Toward optimized surveillance of adventive *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) based on aspects of its foraging ecology

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science, Life Sciences
In
Entomology

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December 9, 2021
Winchester, VA

Keywords: Brown marmorated stink bug, biological control, samurai wasp, parasitoid

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ABSTRACT

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), an invasive agricultural pest from Asia, has established in much of the USA, where it has had significant, deleterious impacts on the production of various specialty crops. *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) is the predominant egg parasitoid of *H. halys* in Asia and considered a potential agent for classical biological control. Adventive *T. japonicus* populations have been detected in 15 US states since 2014 and its continued spread is anticipated, creating the impetus for developing optimized sampling methods for it that are informed by its foraging ecology. Yellow sticky cards (YSC) deployed in the lower and mid-canopy of *Ailanthus altissima* (Mill.) Swingle trees in Virginia captured statistically equivalent numbers of *T. japonicus*. Season-long monitoring of *T. japonicus* and *H. halys* over two seasons resulted in significant correlations between captures of *T. japonicus* and *H. halys* adults, second instar nymphs, and all life stages combined, revealing spatiotemporal synchrony between the parasitoid and its host. Inspection of felled pheromone-baited and non-baited *A. altissima* showed no effect of baiting with the *H. halys* aggregation pheromone on *H. halys* egg mass abundance. Similarly, captures of *T. japonicus* in pheromone-baited and non-baited trees were not significantly different. Augmenting YSC with *H. halys* egg masses or *n*-tridecane did not improve *T. japonicus* captures. Together, these results should improve the efficiency of *T. japonicus* surveillance efforts and provide important guidance for future research toward enhancing the sensitivity of sampling tools for it and the interpretation of its impacts on *H. halys.*

Toward optimized surveillance of adventive *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) based on aspects of its foraging ecology

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GENERAL AUDIENCE ABSTRACT

The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), an invasive species from Asia, has become widely distributed throughout the United States as a serious pest of many important crops. Parasitic wasps that develop on eggs are often key natural enemies for reducing stink bug populations. The samurai wasp, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), is the primary parasitic wasp of BMSB in Asia and is under evaluation for potential use in biological control programs. Populations of samurai wasps have been detected in the United States since 2014 without intentional introduction. Its continued spread is anticipated, creating the need for effective sampling methods to track its establishment. Yellow sticky cards (YSC) deployed in the lower and mid-canopy of tree of heaven in Virginia captured similar numbers of samurai wasps. Season-long monitoring of samurai wasps and BMSB over two seasons resulted in significant correlations between captures of samurai wasps and BMSB adults, second instar nymphs, and all life stages combined, revealing spatiotemporal overlap between the wasp and its host. Inspection of felled pheromone-baited and non-baited tree of heaven showed no effect of baiting with the BMSB aggregation pheromone on numbers of BMSB egg masses. Similarly, captures of samurai wasps in pheromone-baited and non-baited trees were not significantly different. Combining YSC with BMSB egg masses or *n*-tridecane did not improve samurai wasp captures. Together, these results provide important guidance for future research toward enhancing the sensitivity of sampling tools for samurai wasps and the interpretation of its impacts on BMSB.

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Acknowledgements

First and foremost, I would like to express my gratitude and sincerest thanks to my advisors, Dr. Chris Bergh and Dr. Tracy Leskey, for their guidance and support throughout my Master's program. I'd also like to thank Dr. Thomas Kuhar for serving on my thesis committee and providing vital insights. Additionally, I would like to thank Dr. Elijah Talamas for his invaluable expertise and much appreciated service providing species ID confirmations for the many parasitoid specimens collected throughout this thesis. I am also grateful for Dr. Mizuho Nita for his statistical assistance regarding Chapter 2 of this thesis. Many members of the Bergh and Leskey Labs contributed to the work of data collection, especially during the 2020 field season, and I'd like to extend deep gratitude to all those involved including Nate Brandt, Brian Ruether, Whitney Hadden, John Cullum, Laura Nixon, Anthony Rugh, Lee Carper, Chris Hott, and Sharon Jones. I would also like to extend special thanks to the many growers in Frederick and Warren Counties for allowing me to conduct these studies on their land. Lastly, this research was made possible through the support of awards from USDA #2016-51181-25409 and the Virginia Department of Agriculture and Consumer Services.

Chapter 1

Introduction and Literature Review

1.1 Invasive Species

The introduction of invasive, alien species to novel environments has had significant negative impacts on biodiversity (Doherty et al. 2016), food production (Paini et al. 2016), and human well-being (Jones 2017). Invasive species have been identified as a major driver of species extinctions (Bellard et al. 2016), and can impair ecosystem services (Pejchar and Mooney 2009). The global economic cost of damages and management associated with biological invasions has been estimated at $162.7 billion USD annually (Diagne et al. 2021). In the United States alone, approximately 50,000 invasive species, including 4,500 arthropods, have become established (Pimentel et al. 2005). Many invasive species possess a suite of *r*-selected life history traits (e.g., high dispersal capacity, fast development, high fecundity) which contribute to their success in novel environments (Sakai et al. 2001). Additionally, it has been proposed that many invasive species experience "release" from their co-evolved natural enemies when introduced to new territory (Keane and Crawley 2002). Lack of top-down regulation can enable invasive species to outcompete native species and rapidly proliferate across the landscape in the absence of effective constraints on their population growth (Liu and Stiling 2006). Thus, one strategy implemented for the management of invasive species has been the use of classical biological control.

Classical biological control (CBC) has been defined as, "The intentional introduction of an exotic, usually co-evolved, biological control agent for permanent establishment and long-term pest control" (Eilenberg et al. 2001). The modern practice of CBC began in 1888 with the introduction of the vedalia beetle, *Rodolia cardinalis* (Mulsant), against cottony cushion scale, *Icerya purchase* (Maskell), and was the first successful suppression of a pest population using this management approach in the United States (Caltagirone and Doutt 2003). Since then, over 2,300 biological control agents have been introduced through CBC programs to control over 500 pest species worldwide (Cock et al. 2016). By introducing natural enemies from the native range of an invasive pest, CBC follows the base assumption of the enemy release hypothesis and seeks to reestablish the top-down regulation lost when an invasive pest was initially introduced to the novel environment. In the modern era of CBC, candidate biological control agents are subject to extensive risk-assessment protocols involving centrifugal phylogenetic host range tests, among other measures, to ensure their safety (Heimpel and Cock 2018). As a result, relatively few negative effects on non-target species have been observed from CBC throughout its >120 year history (van Lenteren et al. 2005). Biological control remains one of the safest and most cost-effective forms of providing long-term management of insect pests (Bale et al. 2007), and can be used to address new and ongoing biological invasions.

1.2 Invasion Biology of Brown Marmorated Stink Bug, *Halyomorpha halys*

A relatively recent and important invasive species is the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), a highly polyphagous, herbivorous pest native to northeast Asia (Lee et al. 2013). *Halyomorpha halys* was first detected beyond its native range in the late 1990s in Allentown, Pennsylvania, although its identity was not confirmed until 2001 (Hoebeke and Carter 2003). In 2007, the first detection of *H. halys* in Europe was reported from Switzerland (Wermelinger et al. 2008). Genetic analyses indicated that invasive populations of *H. halys* originated from populations in China, which were sources of at least four independent introductions to North America and three in Europe (Valentin et al. 2017). Currently, *H. halys* has been detected in 47 US states (www.stopbmsb.org), Canada (Fogain and Graff 2011), central and eastern Europe (Haye et al. 2015a, Gapon 2017), and most recently in Chile (Faúndez and Rider 2017). Additionally, multiple interceptions of *H. halys* have been made at ports of entry in New Zealand and Australia. In the United States, *H. halys* is estimated to threaten $21 billion worth of crops (ODA 2020), and with a presence on multiple continents, could be considered a pest of global importance.

Halyomorpha halys possesses many of the *r*-selected life history traits of a successful invasive species (Sakai et al. 2001). It utilizes over 170 known wild and cultivated hosts for feeding and/or reproduction, including many economically-important crops such as apples, peaches, some vegetables, and soybean (Leskey and Nielsen 2018). Additionally, *H. halys* nymphs and adults show strong dispersal capacity within and across landscapes via walking and adult flight (Lee et al. 2014a, Lee and Leskey 2015). *Halyomorpha halys* overwinter as adults, often as aggregations in concealed, sheltered locations. In natural areas, this can include rocky outcroppings and beneath the bark of dead trees (Lee et al. 2014b), but it also commonly invades human-made structures, thus becoming an important nuisance pest (Inkley 2012, Lee et al.

2014b, Bergh and Quinn 2018, Hancock et al. 2019). In Asia, overwintering adults begin to emerge in early spring (Lee et al. 2013), a pattern also observed in the Mid-Atlantic United States, where peak emergence occurs from early May through early June (Bergh et al. 2017). Following emergence from overwintering, female *H. halys* are synovigenic and undergo a lengthy pre-oviposition period, during which they become reproductively mature (Nielsen et al. 2008), and can mate and oviposit for the duration of their lifespan (Kawada and Kitamura 1983). Females will produce an average of 9.3 egg masses throughout their lifespan (Nielsen and Hamilton 2009), typically ovipositing on the underside of host plant leaves in median clutch sizes of 28 eggs per mass (Nielsen et al. 2008). Phenological modeling predicts bivoltinism for *H. halys* throughout most of the United States (Nielsen et al. 2016a), with numbers of adults peaking in September and October (Bakken et al. 2015, Leskey et al. 2015, Acebes-Doria et al. 2020).

