It is expected that *T. japonicus* will be able to coexist with native parasitoids of *H. halys* (Konopka et al. 2017), but the long-term effects of the addition of *T. japonicus* to the ecological landscape on pentatomid parasitoid communities have yet to be understood.

In the context of previous findings on the spatial distribution of *T. japonicus* detections and activity in the field (Chapters 2, 3), the data presented here suggest that it is more important to consider the height of the trap, and potentially the habitat type, rather than the particular host plant in which the trap is deployed for *T. japonicus* monitoring. This may be useful in areas where tree of heaven is relatively uncommon or for other scenarios requiring sampling flexibility. It is important to note however that yellow sticky traps provide a measure of relative abundance of the insects rather than a direct measure of biological control activity. There may be differential host plant effects on egg mass detection and parasitization by *T. japonicus*, but those

data are not captured here. Our findings suggest that yellow sticky traps are better suited for the study of *T. japonicus* detection and foraging ecology than for the exploration of questions pertaining to direct biological control impacts.

## 5.6 References

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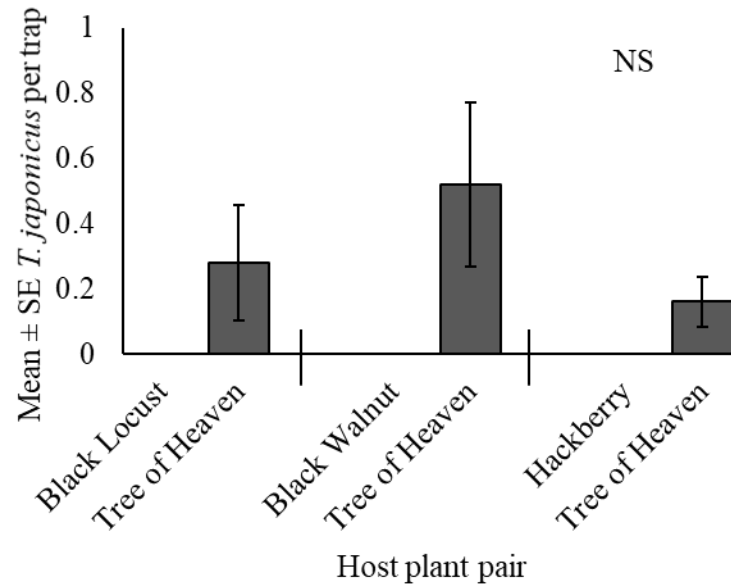
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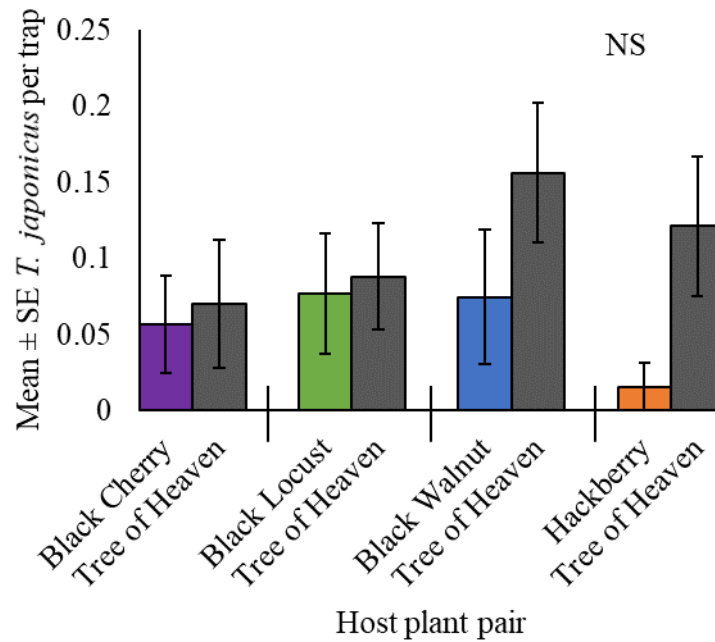
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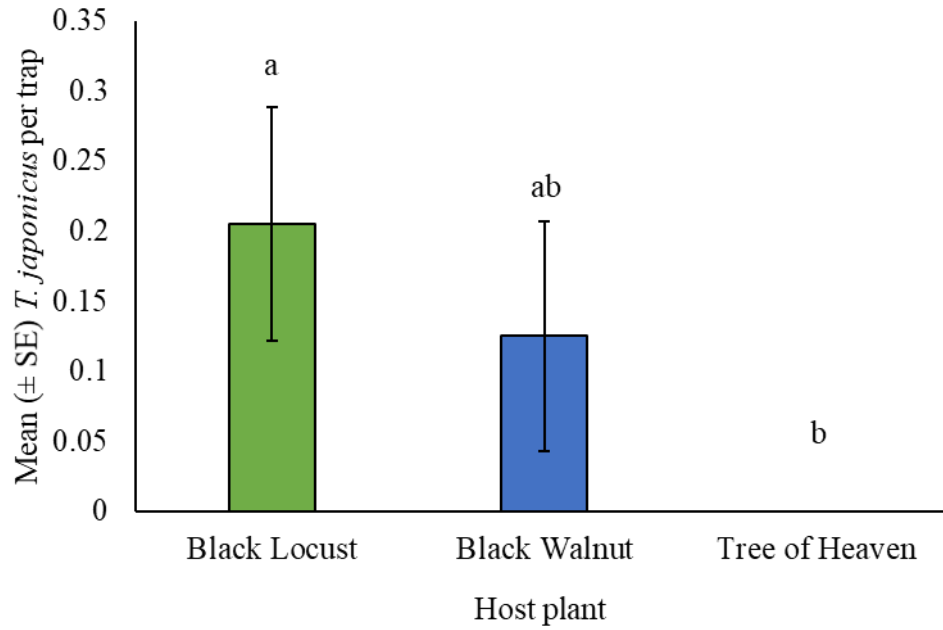
## 5.7 Figures and tables