1.3 Pest Status, Monitoring, and Management of *H. halys*

Pest status

In tree fruits, feeding injury from *H. halys* is expressed as discolored depressions and/or distortions on external surfaces and discolored necrosis of internal tissues beneath feeding sites (Joseph et al. 2015, Acebes-Doria et al. 2016, Kuhar et al. 2012). Adult and late-instar nymphs in particular can inflict severe damage to apple and peaches as fruit mature prior to harvest (Acebes-Doria et al. 2016), but peaches are vulnerable to feeding damage from adults as soon as the crop is set (Joseph et al. 2015, Acebes-Doria et al. 2016). In 2010, a severe and widespread outbreak of *H. halys* in the Mid-Atlantic region of the US caused over $37 million in losses to the apple crop (Leskey et al. 2012a) and the loss of > 90% of stone fruit crop in some orchards (Leskey et al. 2012b).

Halyomorpha halys is considered a perimeter-driven pest in many specialty crop systems (Leskey et al. 2012b, Joseph et al. 2014, Venugopal et al. 2014, Aigner et al. 2017, Blaauw et al. 2016, Hadden et al. 2021), moving into crop fields from adjacent habitats, of which unmanaged woodlands adjoining crop borders are a major source of pressure from *H. halys* (Aigner et al. 2017, Bergh et al. 2021).

Conventional management

In Asia, *H. halys* outbreaks have been managed primarily through repeated applications of pyrethroid and neonicotinoid insecticides (Lee et al. 2013). Likewise, management of invasive *H. halys* populations in the United States has relied heavily on insecticides (Leskey et al. 2012a, Kuhar and Kamminga 2017), and specifically on a few broad-spectrum products from the pyrethroid, neonicotinoid, and carbamate classes (Leskey et al. 2012a, Rice et al. 2014). Pyrethroids or premixtures of a pyrethroid and a neonicotinoid have been used most widely for *H. halys* control, but are also very toxic to arthropod natural enemies and have led to secondary pest outbreaks (Leskey et al. 2012a). Consequently, there has been great interest in the development of monitoring and management tools and programs that would enable sustainable, long-term control of *H. halys* within an Integrated Pest Management (IPM) framework.

Monitoring

Khrimian et al. (2014) identified the male-produced aggregation pheromone of *H. halys,* (3S,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol (3) and (3R,6S,7R,10S)-10,11-epoxy-1-bisabolen-3-ol. Subsequently, Weber et al. (2014) showed syngergized captures of *H. halys* when traps were baited with its aggregation pheromone in combination with methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate (MDT), the aggregation pheromone of another pentatomid species, *Plautia stali* (Scott). Together, these findings enabled the development of commercial lures for *H. halys* monitoring. Black pyramid traps baited with these lures were effective for monitoring *H. halys* adults and nymphs in the United States (Leskey et al. 2015, Acebes-Doria et al. 2018, 2020). Subsequently, clear sticky traps mounted on stakes with *H. halys* lures were found to be equally effective as pyramid traps for monitoring *H. halys* across differing population densities and landscapes (Acebes-Doria et al. 2018, 2020, Leskey et al. 2021). These *H. halys* monitoring tools have been used to develop IPM tactics, such as an action threshold based on cumulative captures in pheromone-baited traps (Short et al. 2017), intended to inform decision-making about its management by growers and crop consultants. Moreover, pheromone-based tactics informed by its response to the pheromone and by the distribution of its density and injury in orchards have led to the development of an attract-and-kill management approach in individual border row trees in apple orchards (Morrison et al. 2019, Leskey et al. 2020), toward reducing insecticide applications against it to entire orchard blocks.

Biological control

As a natural response to the *H. halys* invasion, biological control of *H. halys* has received considerable attention (Herlihy et al. 2016, Ogburn et al. 2016, Dieckhoff et al. 2017, Tillman et al. 2020). In North America, a number of native generalist predators feed on *H. halys* eggs, including species from the families Tettigoniidae, Carabidae, and Gryllidae (Morrison et al. 2016), and others that prey on adults and/or nymphs (Abram et al. 2017a). However, their combined effects have been insufficient to suppress *H. halys* populations adequately (Ogburn et al. 2016, Abram et al. 2017a). In its native range, *H. halys* eggs are attacked by a community of eggs parasitoids (Lee et al. 2013, Zhang et al. 2017), and much attention has been given to both native and exotic parasitoids within invaded ranges. In North America, *H. halys* eggs are attacked by three families of hymenopteran parasitoids—Scelionidae, Eupelmidae, and Encyrtidae (Abram et al. 2017a). Additionally, a few species of Tachinidae (Diptera) have been reported to parasitize *H. halys* adults (Abram et al. 2017a), such as *Pentamorphaga latifascia* (Villeneuve) in China (Chen et al. 2020).

Documented parasitism of *H. halys* eggs by native parasitoids, based on different sampling methodologies, habitats, and locations, has varied widely, ranging from 3% to 55% among field studies (Jones et al. 2014, Haye et al. 2015b, Cornelius et al. 2016a,b, Herlihy et al. 2016, Ogburn et al. 2016). While many native parasitoids attack *H. halys* eggs, rates of successful parasitism have typically been low, with many species failing to develop or emerge from *H. halys* eggs (Cornelius et al. 2016b, Ogburn et al. 2016). Parasitoid-induced mortality of *H. halys* eggs by native parasitoids can contribute to overall egg mortality and may be a useful component of some biological control regimes (Abram et al. 2016), but also may constitute an “evolutionary trap” by diminishing their ecological services (Abram et al. 2014). Compounds on the surface of *H. halys* eggs, for example, interfere with host recognition and reduce parasitism by the native parasitoids, *Telenomus podisi* (Ashmead) and *Trissolcus erugatus* (Johnson) (Tognon et al. 2017). While some generalist egg parasitoids that did not evolve with *H. halys*, such as the European species, *Anastatus bifasciatus* (Geoffroy), have been shown to respond to *H. halys* adult volatiles and herbivore-induced plant volatiles (Rondoni et al. 2017), the apparent inability of native parasitoids to utilize *H. halys* associated cues has limited their success at providing adequate biological control.

Following initial detections of *H. halys* in PA and NJ, field surveys in its native Asian range were conducted to identify potential agents for use in classical *H. halys* biological control programs (Lee et al. 2013). In Asia, *H. halys* is attacked by a guild of hymenopteran egg parasitoid species that are similar to those in the USA, including species of *Trissolcus* (Hymenoptera: Scelionidae), *Telenomus* (Hymenoptera: Scelionidae), *Anastatus* (Hymenoptera: Eupelmidae), and *Ooencyrtus* (Hymenoptera: Encyrtidae), (Arakawa and Namura 2002, Yang et al. 2009, Lee et al. 2013). Among the members of this egg parasitoid guild, species of *Trissolcus* have shown promise as potential biological control agents for *H. halys* (Lee et al. 2013), and *Trissolcus japonicus* (Ashmead) (syn. *T. halyomorphae*) was identified as a key natural enemy for *H. halys*.

1.4 *Trissolcus japonicus*, the Samurai wasp

The accepted common name of *T. japonicus* is samurai wasp. It is a solitary endoparasitoid of pentatomid eggs, that like *H. halys*, is native to Asia and is the predominant *H. halys* parasitoid there (Yang et al. 2009, Zhang et al. 2017). In China, *T. japonicus* shows annual parasitism rates of *H. halys* eggs ranging from 50% to 80% (Yang et al. 2009). The species has a female-biased sex ratio (Yang et al. 2009) and can complete several generations per year (Qiu et al. 2007), characteristics which make it a promising candidate for classical biological control.

The lifetime fecundity of female *T. japonicus* averages 44.2 eggs, and it often parasitizes nearly all eggs in a mass (Yang et al. 2009). Female *T. japonicus* prefer to oviposit in egg masses not more than 72-hr old (Qiu et al. 2007). Like many egg parasitoids, *T. japonicus* host acceptance involves a process of examination via antennal "drumming," probing, drilling, and oviposition (Godfray 1994, Botch and Delfosse 2018). Scelionids such as *T. japonicus* often exhibit aggressive egg-guarding behavior in which female wasps will remain on parasitized masses for several hours (Field 1998). In Europe, *T. japonicus* parasitism has been observed to facilitate successful multi-parasitism of *H. halys* eggs by the European native, *Trissolcus cultratus* (Mayr) (Konopka et al. 2017a). To complete development. *T. japonicus* requires the accumulation of 132.5 DD at a minimum developmental threshold of 12.2 C (Qiu et al. 2007). Fully developed wasps emerge by chewing a hole through the egg operculum. Males typically emerge first, then remain on the egg mass to mate with emerging females (Yang et al. 2009).

Explorations in Asia identified *T. japonicus* as a prime candidate for classical biological control, and populations from Beijing were studied in quarantine facilities in the USA for host-range testing and risk evaluation (Talamas et al. 2015, Dieckhoff et al. 2017). However, in 2014 an adventive population of *T. japonicus* was discovered in Beltsville, MD during surveys of native *H. halys* parasitoids via *H. halys* sentinel egg masses (Talamas et al. 2015, Herlihy et al. 2016). Genetic analyses confirmed that this population was distinct from those in quarantine at the USDA facility in nearby Newark, DE, and therefore not due to a security breach (Bon et al. 2017). Subsequently, *T. japonicus* has been detected in 15 US states (and DC) (www.stopbmsb.org), Canada (Abram et al. 2019, Gariepy and Talamas 2019), Switzerland (Stahl et al. 2019), Italy (Peverieri et al. 2018), and Germany (Dieckhoff et al. 2021), and niche models predict the potential for its establishment where *H. halys* has established (Avila and Charles 2018, Yonow et al. 2021).

In 2020, Italy became the first European country to authorize *T. japonicus* releases, following confirmation of adventive populations within its borders (Conti et al. 2021). In New Zealand, regulatory agencies have taken pre-emptive, risk assessment measures to approve the conditional release of *T. japonicus* if *H. halys* establishes there (Charles et al. 2019, Conti et al. 2021). Some states in the US have permitted the redistribution of adventive *T. japonicus* within their borders, using wasps reared from specimens collected within the respective states, and several such efforts are in progress (Bergh unpublished, Jentsch 2017, Lowenstein et al. 2019). Ludwick et al. (2020) demonstrated the potential for *T. japonicus* to be integrated into conventional orchard management practices.

A number of studies concerning *T. japonicus* have been conducted in controlled laboratory settings (Konopka 2017a,b, Zhong et al. 2017, Bertoldi et al. 2019, Boyle et al. 2020a,b, Malek et al. 2021, Miura et al. 2021), but the discovery of adventive populations presented important opportunities to study its biology and ecology in the field and to address questions concerning its establishment, range expansion, and foraging behavior.

1.5 Foraging behavior and ecology of *T. japonicus*

As mentioned previously, *T. japonicus* is the predominant parasitoid of *H. halys* in Asia, with reported parasitism rates in the field ranging from 50-80% (Yang et al. 2009) and 70% of parasitized eggs yielding wasps under laboratory conditions (Zhang et al. 2017). Similar rates of parasitism under experimental conditions have been reported in the US and Europe, ranging from 50-90% (Hedstrom et al. 2017, Haye et al. 2020). No-choice laboratory assays with nontarget hemipteran hosts have revealed a rather broad physiological host range for *T. japonicus* (Hedstrom et al. 2017, Lara et al. 2019, Haye et al. 2020). Hedstrom et al. (2017) reported that *T. japonicus* parasitized 7 of 10 stink bug species tested, including *Banasa dimidiata* (Say) and *Holcostethus abbreviatus* (Uhler), whose parasitism rates were not significantly different from *H. halys*. Similarly, Lara et al. (2019) found that *T. japonicus* developed in 6 of 10 non-target species in California, and in Europe, successful emergence of *T. japonicus* was observed in 11 of 13 non-target species in no-choice assays (Haye et al. 2020), including the beneficial, predatory pentatomid, *Podisus maculiventris* (Say) (Abram et al. 2014). However, while *T. japonicus* can develop on a number of non-target species, parasitism of *H. halys* eggs was typically higher than those from non-target species in paired-host assays (Hedstrom et al. 2017, Lara et al. 2019, Haye et al. 2020). Additionally, *T. japonicus* reared on native hosts are smaller and produce fewer offspring than those reared on *H. halys* (Medal and Smith 2015, Botch and Delfosse 2018), suggesting that *H. halys* is a superior reproductive host for *T. japonicus*. Zhang et al. (2017) reported that while *T. japonicus* did parasitize non-target pentatomids in the field, a level of niche partitioning was observed among pentatomid egg parasitoids, with each host species having a dominant parasitoid species. Evidence from Oregon supported a narrower realized host range for *T. japonicus*, as observed levels of non-target parasitism were significantly less than parasitism of *H. halys* (Milnes and Beers 2019). In combination, these data suggest that *T. japonicus* is unlikely to cause significant negative impacts on non-target hemipterans.

Under experimental conditions, female *T. japonicus* have exhibited positive behavioral responses to a number of *H. halys* associated stimuli. In Y-tube assays, they responded to volatiles from plants with *H. halys* herbivory and oviposition, but not plants with only *H. halys* herbivory or plants attacked by the native, *P. maculiventris* (Bertoldi et al. 2019). Female *T. japonicus* are also responsive to the tarsal residues, or “footprints”, left by *H. halys* adults on various substrates (Boyle et al. 2020a, Arif et al. 2021, Malek et al. 2021), and the use of such footprints during host location by Scelionidae is well documented (Colazza et al. 2009, Abram et al. 2017b, Gomes Lagôa et al. 2020). Boyle et al. (2020a) reported that *T. japonicus* exposed to a substrate contaminated by *H. halys* showed residence times that were twice as long and increased linear walking velocity compared with controls or a non-target species (*P. maculiventris*). Parental host species did not significantly effect residence times on patches contaminated by either species, suggesting a degree of host fidelity in *T. japonicus* (Boyle et al. 2020b). Similar positive responses to *H. halys* adult and nymph tarsal prints were reported by Malek et al. (2021). Furthermore, *T. japonicus* can distinguish between *H. halys* and native host footprints and between female and male *H. halys* footprints (Arif et al. 2021). Tarsal residues left by *H. halys* can remain bioactive for at least 4 days (Malek et al. 2021), but their role in host location under field conditions has not been explored.

The response of female *T. japonicus* to *H. halys* tarsal prints may be related to the presence of *n*-tridecane, identified as a main component of *H. halys* tarsal residues by Malek et al. (2020). This compound is a defensive secretion from the metathorax that has elicited electrophysiological and behavioral responses in female *T. japonicus* (Zhong et al. 2017, Malek et al. 2021) in addition to behavioral responses in other pentatomid natural enemies, such as the minute pirate bug, *Orius insidiosus* (Say) (Fraga et al. 2017) and *T. podisi* (Laumann et al. 2009). In *H. halys*, the production of *n*-tridecane increased with *H. halys* density while the production of aggregation pheromone decreased (Harris et al. 2015). Thus, it has been hypothesized that *n*-tridecane may function in regulating chemical communications between male *H. halys* for aggregation and mating (Harris et al. 2015, Zhong et al. 2017). Zhong et al. (2017) reported that *T. japonicus* was attracted to 40 ng of *n*-tridecane and showed reduced host search time when it was applied to egg masses. While behavioral responses to n-tridecane have been observed under laboratory conditions, attraction to *n*-tridecane in field settings has not been examined.

1.6 Monitoring adventive *T. japonicus*

Initial surveys to monitor the parasitism of *H. halys* eggs by *T. japonicus* and other parasitoids used sentinel *H. halys* eggs masses (Herlihy et al. 2016, Hedstrom et al. 2017, Morrison et al. 2018, Abram et al. 2019, Milnes and Beers 2019). While essential for addressing questions about the taxa that attack them and parasitism rates, sentinel eggs are limited in their effectiveness for intensive and extensive surveillance of *T. japonicus* across large geographic scales. The handling and manipulation of sentinel eggs may remove semiochemical cues used for host location (Godfray 1994) and may contribute to their tendency to underestimate actual parasitism (Jones et al. 2014). Additionally, the acceptability of *H. halys* eggs to *T. japonicus* declines after about 72 hr (Qiu et al. 2007) and they are prone to predation during deployment (Abram et al. 2015).

Moreover, sentinel egg deployment requires maintaining an *H. halys* colony and daily collection of egg masses (Medal et al. 2012), both rather labor-intensive processes. Collected masses not immediately used in field studies must be frozen, and McIntosh et al. (2019) reported that parasitism of frozen egg masses was reduced by 1-3% per month in storage and caused a delay in wasp emergence after 5-6 days of freezing.

Yellow sticky cards (YSC) are an efficient alternative for addressing questions about the presence, abundance, and range expansion of adventive *T. japonicus* (Quinn et al. 2019a). An innate preference for yellow has been observed in other scelionid wasps (Ferreira Santos de Aquino et al. 2012) and YSC have been used effectively to monitor adventive *T. japonicus* in Virginia (Quinn et al. 2021) and Pennsylvania (Peterson et al. 2021), and to establish state records of *T. japonicus* (e.g. Utah, Holthouse et al. 2021). Quinn et al. (2019b) captured significantly more *H. halys* adults and nymphs in the mid- and upper canopy of large *A. altissima* trees, found most *H. halys* egg masses in the mid-canopy of felled trees, and showed that most masses parasitized by *T. japonicus* were in mid-canopy. However, most surveys, whether via sentinel eggs or YSC, have been conducted from ground level. Based on the findings of Quinn et al. (2019a,b) this practice may lead to an underestimation of *H. halys* egg mass density and levels of egg parasitism.

1.7 Justification for research

Halyomorpha halys continues to threaten global crop production, and additional invasive populations are likely to establish in new areas and/or countries. At the same time, first records of adventive populations of *T. japonicus* continue to be reported in Europe and North America. Although the impact of these adventive populations on *H. halys* has yet to be determined (Abram et al. 2020), developing effective and efficient methods for monitoring *T. japonicus* will be critical for tracking the spread, growth, and distribution of these adventive populations. The discovery of adventive *T. japonicus* populations presents important opportunities to study its biology and ecology in the field and to address questions concerning the establishment, expansion, and foraging behavior of this exotic egg parasitoid. This thesis aims to improve the efficiency and sensitivity of *T. japonicus* surveillance efforts and to establish a basis upon which future research can proceed. Furthermore, the development of optimized surveillance informed by aspects of *T. japonicus* foraging ecology will provide valuable insights into the behavior of adventive populations that will aid the interpretation of future studies, and particularly those involving *T. japonicus* releases, if eventually approved by USDA APHIS. In Chapter 2, captures of *T. japonicus* in YSC deployed in the mid- and lower canopy of host trees were compared to resolve ongoing questions about the effect of trap location in the tree canopy. In Chapter 3, the spatial and temporal synchrony of *H. halys* and *T. japonicus* was examined, using seasonal captures in pheromone traps and YSC, respectively. In Chapter 4, the effect of baiting the canopy of host trees with *H. halys* aggregation pheromone lures on *H. halys* egg mass abundance and captures of *T. japonicus* in YSC was tested to understand the role of *H. halys* aggregation on the behavior of *T. japonicus* and its host. Additionally, YSC were augmented with *H. halys* egg masses and *n*-tridecane to determine if detections of *T. japonicus* in YSC could be enhanced with *H. halys* associated stimuli.