**Figure 5.1.** Mean *T. japonicus* captures per yellow sticky trap deployed in mid-canopy of paired hosts ( $n = 5$  per pairing) at the forest edge in Frederick Co., VA, from 31 July until 29 August 2017. Traps were collected and replaced at  $7 \pm 2$ -day intervals. There were no significant differences in *T. japonicus* captures within host plant pairs as indicated by “NS” (Wilcoxon signed-rank test,  $P > 0.05$ ).



**Figure 5.2.** Mean *T. japonicus* captures per yellow sticky trap deployed in mid-canopy of paired hosts ( $n = 5$  per pairing) at the forest edge in Frederick Co., VA, from 13 June until 20 September, 2018. Traps were collected and replaced at  $7 \pm 2$ -day intervals. There were no significant differences in *T. japonicus* captures within host plant pairs as indicated by “NS” (Wilcoxon signed-rank test,  $P > 0.05$ ).



**Figure 5.3.** Mean captures of *T. japonicus* in yellow sticky traps deployed in mid-canopy of host trees in windbreaks (n = 5) in Frederick Co., VA, from 17 June until 11 August, 2019. Traps were collected and replaced at  $7 \pm 2$  day intervals. Bars with the same letter are not significantly different from one another (Kruskal-Wallis test and Bonferroni corrected Dunn's test,  $P > 0.05$ ).

**Table 5.1.** Adventive and native parasitoids of *H. halys* captured in yellow sticky traps deployed in mid-canopy of paired hosts (n = 5 per pairing) at the forest edge in Frederick Co., VA, from 31 July until 29 August 2017. Individual host pairs are indicated by shading. All parasitoids listed are known to emerge from *H. halys* sentinel egg masses (Abram et al. 2017)

Host plant	<i>Trissolcus</i>			<i>Telenomus</i>	<i>Gryon</i>
	<i>japonicus</i>	<i>brochymenae</i>	<i>euschisti</i>	spp.	spp.
Black Locust	0	1	1	3	1
Tree of Heaven	7	1	1	0	0
Black Walnut	0	0	0	0	0
Tree of Heaven	13	1	1	0	0
Hackberry	0	0	0	0	0
Tree of Heaven	4	0	2	0	0
<i>Total</i>	<i>24</i>	<i>3</i>	<i>5</i>	<i>3</i>	<i>1</i>

**Table 5.2.** Adventive and native parasitoids of *H. halys* captured in yellow sticky traps deployed in mid-canopy of paired hosts (n = 5 per pairing) at the forest edge in Frederick Co., VA, from 13 June until 20 September, 2018. Individual host pairs are indicated by shading. All parasitoids listed are known to emerge from *H. halys* sentinel egg masses (Abram et al. 2017).