1.8 Objectives

Objective 1 (Chapter 2). Evaluate the effect of trap location in the canopy of an *H. halys* tree host on captures of *T. japonicus*.

Objective 2 (Chapter 3). Determine the spatial and temporal relationship between *H. halys* and adventive *T. japonicus* through season-long monitoring of each.

Objective 3 (Chapter 4). Assess the effect of stimuli associated with *H. halys* on the abundance of its egg masses in the tree canopy and captures of *T. japonicus*.

1.9 References

Abram, P. K., J. Brodeur, V. Burte, and G. Boivin. 2016. Parasitoid-induced host egg abortion: an underappreciated component of biological control services provided by egg parasitoids. Biol. Control 98: 52–60.

Abram, P. K., J. Doyon, J. Brodeur, T. D. Gariépy, and G. Boivin. 2015. Susceptibility of *Halyomorpha halys* (Hemiptera: Pentatomidae) eggs to different life stages of three generalist predators. Can. Entomol. 147: 222–226.

Abram, P. K., N. J. Mills, and E. H. Beers. **2020**. Review: classical biological control of invasive stink bugs with egg parasitoids – what does success look like? Pest Manag. Sci. 76: 1980–1992.

Abram, P. K., T. D. Gariepy, G. Boivin, and J. Brodeur. 2014. An invasive stink bug as an evolutionary trap for an indigenous egg parasitoid. Biol. Invasions 16: 1387–1395.

Abram, P. K., K. A. Hoelmer, A. Acebes-Doria, H. Andrews, E. H. Beers, J. C. Bergh, R. Bessin, D. Biddinger, P. Botch, M. L. Buffington, M. L. Cornelius, E. Costi, E. S. Delfosse, C. Dieckhoff, R. Dobson, Z. Donais, M. Grieshop, G. Hamilton, T. Haye, C. Hedstrom, M. V. Herlihy, M. S. Hoddle, C. R. R. Hooks, P. Jentsch, N. K. Joshi, T. P. Kuhar, J. Lara, J. C. Lee, A. Legrand, T. C. Leskey, D. Lowenstein, L. Maistrello, C. R. Mathews, J. M. Milnes, W. R. Morrison, A. L. Nielsen, E. C. Ogburn, C. H. Pickett, K. Poley, J. Pote, J. Radl, P. M. Shrewsbury, E. Talamas, L. Tavella, J. F. Walgenbach, R. Waterworth, D. C. Weber, C. Welty, and N. G. Wiman. 2017a. Indigenous arthropod natural enemies of the invasive brown marmorated stink bug in North America and Europe. J. Pest Sci. 90: 1009-1020.

Abram, P. K., A. Cusumano, K. Abram, S. Colazza, and E. Peri. 2017b. Testing the habituation assumption underlying models of parasitoid foraging behavior. PeerJ. 5: e3097.

Abram, P. K., E. J. Talamas, S. Acheampong, P. G. Mason, and T. D. Gariepy. 2019. First detection of the samurai wasp, *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae), in Canada. J. Hymenopt. Res. 68: 29–36.

Acebes-Doria, A. L., A. M. Agnello, D. G. Alston, H. Andrews, E. H. Beers, J. C. Bergh, R. Bessin, B. R. Blaauw, G. D. Buntin, E. C. Burkness, S. Chen, T. E. Cottrell, K. M. Daane, L. E. Fann, S. J. Fleischer, C. Guédot, L. J. Gut, G. C. Hamilton, R. Hilton, K. A. Hoelmer, W. D. Hutchison, P. Jentsch, G. Krawczyk, T. P. Kuhar, J. C. Lee, J. M. Milnes, A. L. Nielsen, D. K. Patel, B. D. Short, A. A. Sial, L. R. Spears, K. Tatman, M. D. Toews, J. D. Walgenbach, C. Welty, N. G. Wiman, J. Van Zoeren, and T. C. Leskey. 2020. Season-long monitoring of the brown marmorated stink bug (Hemiptera: Pentatomidae) throughout the United States using commercially available traps and lures. J. Econ. Entomol. 113: 159-171.

Acebes-Doria, A. L., T. C. Leskey, and J. C. Bergh. 2016. Injury to apples and peaches at harvest from feeding by *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) nymphs early and late in the season. Crop Prot. 89: 58–65.

Acebes-Doria, A., W. Morrison, B. Short, K. Rice, H. Bush, T. Kuhar, C. Duthie, and T. Leskey. 2018. Monitoring and biosurveillance tools for the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). Insects 9: 82.

Aigner, B. L., T. P. Kuhar, D. A. Herbert, C. C. Brewster, J. W. Hogue, and J. D. Aigner. 2017. Brown marmorated stink bug (Hemiptera: Pentatomidae) infestations in tree borders and subsequent patterns of abundance in soybean fields. J. Econ. Entomol. 110: 487–490.

Arakawa, R., and Y. Namura. 2002. Effects of temperature on development of three *Trissolcus* spp. (Hymenoptera: Scelionidae), egg parasitoids of the brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae). Entomol. Sci. 5: 215–218.

Arif, M. A., T. Alınç, S. Guarino, S. Colazza, A. Cusumano, and E. Peri. 2021. Only females oviposit: chemical discrimination of adult stink bug sex by the egg parasitoid *Trissolcus japonicus*. Front. Ecol. Evol. 9: 213.

Avila, G. A., and J. G. Charles. 2018. Modelling the potential geographic distribution of *Trissolcus japonicus*: a biological control agent of the brown marmorated stink bug, *Halyomorpha halys*. BioControl 63: 505–518.

Bakken, A. J., S. C. Schoof, M. Bickerton, K. L. Kamminga, J. C. Jenrette, S. Malone, M. A. Abney, D. A. Herbert, D. Reisig, T. P. Kuhar, and J. F. Walgenbach. 2015. Occurrence of brown marmorated stink bug (Hemiptera: Pentatomidae) on wild hosts in non-managed woodlands and soybean fields in North Carolina and Virginia. Environ. Entomol. 44: 1011–1021.

Bale, J., J. van Lenteren, and F. Bigler. 2007. Biological control and sustainable food production. Philos. Trans. R. Soc. B Biol. Sci. 363: 761–776.

Bellard, C., P. Cassey, and T. M. Blackburn. 2016. Alien species as a driver of recent extinctions. Biol. Lett. 12: 20150623.

Bergh, J. C., W. R. Morrison, S. V. Joseph, and T. C. Leskey. 2017. Characterizing spring emergence of adult *Halyomorpha halys* using experimental overwintering shelters and commercial pheromone traps. Entomol. Exp. Appl. 162: 336–345.

Bergh, J. C., W. R. Morrison, J. W. Stallrich, B. D. Short, J. P. Cullum, and T. C. Leskey. 2021. Border habitat effects on captures of *Halyomorpha halys* (Hemiptera: Pentatomidae) in pheromone traps and fruit injury at harvest in apple and peach orchards in the Mid-Atlantic, USA. Insects 12: 419.

Bergh, J. C., and N. F. Quinn. 2018. Can the dispersal behavior of *Halyomorpha halys* (Hemiptera: Pentatomidae) inform the use of insecticide-treated netting to mitigate homeowner issues from its fall invasion? Environ. Entomol. 47: 1501–1508.

Bertoldi, V., G. Rondoni, J. Brodeur, and E. Conti. 2019. An egg parasitoid efficiently exploits cues from a coevolved host but not those from a novel host. Front. Physiol. 10: 746.

Blaauw, B. R., V. P. Jones, and A. L. Nielsen. 2016. Utilizing immunomarking techniques to track *Halyomorpha halys* (Hemiptera: Pentatomidae) movement and distribution within a peach orchard. PeerJ. 2016: e1997.

Bon, M.-C., K. Hoelmer, E. J. Talamas, M. L. Buffington, F. Guermache, and D. C. Weber. 2017. Genetic diversity and origins of *Halyomorpha halys* in the U.S. and of its potential biological control agent unexpectedly recovered from the wild in the United States, pp. 240–242. In: PG, M., DR, G., C, V. (eds.), Proc. 5th Int. Symp. Biol. Control Arthropods. CAB International, Wallingford, United Kingdom., Langkawi, Malaysia.

Botch, P. S., and E. S. Delfosse. 2018. Host-acceptance behavior of *Trissolcus japonicus* (Hymenoptera: Scelionidae) reared on the invasive *Halyomorpha halys* (Heteroptera: Pentatomidae) and nontarget species. Environ. Entomol. 47: 403–411.

Boyle, S. M., D. C. Weber, J. Hough-Goldstein, and K. A. Hoelmer. 2020a. Host kairomones influence searching behavior of *Trissolcus japonicus* (Hymenoptera: Scelionidae), a parasitoid of *Halyomorpha halys* (Heteroptera: Pentatomidae). Environ. Entomol. 49: 15–20.

Boyle, S. M., D. C. Weber, J. Hough-Goldstein, and K. A. Hoelmer. 2020b. Parental host species affects behavior and parasitism by the pentatomid egg parasitoid, *Trissolcus japonicus* (Hymenoptera: Scelionidae). Biol. Control 149: 104324.

Caltagirone, L. E. and R. L. Doutt. 2003. The history of the vedalia beetle importation to California and its impact on the development of biological control. Ann. Rev. Entomol. 34:1-16.

Charles, J. G., G. A. Avila, K. A. Hoelmer, S. Hunt, R. Gardner-Gee, F. MacDonald, and V. Davis. 2019. Experimental assessment of the biosafety of *Trissolcus japonicus* in New Zealand, prior to the anticipated arrival of the invasive pest *Halyomorpha halys*. BioControl 64: 367–379.