Host plant	<i>Trissolcus</i>			<i>Telenomus</i>		<i>Gryon</i>
	<i>japonicus</i>	<i>thyantae</i>	<i>euschisti</i>	<i>podisi</i>	spp.	spp.
Black Cherry	3	1	6	1	1	0
Tree of Heaven	4	0	1	0	0	0
Black Locust	5	0	4	1	1	0
Tree of Heaven	6	0	3	6	0	0
Black Walnut	5	0	3	0	0	0
Tree of Heaven	10	0	5	0	0	0
Hackberry	1	1	5	2	0	1
Tree of Heaven	8	0	8	1	1	0
<i>Total</i>	<i>42</i>	<i>2</i>	<i>35</i>	<i>11</i>	<i>3</i>	<i>1</i>

**Table 5.3.** Adventive and native parasitoids of *H. halys* captured in yellow sticky traps deployed in mid-canopy of host trees in windbreaks (n = 5) in Frederick Co., VA, from 17 June until 11 August, 2019. All parasitoids listed are known to emerge from *H. halys* sentinel egg masses (Abram et al. 2017)

Host plant	<i>Trissolcus</i>					<i>Telenomus</i>	
	<i>japonicus</i>	<i>brochymenae</i>	<i>thyantae</i>	<i>euschisti</i>	<i>edessae</i>	<i>podisi</i>	spp.
Tree of Heaven	0	1	2	6	1	9	2
Black Locust	8	1	0	9	0	2	0
Black Walnut	5	1	0	6	0	6	2
<i>Total</i>	<i>13</i>	<i>3</i>	<i>2</i>	<i>21</i>	<i>1</i>	<i>17</i>	<i>4</i>

## Chapter 6

### Summary, Implications, and Suggestions for Future Research

#### 6.1 Results Summary and Implications

The studies reported in this dissertation revealed many important insights into the foraging ecology and sampling techniques for *T. japonicus* and its host, *H. halys*, in wild host trees. As an important natural enemy of one of the most injurious invasive tree fruit pests in recent years, a thorough understanding of adventive *T. japonicus* ecology is essential. As this work has demonstrated, *T. japonicus* appears to be well-established where these studies were conducted (Frederick County, VA), for reasons that remain to be determined. This fortunate circumstance enabled research on adventive *T. japonicus* that, based on current knowledge, was not possible in most other locations in the USA where it has been detected thus far. This dissertation has addressed critical knowledge gaps while laying the groundwork for future studies, as described below.

The impetus for the evaluation of the distribution of *H. halys* life stages in the canopy of its wild host trees was prompted by the detection of *T. japonicus* at the Alson H. Smith, Jr. Agricultural Research and Extension Center in 2015. Prior to the initiation of this research, all sampling for *H. halys* had occurred via ground-based traps or traps in the canopy of smaller trees (e.g., apple). The only information about its spatial distribution within the canopy of wild tree hosts was based on anecdotal reports of high bug density at the top of tree of heaven, measured via visual observations from a mechanical lift (BD Short, unpublished data). Additionally, Joseph et al. (2014) documented significantly greater fruit injury at the top of apple tree canopies than the middle and lower canopy. It was posited that if the density of *H. halys* adults is actually higher in the upper than lower portions of wild host trees, then the distribution of its egg masses may also be similarly stratified. If true, then by extension, *T. japonicus* may also be more prevalent in the upper portions of the tree canopy.

In Chapter 2, using female and male tree of heaven as the model host and hackberry as an alternate host, vertical transects of traps baited with a low-dose pheromone lure to capture *H. halys* were deployed, and used captures as a proxy for differences in bug density among canopy locations. Using this approach, it was determined that *H. halys* adults and nymphs were caught in the greatest numbers at the top of the tree canopy, regardless of host sex or species. This may be due to the negatively gravitactic and positively phototactic nature of *H. halys* movement (Acebes-Doria et al. 2016), resulting in greater densities higher up in the tree. Supporting this hypothesis, the mid-canopy foliage from felled tree of heaven contained the greatest number of *H. halys* egg masses. Moreover, *T. japonicus* emerged only from egg masses collected from the mid- and upper canopy of felled trees, suggesting that previous studies in which all sampling for *H. halys* egg masses occurred via visual observations from the ground or sentinel egg masses in the lower canopy may have underestimated *T. japonicus* activity. Laying egg masses at mid-canopy may be an evolutionary strategy to increase survivorship by making them more difficult for natural enemies to find due to increased habitat complexity (Gols et al. 2005, Martijn Bezemer et al. 2010) and protecting them shifting abiotic conditions (Ferro et al. 1979). The data presented here indicate that despite this, *T. japonicus* is still capable of finding the egg masses in these locations.