Chen, J., W. Li, Q. Mi, F. Zhang, S. Shi, and J. Zhang. 2020. A newly reported parasitoid, *Pentatomophaga latifascia* (Diptera: Tachinidae), of adult *Halyomorpha halys* in Beijing, China. Insects 11: 1–8.

Cock, M. J. W., S. T. Murphy, M. T. K. Kairo, E. Thompson, R. J. Murphy, and A. W. Francis. 2016. Trends in the classical biological control of insect pests by insects: an update of the BIOCAT database. BioControl 61: 349–363.

Colazza, S., M. Lo Bue, D. Lo Giudice, and E. Peri. 2009. The response of *Trissolcus basalis* to footprint contact kairomones from *Nezara viridula* females is mediated by leaf epicuticular waxes. Naturwissenschaften 96: 975–981.

Conti, E., G. Avila, B. Barratt, F. Cingolani, S. Colazza, S. Guarino, K. Hoelmer, R. A. Laumann, L. Maistrello, G. Martel, E. Peri, C. Rodriguez-Saona, G. Rondoni, M. Rostás, P. F. Roversi, R. F. H. Sforza, L. Tavella, and E. Wajnberg. 2021. Biological control of invasive stink bugs: review of global state and future prospects. Entomol. Exp. Appl. 169: 28–51.

Cornelius, M. L., C. Dieckhoff, K. A. Hoelmer, R. T. Olsen, D. C. Weber, M. V. Herlihy, E. J. Talamas, B. T. Vinyard, and M. H. Greenstone. 2016a. Biological control of sentinel egg masses of the exotic invasive stink bug *Halyomorpha halys* (Stål) in Mid-Atlantic USA ornamental landscapes. Biol. Control 103: 11–20.

Cornelius, M. L., C. Dieckhoff, B. T. Vinyard, and K. A. Hoelmer. 2016b. Parasitism and predation on sentinel egg masses of the brown marmorated stink bug (Hemiptera: Pentatomidae) in three vegetable crops: importance of dissections for evaluating the impact of native parasitoids on an exotic pest. Environ. Entomol. 45: 1536–1542.

Diagne, C., B. Leroy, A.-C. Vaissière, R. E. Gozlan, D. Roiz, I. Jarić, J.-M. Salles, C. J. A. Bradshaw, and F. Courchamp. 2021. High and rising economic costs of biological invasions worldwide. Nature 592: 571–576.

Dieckhoff, C., K. M. Tatman, and K. A. Hoelmer. 2017. Natural biological control of *Halyomorpha halys* by native egg parasitoids: a multi-year survey in northern Delaware. J. Pest Sci. 90: 1143–1158.

Dieckhoff, C., S. Wenz, M. Renninger, A. Reißig, H. Rauleder, C. P. W. Zebitz, J. Reetz, and O. Zimmermann. 2021. Add Germany to the list—adventive population of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) emerges in Germany. Insects 12: 414.

Doherty, T. S., A. S. Glen, D. G. Nimmo, E. G. Ritchie, and C. R. Dickman. 2016. Invasive predators and global biodiversity loss. Proc. Natl. Acad. Sci. 113: 11261–11265.

Eilenberg, J., A. Hajek, and C. Lomer. 2001. Suggestions for unifying the terminology in biological control. BioControl 46: 387–400.

Faúndez, E., and D. Rider. 2017. The brown marmorated stink bug *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) in Chile. Arq. Entomolóxicos 17: 305–307.

Ferreira Santos de Aquino, M., A. M. Dias, M. Borges, M. C. B. Moraes, and R. A. Laumann. 2012. Influence of visual cues on host-searching and learning behaviour of the egg parasitoids *Telenomus podisi* and *Trissolcus basalis*. Entomol. Exp. Appl. 145: 162–174.

Field, S. A. 1998. Patch exploitation, patch-leaving and pre-emptive patch defence in the parasitoid wasp *Trissolcus basalis* (Insecta: Scelionidae). Ethology 104: 323–338.

Fogain, R., and S. Graff. 2011. First records of the invasive pest, *Halyomorpha halys* (Hemiptera: Pentatomidae), in Ontario and Quebec. J. Entomol. Soc. Ontario 142: 45-48.

Fraga, D. F., J. Parker, A. C. Busoli, G. C. Hamilton, A. L. Nielsen, and C. Rodriguez-Saona. 2017. Behavioral responses of predaceous minute pirate bugs to tridecane, a volatile emitted by the brown marmorated stink bug. J. Pest Sci. 90: 1107–1118.

Gapon, D. A. 2017. First records of the brown marmorated stink bug *Halyomorpha halys* (Stål, 1855) (Heteroptera, Pentatomidae) in Russia, Abkhazia, and Georgia. Entomol. Rev. 96: 1086–1088.

Gariepy, T. D., and E. J. Talamas. 2019. Discovery of *Trissolcus japonicus* (Hymenoptera: Scelionidae) in Ontario, Canada. Can. Entomol. 151: 824-826.

Godfray, H. C. J. 1994. Parasitoids: behavioral and evolutionary ecology. Princeton University Press, Princeton, NJ.

Gomes Lagôa, A. C., M. C. Blassioli Moraes, M. Borges, and R. A. Laumann. 2020. Selective responses of *Trissolcus basalis* and *Telenomus podisi* to chemical footprints of preferred hosts. Physiol. Entomol. 45: 60–71.

Hadden, W. T., L. J. Nixon, T. C. Leskey, and J. C. Bergh. 2021. Seasonal distribution of *Halyomorpha halys* (Hemiptera: Pentatomidae) captures in woods-to-orchard pheromone trap transects in Virginia. J. Econ. Entomol. (in press).

Hancock, T. J., D. H. Lee, J. C. Bergh, W. R. Morrison III, and T. C. Leskey. 2019. Presence of the invasive brown marmorated stink bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) on home exteriors during the autumn dispersal period: results generated by citizen scientists. Agric. For. Entomol. 21: 99–108.

Harris, C., S. Abubeker, M. Yu, T. Leskey, and A. Zhang. 2015. Semiochemical production and laboratory behavior response of the brown marmorated stink bug, *Halyomorpha Halys*. PLoS One 10: e0140876.

Haye, T., T. Gariepy, K. Hoelmer, J.-P. Rossi, J.-C. Streito, X. Tassus, and N. Desneux. 2015a. Range expansion of the invasive brown marmorated stinkbug, *Halyomorpha halys*: an increasing threat to field, fruit and vegetable crops worldwide. J. Pest Sci. 88: 665–673.

Haye, T., S. Fischer, J. Zhang, and T. Gariepy. 2015b. Can native egg parasitoids adopt the invasive brown marmorated stink bug, *Halyomorpha halys* (Heteroptera: Pentatomidae), in Europe? J. Pest Sci. 88: 693–705.

Haye, T., S. T. Moraglio, J. Stahl, S. Visentin, T. Gregorio, and L. Tavella. 2020. Fundamental host range of *Trissolcus japonicus* in Europe. J. Pest Sci. 93: 171–182.

Hedstrom, C., D. Lowenstein, H. Andrews, B. Bai, and N. Wiman. 2017. Pentatomid host suitability and the discovery of introduced populations of *Trissolcus japonicus* in Oregon. J. Pest Sci. 90: 1169–1179.

Heimpel, G. E., and M. J. W. Cock. 2018. Shifting paradigms in the history of classical biological control. BioControl 63: 27–37.

Herlihy, M. V., E. J. Talamas, and D. C. Weber. 2016. Attack and success of native and exotic parasitoids on eggs of *Halyomorpha halys* in three Maryland habitats. PLoS One 11: e0150275.

Hoebeke, E., and M. Carter. 2003. *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. Proc. Entomol. Soc. Wash. 105: 225–237.

Holthouse, M. C., L. R. Spears, and D. G. Alston. **2021**. Comparison of yellow and blue sticky cards for detection and monitoring parasitoid wasps of the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae). J. Insect Sci. 21: 1–1.

Inkley, D. B. 2012. Characteristics of home invasion by the brown marmorated stink bug (Hemiptera: Pentatomidae). J. Entomol. Sci. 47: 125–130.

Jentsch, P. J. 2017. Expanding the range of the samurai wasp, *Trissolcus japonicus*, in New York orchards. Fruit Q. 25: 31–35.

Jones, A. L., D. E. Jennings, C. R. R. Hooks, and P. M. Shrewsbury. 2014. Sentinel eggs underestimate rates of parasitism of the exotic brown marmorated stink bug, *Halyomorpha halys*. Biol. Control 78: 61–66.

Jones, B. A. 2017. Invasive species impacts on human well-being using the life satisfaction index. Ecol. Econ. 134: 250–257.

Joseph, S. V., M. Nita, T. C. Leskey, and J. C. Bergh. 2015. Temporal effects on the incidence and severity of brown marmorated stink bug (Hemiptera: Pentatomidae) feeding injury to peaches and apples during the fruiting period in Virginia. J. Econ. Entomol. 108: 592–599.

Joseph, S. V., J. W. Stallings, T. C. Leskey, G. Krawczyk, D. Polk, B. Butler, and J. C. Bergh. 2014. Spatial distribution of brown marmorated stink bug (Hemiptera: Pentatomidae) injury at harvest in Mid-Atlantic apple orchards. J. Econ. Entomol. 107: 1839–1848.

Kawada, H., and C. Kitamura. 1983. The reproductive behavior of the brown marmorated stink bug, *Halyomorpha mista* UHLER (Heteroptera : Pentatomidae) I. Observation of mating behavior and multiple copulation. Appl. Entomol. Zool. 18: 234–242.

Keane, R. M., and M. J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. Trends Ecol. Evol. 17: 164–170.