Primarily for convenience, most sampling for *T. japonicus* and native egg parasitoids of *H. halys* and other pentatomids has used either ground-based sampling for wild egg masses or sentinel egg masses deployed on host tree foliage at ~2 m above the ground. Following preliminary information showing that *T. japonicus* was captured in yellow sticky traps, a comparison of detections of *T. japonicus* using sentinel egg masses attached to cut foliage and yellow sticky traps deployed in vertical tree transects in the upper, middle, and lower tree canopy was performed (Chapter 3). Confirming earlier findings, the majority of *T. japonicus* were detected at mid-canopy using both sampling methods. It was also determined that yellow sticky traps were a much more resource-efficient sampling method. Informed by the findings reported in Chapters 2 and 3, a novel approach for sampling *T. japonicus* was developed, involving the deployment of yellow sticky traps at the apparently optimal mid-canopy location. The use of 4.8 m bamboo poles to enable sticky trap placement at greater heights proved to be a simple, cost-effective

method by which to detect *T. japonicus*, and can be adapted or modified to a variety of situations and ecosystems, as needed.

In Chapters 4 and 5, this sampling approach was employed to address questions about *T. japonicus* foraging ecology among habitats and hosts. Sampling in two consecutive seasons did not reveal consistent effects of habitat type (Chapter 4) or host tree species (Chapter 5) on *T. japonicus* captures. This suggests that sampling for *T. japonicus* need not be restricted to any single habitat or host plant species. However, the seasonal trends in captures were remarkably consistent between years. Captures across two seasons occurred on most weeks from mid-May through mid-September and showed two peaks annually, in about mid-July and early August (Chapter 4). Furthermore, for the purposes of *T. japonicus* surveillance in areas where it has not yet been detected and/or where it has been released, deploying yellow sticky traps in the mid-canopy of trees from July through about early August should optimize the likelihood and efficiency of detecting it. Across all studies in Frederick County, VA, native parasitoids of *H. halys* were collected or captured, albeit in lower numbers than *T. japonicus*. This indicated that *T. japonicus* is well-established locally and provided important baseline information for tracking the establishment and population dynamics of *T. japonicus* and native *H. halys* parasitoids here and elsewhere within its expanding range. However, overall low numbers prevent support for strong conclusions at this time.

## **6.2 Suggestions for Future Research**

The findings described here improve our understanding of the ecology of *T. japonicus* and *H. halys*, but may have the most immediate utility in improving detection rates of *T. japonicus* as it continues to expand its range. In several USA states in which *T. japonicus* has been detected, including Virginia, the respective Departments of Agriculture have approved its redistribution within state borders, and such efforts have been initiated. The sampling tool and protocol demonstrated by this work has already been adopted for surveillance by some researchers and are expected to improve the effectiveness and efficiency documenting the success of release and redistribution efforts. Based on the data reported, it is recommended that *T. japonicus* sampling should occur at mid-canopy to increase the likelihood of *T. japonicus* detection during

surveillance efforts. The habitats and host plants sampled here were found to be equally likely to yield *T. japonicus*, suggesting that for surveillance purposes, habitat and host plant deployment have limited impact on detection. Ultimately this benefits surveillance efforts, allowing for greater flexibility to accommodate ecosystem differences and researcher needs. Adoption of yellow sticky traps deployed at mid-canopy of wild host trees will enable efficient detection of *T. japonicus* across a variety of ecosystems, both cultivated and wild. However, there is a need to improve the selectivity of sticky traps as a monitoring method, as capture of non-target insects can reduce visible trap area (pers. obs.), potentially reducing detection rates. To this end, further exploration of trap colors could be informative. Yellow is widely accepted as a broad-spectrum insect attractant, including for parasitoids (Weseloh 1986), but there may be other colors that are equally or more effective while reducing nontarget captures. In 2019, a field study in which *T. japonicus* captures were compared among yellow, green, white, blue, and red traps, captures were too low to make definitive statements about differences among the color treatments. Future trap color studies may be best conducted in the lab to ensure sufficient replication, prior to field testing. Another approach would be to reduce the ability of non-target insects to become trapped by adding netting or mesh to the trap to allow larger insects to escape, as described by Sétamou et al. (2019).