Khrimian, A., A. Zhang, D. C. Weber, H. Y. Ho, J. R. Aldrich, K. E. Vermillion, M. A. Siegler, S. Shirali, F. Guzman, and T. C. Leskey. 2014. Discovery of the aggregation pheromone of the brown marmorated stink bug (*Halyomorpha halys*) through the creation of stereoisomeric libraries of 1-bisabolen-3-ols. J. Nat. Prod. 77: 1708–1717.

Konopka, J. K., T. Haye, T. Gariepy, P. Mason, D. Gillespie, and J. N. McNeil. 2017a. An exotic parasitoid provides an invasional lifeline for native parasitoids. Ecol. Evol. 7: 277–284.

Konopka, J. K., T. Haye, T. D. Gariepy, and J. N. McNeil. 2017b. Possible coexistence of native and exotic parasitoids and their impact on control of *Halyomorpha halys*. J. Pest Sci. 90: 1119–1125.

Kuhar, T. P., and K. Kamminga. 2017. Review of the chemical control research on *Halyomorpha halys* in the USA. J. Pest Sci. 90: 1021–1031.

Kuhar T. P., Kamminga K. L., Whalen J. Dively, G. P. Brust, G. Hooks, C.R.R. Hamilton, G. Herbert, D. A. 2012. The pest potential of brown marmorated stink bug on vegetable crops. Online. Plant Health Progress doi:10.1094/PHP-2012-0523-01-BR.

Lara, J. R., C. H. Pickett, M. T. Kamiyama, S. Figueroa, M. Romo, C. Cabanas, V. Bazurto, V. Strode, K. Briseno, M. Lewis, J. Oliva, G. Hernandez, and M. S. Hoddle. 2019. Physiological host range of *Trissolcus japonicus* in relation to *Halyomorpha halys* and other pentatomids from California. BioControl 64: 513–528.

Laumann, R. A., M. F. S. Aquino, M. C. B. Moraes, M. Pareja, and M. Borges. 2009. Response of the egg parasitoids *Trissolcus basalis* and *Telenomus podisi* to compounds from defensive secretions of stink bugs. J. Chem. Ecol. 35: 8–19.

Lee, D.-H., and T. C. Leskey. 2015. Flight behavior of foraging and overwintering brown marmorated stink bug, *Halyomorpha halys* (Hemiptera: Pentatomidae). Bull. Entomol. Res. 105: 566–573.

Lee, D.-H., A. L. Nielsen, and T. C. Leskey. 2014a. Dispersal capacity and behavior of nymphal stages of *Halyomorpha halys* (Hemiptera: Pentatomidae) evaluated under laboratory and field conditions. J. Insect Behav. 27: 639–651.

Lee, D.-H., J. P. Cullum, J. L. Anderson, J. L. Daugherty, L. M. Beckett, and T. C. Leskey. 2014b. Characterization of overwintering sites of the invasive brown marmorated stink bug in natural landscapes using human surveyors and detector canines. PLoS One 9: e91575.

Lee, D. H., B. D. Short, S. V. Joseph, J. C. Bergh, and T. C. Leskey. 2013. Review of the biology, ecology, and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. Environ. Entomol. 42: 627-641.

Leskey, T. C., A. Agnello, J. C. Bergh, G. P. Dively, G. C. Hamilton, P. Jentsch, A. Khrimian, G. Krawczyk, T. P. Kuhar, D. H. Lee, W. R. Morrison, D. F. Polk, C. Rodriguez-Saona, P. W. Shearer, B. D. Short, P. M. Shrewsbury, J. F. Walgenbach, D. C. Weber, C. Welty, J. Whalen, N. Wiman, and F. Zaman. 2015. Attraction of the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae) to traps baited with semiochemical stimuli across the United States. J. Econ. Entomol. 44: 746–756.

Leskey, T. C., H. Andrews, A. Bády, L. Benvenuto, I. Bernardinelli, B. Blaauw, P. P. Bortolotti, L. Bosco, E. Di Bella, G. Hamilton, T. Kuhar, D. Ludwick, L. Maistrello, G. Malossini, R. Nannini, L. J. Nixon, E. Pasqualini, M. Preti, B. D. Short, L. Spears, L. Tavella, G. Vétek, and N. Wiman. 2021. Refining pheromone lures for the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae) through collaborative trials in the United States and Europe. J. Econ. Entomol. 114: 1666–1673.

Leskey, T. C., and A. L. Nielsen. 2018. Impact of the invasive brown marmorated stink bug in North America and Europe: history, biology, ecology, and management. Annu. Rev. Entomol. 63: 599–618.

Leskey, T. C., B. D. Short, B. R. Butler, and S. E. Wright. 2012a. Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in Mid-Atlantic tree fruit orchards in the United States: case studies of commercial management. Psyche 2012: 1–14.

Leskey, T. C., G. C. Hamilton, A. L. Nielsen, D. F. Polk, C. Rodriguez-Saona, J. Christopher Bergh, D. Ames Herbert, T. P. Kuhar, D. Pfeiffer, G. P. Dively, C. R. R. Hooks, M. J. Raupp, P. M. Shrewsbury, G. Krawczyk, P. W. Shearer, J. Whalen, C. Koplinka-Loehr, E. Myers, D. Inkley, K. A. Hoelmer, D. H. Lee, and S. E. Wright. 2012b. Pest status of the brown marmorated stink bug, *Halyomorpha halys* in the USA. Outlooks Pest Manag. 23: 218–226.

Leskey, T. C., B. D. Short, and D. Ludwick. 2020. Comparison and refinement of integrated pest management tactics for *Halyomorpha halys* (Hemiptera: Pentatomidae) management in apple orchards. J. Econ. Entomol. 113: 1725–1734.

Liu, H., and P. Stiling. 2006. Testing the enemy release hypothesis: a review and meta-analysis. Biol. Invasions 8: 1535–1545.

Lowenstein, D. M., H. Andrews, R. J. Hilton, C. Kaiser, and N. G. Wiman. 2019. Establishment in an introduced range: dispersal capacity and winter survival of *Trissolcus japonicus*, an adventive egg parasitoid. Insects 10: 443.

Ludwick, D. C., J. Patterson, L. B. Leake, L. Carper, and T. C. Leskey. 2020. Integrating *Trissolcus japonicus* (Ashmead, 1904) (Hymenoptera: Scelionidae) into management programs for *Halyomorpha halys* (Stål, 1855) (Hemiptera: Pentatomidae) in apple orchards: impact of insecticide applications and spray patterns. Insects 11: 833.

Malek, R., J. M. Kaser, G. Anfora, M. Ciolli, A. Khrimian, D. C. Weber, and K. A. Hoelmer. 2021. *Trissolcus japonicus* foraging behavior: implications for host preference and classical biological control. Biol. Control 161: 104700.

McIntosh, H., D. M. Lowenstein, N. G. Wiman, J. S. Wong, and J. C. Lee. 2019. Parasitism of frozen *Halyomorpha halys* eggs by *Trissolcus japonicus*: applications for rearing and experimentation. Biocontrol Sci. Technol. 29: 478–493.

Medal, J., and T. Smith. 2015. Reversible polyphenism in *Trissolcus japonicus* (Hymenoptera: Platygastridae) induced by stink bug egg size. J. Entomol. Sci. 50: 363–366.

Medal, J., T. Smith, A. Fox, A. S. Cruz, A. Poplin, and A. Hodges. 2012. Rearing the brown marmorated stink bug *Halyomorpha halys* (Heteroptera: Pentatomidae). Florida Entomol. 95: 800–802.

Milnes, J. M., and E. H. Beers. 2019. *Trissolcus japonicus* (Hymenoptera: Scelionidae) causes low levels of parasitism in three North American pentatomids under field conditions. J. Insect Sci. 19: 15.

Miura, A., M. Furuno, D. Kuki, and K. Itoyama. 2021. Comparisons of parasitism and developmental properties of *Trissolcus japonicus* (Hymenoptera: Scelionidae) reared on eggs of three species of fruit-piercing stink bugs. Appl. Entomol. Zool. 56: 379–383.

Morrison, W. R., B. R. Blaauw, A. L. Nielsen, E. Talamas, and T. C. Leskey. 2018. Predation and parasitism by native and exotic natural enemies of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) eggs augmented with semiochemicals and differing host stimuli. Biol. Control 121: 140–150.

Morrison, W. R., B. R. Blaauw, B. D. Short, A. L. Nielsen, J. C. Bergh, G. Krawczyk, Y. L. Park, B. Butler, A. Khrimian, and T. C. Leskey. 2019. Successful management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in commercial apple orchards with an attract-and-kill strategy. Pest Manag. Sci. 75: 104–114.

Morrison, W. R., C. R. Mathews, and T. C. Leskey. 2016. Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. Biol. Control 97: 120–130.

Nielsen, A. L., S. Chen, and S. J. Fleischer. 2016. Coupling developmental physiology, photoperiod, and temperature to model phenology and dynamics of an invasive heteropteran, *Halyomorpha halys*. Front. Physiol. 7: 165.

Nielsen, A. L., and G. C. Hamilton. 2009. Life history of the invasive species *Halyomorpha halys* (Hemiptera: Pentatomidae) in Northeastern United States. Ann. Entomol. Soc. Am. 102: 608–616.

Nielsen, A. L., G. C. Hamilton, and D. Matadha. 2008. Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). Environ. Entomol. 37: 348–355.

Ogburn, E. C., R. Bessin, C. Dieckhoff, R. Dobson, M. Grieshop, K. A. Hoelmer, C. Mathews, J. Moore, A. L. Nielsen, K. Poley, J. M. Pote, M. Rogers, C. Welty, and J. F. Walgenbach. 2016. Natural enemy impact on eggs of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in organic agroecosystems: a regional assessment. Biol. Control 101: 39–51.

(ODA) Oregon Department of Agriculture. 2020. Pest alert: Brown marmorated stink bug, *Halyomorpha halys*. Salem, OR.

Paini, D. R., A. W. Sheppard, D. C. Cook, P. J. De Barro, S. P. Worner, and M. B. Thomas. 2016. Global threat to agriculture from invasive species. Proc. Natl. Acad. Sci. 113: 7575–7579.

Pejchar, L., and H. A. Mooney. 2009. Invasive species, ecosystem services and human well-being. Trends Ecol. Evol. 24: 497–504.

Peterson, H. M., E. Talamas, and G. Krawczyk. 2021. Survey for adventive populations of the samurai wasp, *Trissolcus japonicus* (Hymenoptera: Scelionidae) in Pennsylvania at commercial fruit orchards and the surrounding forest. Insects 12: 258.

Peverieri, G. S., E. Talamas, M. Claude Bon, L. Marianelli, I. Bernardinelli, G. Malossini, L. Benvenuto, P. F. Roversi, and K. Hoelmer. 2018. Two Asian egg parasitoids of *Halyomorpha halys* (Stål) (Hemiptera, Pentatomidae) emerge in northern Italy: *Trissolcus mitsukurii* (Ashmead) and *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae). J. Hymenopt. Res. 67: 37–53.

Pimentel, D., R. Zuniga, and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. 52: 273–288.

Qiu, L.-F., Z.-Q. Yang, and W. Q. Tao. 2007. Biology and population dynamics of *Trissolcus halyomorphae*. Sci. Silvae Sin. 43: 62–65.

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

Quinn, N. F., E. J. Talamas, A. L. Acebes-Doria, T. C. Leskey, and J. C. Bergh. 2019b. Vertical sampling in tree canopies for *Halyomorpha halys* (Hemiptera: Pentatomidae) life stages and its egg parasitoid, *Trissolcus japonicus* (Hymenoptera: Scelionidae). Environ. Entomol. 48: 173–180.

Quinn, N. F., E. J. Talamas, T. C. Leskey, and J. C. Bergh. 2021. Seasonal captures of *Trissolcus japonicus* (Ashmead) (hymenoptera: Scelionidae) and the effects of habitat type and tree species on detection frequency. Insects 12: 1–12.

Rice, K. B., C. J. Bergh, E. J. Bergmann, D. J. Biddinger, C. Dieckhoff, G. Dively, H. Fraser, T. Gariepy, G. Hamilton, T. Haye, A. Herbert, K. Hoelmer, C. R. Hooks, A. Jones, G. Krawczyk, T. Kuhar, H. Martinson, W. Mitchell, A. L. Nielsen, D. G. Pfeiffer, M. J. Raupp, C. Rodriguez-Saona, P. Shearer, P. Shrewsbury, P. D. Venugopal, J. Whalen, N. G. Wiman, T. C. Leskey, and J. F. Tooker. 2014. Biology, ecology, and management of brown marmorated stink bug (Hemiptera: Pentatomidae). J. Integr. Pest Manag. 5: 1–13.

Rondoni, G., V. Bertoldi, R. Malek, M. C. Foti, E. Peri, L. Maistrello, T. Haye, and E. Conti. 2017. Native egg parasitoids recorded from the invasive *Halyomorpha halys* successfully exploit volatiles emitted by the plant–herbivore complex. J. Pest Sci. 90: 1087–1095.

Sakai, A. K., F. W. Allendorf, J. S. Holt, D. M. Lodge, J. Molofsky, K. A. With, S. Baughman, R. J. Cabin, J. E. Cohen, N. C. Ellstrand, D. E. McCauley, P. O'Neil, I. M. Parker, J. N. Thompson, and S. G. Weller. 2001. The population biology of invasive species. Annu. Rev. Ecol. Syst. 32: 305-332.

Short, B. D., A. Khrimian, and T. C. Leskey. 2017. Pheromone-based decision support tools for management of *Halyomorpha halys* in apple orchards: development of a trap-based treatment threshold. J. Pest Sci. 90: 1191–1204.

Stahl, J., F. Tortorici, M. Pontini, M. C. Bon, K. Hoelmer, C. Marazzi, L. Tavella, and T. Haye. 2019. First discovery of adventive populations of *Trissolcus japonicus* in Europe. J. Pest Sci. 92: 371–379.

Talamas, E. J., M. V. Herlihy, C. Dieckhoff, K. A. Hoelmer, M. L. Buffington, M. C. Bon, and D. C. Weber. 2015. *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae) emerges in North America. J. Hymenopt. Res. 43: 119–128.

Tillman, G., M. Toews, B. Blaauw, A. Sial, T. Cottrell, E. Talamas, D. Buntin, S. Joseph, R. Balusu, H. Fadamiro, S. Lahiri, and D. Patel. 2020. Parasitism and predation of sentinel eggs of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in the southeastern US. Biol. Control 145: 104247.

Tognon, R., J. R. Aldrich, M. L. Buffington, E. J. Talamas, J. Sant'Ana, and F. G. Zalom. 2017. *Halyomorpha halys* (Heteroptera: Pentatomidae) egg surface chemicals inhibit North American *Telenomus* and *Trissolcus* (Hymenoptera: Scelionidae) parasitism. Biol. Control 114: 39–44.

Valentin, R. E., A. L. Nielsen, N. G. Wiman, D.-H. Lee, and D. M. Fonseca. 2017. Global invasion network of the brown marmorated stink bug, *Halyomorpha halys*. Sci. Reports 7: 1–12.

Van Lenteren, J. C., J. Bale, F. Bigler, H. M. T. Hokkanen, and A. J. M. Loomans. 2005. Assessing risks of releasing exotic biological control agents of arthropod pests. Annu. Rev. Entomol. 51: 609-634.

Venugopal, P. D., P. L. Coffey, G. P. Dively, and W. O. Lamp. 2014. Adjacent habitat influence on stink bug (Hemiptera: Pentatomidae) densities and the associated damage at field corn and soybean edges. PLoS One 9: e109917.

Weber, D. C., T. C. Leskey, G. C. Walsh, and A. Khrimian. 2014. Synergy of aggregation pheromone with methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate in attraction of *Halyomorpha halys* (Hemiptera: Pentatomidae). J. Econ. Entomol. 107: 1061–1068.

Wermelinger, B., D. Wyniger, and B. Forster. 2008. First records of an invasive bug in Europe: *Halyomorpha halys* Stål (Heteroptera: Pentatomidae), a new pest on woody ornamentals and fruit trees? Mit. Sch. Ges. 81: 1–8.

Yang, Z.-Q., Y.-X. Yao, L.-F. Qiu, and Z.-X. Li. 2009. A new species of *Trissolcus* (Hymenoptera: Scelionidae) parasitizing eggs of *Halyomorpha halys* (Heteroptera: Pentatomidae) in China with comments on its biology. Ann. Entomol. Soc. Am. 102: 39–47.

Yonow, T., D. J. Kriticos, N. Ota, G. A. Avila, K. A. Hoelmer, H. Chen, and V. Caron. 2021. Modelling the potential geographic distribution of two *Trissolcus* species for the brown marmorated stink bug, *Halyomorpha halys*. Insects 12: 491.

Zhang, J., F. Zhang, T. Gariepy, P. Mason, D. Gillespie, E. Talamas, and T. Haye. 2017. Seasonal parasitism and host specificity of *Trissolcus japonicus* in northern China. J. Pest Sci. 90: 1127–1141.

Zhong, Y. Z., J. P. Zhang, L. L. Ren, R. Tang, H. X. Zhan, G. H. Chen, and F. Zhang. 2017. Behavioral responses of the egg parasitoid *Trissolcus japonicus* to volatiles from adults of its stink bug host, *Halyomorpha halys*. J. Pest Sci. 90: 1097–1105.

Chapter 2

Influence of trap location in the tree canopy on captures of adventive *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae)

2.1 Abstract

Trissolcus japonicus (Ashmead) (Hymenoptera: Scelionidae) is an egg parasitoid of the invasive agricultural and nuisance pest from Asia, brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). Also native to Asia, adventive *T. japonicus* populations have been detected in North America since 2014, and are currently reported from 15 US states, Washington, DC, and two Canadian provinces. Yellow sticky cards (YSC) have proven effective for monitoring the presence, distribution, and spread of these adventive populations. Although a previous study reported that naturally-laid *H. halys* egg masses parasitized by *T. japonicus* were found exclusively in the mid- and upper canopy of felled tree of heaven, *Ailanthus altissima*, some investigators have captured *T. japonicus* using YSC deployed from the ground in the lower canopy. Given that the effect of YSC placement in *H. halys* host trees on *T. japonicus* captures had not been examined, captures in YSC atop bamboo poles suspended from mid- and lower-canopy branches in individual *A. altissima* were compared in 2020 and 2021. Traps were replaced weekly for five weeks and assessed for Scelionidae species. In both years, *T. japonicus* represented >53% of all Scelionidae captured, and location of YSC in the canopy did not have a significant effect on *T. japonicus* captures. Deploying YSC at either canopy height was effective for measuring the relative abundance of *T. japonicus*, but sampling from the lower canopy substantially improved the efficiency and convenience of surveillance for adventive *T. japonicus*.