Much about the ecology of adventive *T. japonicus* populations remains to be explored. The studies detailed here all occurred in wild host trees adjacent to apple and peach orchards in Virginia. However, its foraging ecology may well differ among regions or crops, e.g. west coast almond groves. Applying the methods detailed in this dissertation to other contexts could reveal additional information about *T. japonicus* foraging ecology. Future studies would also benefit from simultaneous sampling of *H. halys* populations in areas in which *T. japonicus* is monitored to examine the relationship between captures of *T. japonicus* and *H. halys* abundance (Zhang et al. 2017). Additionally, future studies should continue to monitor native parasitoids to record their response to the addition of *T. japonicus* over time.

The dispersal patterns and habitat utilization of *T. japonicus* in the field need additional study, although minute size and relative scarcity of *T. japonicus* pose significant obstacles to this. Future studies could address this in a semi-field context, releasing lab-reared *T. japonicus* in a

large field cage and measuring the number of *T. japonicus* caught on traps at increasing distances from the release point over time. Field studies utilizing mark-release-recapture could also be an excellent approach. Parasitized egg masses could be dusted with protein powder in the lab, which *T. japonicus* would ingest upon emergence, preparing them for gut content analysis upon their recapture. Large numbers of *T. japonicus* would be needed for this to ensure sufficient recapture rates.

The data presented here did not show consistent host plant effects on *T. japonicus*, indicating that this is also an area needing additional study. Lab and field studies comparing the relative attractiveness of different host plants with and without *H. halys* semiochemical cues could yield insights into the foraging ecology of *T. japonicus* in the field. Several iterations of choice experiments exploring the ability of *T. japonicus* to locate *H. halys* egg masses with and without tree foliage were attempted under lab, greenhouse, and semi-field conditions for this dissertation, all of which proved unsuccessful. This was likely due to the absence of adult *H. halys* kairomones, which were not present in any study, but which several recent studies have demonstrated to be important in eliciting a search response from *T. japonicus* (Boyle 2017, Bertoldi et al. 2019). However, it is still possible that host plants, independent of *H. halys* cues, play a role in *T. japonicus* long-distance patch evaluation. Y-tube studies in which *T. japonicus* is exposed to volatile organic compounds from host plant species could be informative. Perhaps the use of clear sticky traps, thereby removing the visual stimulus of the trap, in a semi-field experiment would provide additional information on the constitutive attractiveness of host plants in the absence of *H. halys* cues. However, the efficacy of clear sticky traps for capturing *T. japonicus* has yet to be determined. Ideally, the sticky trap would be paired with a lure to enhance *T. japonicus* captures, but as of yet a long-range semiochemicals attractant of *T. japonicus* has not been identified. Another important aspect of *T. japonicus* host plant utilization that has yet to be determined is their role as food sources. *Trissolcus* species and other parasitoids require sugars, usually in the form of flower nectar, to survive and produce eggs (Rahat et al. 2005). Determining which floral resources enhance their longevity and fecundity may be an essential aspect of supporting their biological control within the landscape. Given the arboreal tendencies of *T. japonicus*, examining the flowers of common host trees, such as tree of

heaven and black cherry, in this regard, though cultivated hosts such as peach may also be of interest.

Another area in need of study is the overwintering biology of *T. japonicus*. Other scelionids have been documented overwintering under tree bark (Lodos 1961), but overwintering *T. japonicus* has not been recovered from hibernacula in the field. Scouting of tree bark in winter 2019 at sites in which *T. japonicus* had previously been captured did not yield any detections (N. F. Quinn unpublished data). It is possible that *T. japonicus* travels deep into tree crevices where it is unlikely to be found, or it may use another habitat entirely, such as leaf litter. Knowing the preferred overwintering habitat of *T. japonicus* would allow growers to provide or preserve such habitat on their farms, potentially increasing their early-season populations of *T. japonicus*. Measuring the winter survival of *T. japonicus* placed in various artificial hibernacula may help address this question.

### 6.3 References

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