2.2 Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an invasive pest from Asia that was first detected in North America in the late 1990s (Hoebeke and Carter 2003) and has now established or been detected in 47 US states (www.stopbmsb.org), many other countries in the northern hemisphere, and in Chile (Leskey and Nielsen 2018). Feeding on >170 wild and cultivated hosts (Leskey and Nielsen 2018), *H. halys* caused tremendous losses to the Mid-Atlantic, USA tree fruit crop during its initial outbreak in 2010 (Leskey et al. 2012), and continues to threaten specialty crop production in the invaded ranges (Hedstrom et al. 2014, Wiman et al. 2015, Moore et al. 2019). Furthermore, adults can be a significant nuisance during their autumn dispersal to overwintering sites, which include human-made structures (Inkley 2012, Bergh and Quinn 2018, Hancock et al. 2019). Management of *H. halys* in agricultural systems in the US has relied on repeated applications of broad-spectrum insecticides (Kuhar and Kamminga 2017b), but these have disrupted Integrated Pest Management programs and increased the incidence of secondary pest outbreaks in some systems (Leskey et al. 2012). Thus, the potential for biological control of *H. halys* has become an important area of research.

In North America, various *H. halys* life stages are attacked by a suite of native predators and parasitoids (Morrison et al. 2016, Morrison et al. 2018), but their combined effects have not yielded sufficient levels of biological control (Abram et al. 2017). Foreign explorations in Asia revealed that the egg parasitoid, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae), is one of the predominant, co-evolved natural enemies of *H. halys* in its native range (Zhang et al. 2017), and parasitism rates from *T. japonicus* of 50-80% have been reported (Yang et al. 2009, Zhang et al. 2017). For these reasons, *T. japonicus* from China was evaluated in quarantine facilities in the US for its potential as a classical biocontrol agent (Talamas et al. 2015a). However, in 2014 an adventive population of *T. japonicus* was detected in Maryland, USA (Talamas et al. 2015a, Herlihy et al. 2016) and subsequent detections have been reported from 15 US states, Washington, DC (www.stopbmsb.org), two Canadian provinces (Abram et al. 2019), and several European countries (Stahl et al. 2019). Niche models suggest that *T. japonicus* may establish in many of the regions where *H. halys* is present (Avila and Charles 2018), and ongoing surveillance efforts are tracking its presence and range expansion.

Initial field surveys for *H. halys* egg parasitoids utilized naturally-occurring *H. halys* egg masses (Jones et al. 2014, Ogburn et al. 2016, Zhang et al. 2017) and/or sentinel *H. halys* eggs from laboratory colonies (Herlihy et al. 2016, Hedstrom et al. 2017, Holthouse et al. 2020, Tillman et al. 2020). While these are essential for generating field data on the taxa that attack *H. halys* eggs and rates of parasitism, for the various reasons discussed by Quinn et al. (2019a), they are not optimally efficient for parasitoid surveillance efforts across large geographic areas. More recently, yellow sticky cards (YSC) also have been used to track the presence, abundance, seasonal phenology, and distribution of *T. japonicus* (Holthouse et al. 2021, Peterson et al. 2021, Quinn et al. 2021). In Virginia, Quinn et al. (2019b) found that *H. halys* adults, nymphs, and egg masses were more abundant in the mid- and upper canopy of *H. halys* host trees than in the lower tree canopy, and that detections of *T. japonicus* occurred most frequently from egg masses found in the mid-canopy. Consequently, most of our subsequent sampling for *T. japonicus* in Virginia has employed YSC deployed in the mid-canopy of host trees. However, investigators in other areas have captured *T. japonicus* in YSC deployed from the ground in the lower canopy (Holthouse et al. 2021, Peterson et al. 2021), raising the question of whether *T. japonicus* captures are affected by the location of traps in the host tree canopy. Here, we report the results of studies in two consecutive seasons that compared *T. japonicus* captures in YSC deployed simultaneously in the lower and mid-canopy of individual host trees.

2.3 Methods

Trissolcus japonicus was sampled from mature, female tree of heaven, *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae) growing in tree lines or isolated patches adjoining fruit orchards in Frederick County, VA, where this parasitoid is well-established. Trees with branches and foliage extending from the lower portion of the trunk to the top were used. Following Quinn et al. (2021), sampling used a backfolding, double-sided YSC (46 × 28 cm; AlphaScents, Inc., West Linn, OR) affixed to the top of a bamboo pole suspended from a branch via a wire hook below the YSC. Deployment of YSC in the lower and mid-canopy, respectively, utilized 1.0 m and 4.6 m poles, and the mean elevation of YSC in the lower and mid-canopy was 2.3 ± 0.14 m and 5.80 ± 0.16 m. The YSC were deployed in pairs in the same tree (Fig. 2.1), using 5 and 7 trees in 2020 and 2021, respectively, and replaced weekly for five weeks between 16 July and 20 August, 2020 and 6 July to 10 August, 2021, coinciding with seasonal peaks in *T. japonicus* abundance recorded in this area (Quinn et al. 2021, Dyer unpublished). Trees used for sampling in 2020 were reused the following year. Upon retrieval, YSC were inspected under a dissecting microscope at 10x magnification, and all scelionid parasitoids captured were removed *in situ* on a small piece of the YSC for identification to species or genus, following Talamas et al. (2015b). The sex of *T. japonicus* specimens was determined based on antennal morphology (Yang et al. 2016).

Statistical analysis

Statistical analyses used SAS® Studio 3.8 (SAS Institute Inc., Cary, NC, USA) with $\alpha = 0.05$. For each year, comparisons of *T. japonicus* captures between trap locations (lower vs. mid-canopy) used a generalized linear mixed model (GLMM) with Laplace method for fit and assumed a Poisson distribution. Trap location in the canopy, sampling site, and the interaction between trap location and sampling site were main effects, and sampling date was treated as a random effect.

2.4 Results

In 2020 and 2021, respectively, 359 and 317 Scelionidae parasitoids were captured (Table 2.1), with *T. japonicus* comprising more than half of all captures in 2020 (52.9%) and 2021 (56.5%). Captures of *T. japonicus* were female-biased in 2020 (70.0% female) and 2021 (70.4% female). Trap location in the tree canopy did not have a significant effect on weekly *T. japonicus* captures in 2020 ($P = 0.3471$, $F_{1,36} = 0.91$) or 2021 ($P = 0.0684$, $F_{1,52} = 3.46$) (Fig. 2.2). While *T. japonicus* was captured in all trees sampled each year, there was a significant effect of sampling site on captures in 2020 ($P < 0.0001$, $F_{4,36} = 19.00$) (Fig. 2.3) and 2021 ($P < 0.0001$, $F_{6,52} = 19.39$) (Fig. 2.4). There was a significant interaction between sampling site and trap location on *T. japonicus* captures in 2020 ($P < 0.0001$, $F_{4,36} = 5.43$), but not in 2021.

2.5 Discussion

Yellow sticky cards deployed in the lower or mid-canopy of mature *A. altissima* were equally effective for capturing the *H. halys* egg parasitoid, *T. japonicus*. This result has important implications for ongoing efforts to track the presence and range expansion of *T. japonicus*, as deploying, retrieving, and replacing these traps in the lower tree canopy is much more efficient and less time-consuming than at higher elevations in the tree. While the deployment of YSC is more time-efficient than sentinel eggs overall (Quinn et al. 2019a), placing them in the mid-canopy of trees has attendant drawbacks. When attached at the top of 4.6 m bamboo poles, YSC must be carefully manipulated to avoid entanglement with canopy leaves and branches, especially during windy conditions, which also may also lead to the long poles being dislodged after having been deployed. These issues were largely mitigated by placing YSC atop short poles suspended from branches in the lower canopy, and yielded detections of *T. japonicus* and other *H. halys* parasitoids that were equivalent to those in mid-canopy.

Although Quinn et al. (2019b) reported that *H. halys* egg masses and egg masses parasitized by *T. japonicus* were most abundant in the mid-canopy of felled *A. altissima*, results from the present study suggest that *T. japonicus* forages throughout the canopy, or at least is attracted to the visual cue from YSC's from various locations in the canopy. Other studies based on visual surveys from the ground have reported the presence of *H. halys* egg masses that were presumably in the lower portions of tree canopies (Bakken et al. 2015, Formella et al. 2020). Moreover, female *T. japonicus* are known to use host-associated olfactory cues for host location, recognition, and acceptance (Zhong et al. 2017, Boyle et al. 2020, Malek et al. 2021). Many insects (Döring and Chittka 2007, Shimoda and Honda 2013, Allan et al. 2020), including Scelionidae (Ferreira Santos de Aquino et al. 2012), are attracted to the color yellow. While neither the distance over which *T. japonicus* responds to YSC nor the relative strength of their response to YSC versus host cues is known, these studies provide further confirmation that the YSC is a sensitive monitoring tool for this species.

Quinn et al. (2019b) also found an uneven distribution of *T. japonicus* in the tree canopy; YSC and sentinel *H. halys* egg masses yielded *T. japonicus* detections only from the mid- and upper canopy. Consequently, our subsequent surveys for adventive populations utilized YSC deployed in mid-canopy (Quinn et al. 2021, Dyer unpublished), assuming an increased likelihood of *T. japonicus* detections. However, others have reported detections of *T. japonicus* from naturally-laid and sentinel *H. halys* egg masses on low-hanging and easily-accessible tree branches (Bakken et al. 2015, Formella et al. 2020), as well as from YSC deployed from ground-level (Holthouse et al. 2021, Peterson et al. 2021). Studies conducted by Quinn et al. (2019a) in 2016 and 2017 in Frederick County, VA resulted in relatively few *T. japonicus* captures (< 20) in YSC, likely due to this adventive population being early in its establishment. By contrast, each year of the present study yielded >170 *T. japonicus* that comprised >50% of all Scelionidae captured. Other studies (Dyer unpublished) also conducted in Frederick County, VA during the same period, yielded a total of 180 and 389 *T. japonicus* captures in YSC in 2020 and 2021, respectively, with captures recorded at every location at which YSC were deployed. Importantly, the present study was conducted at a time when adventive populations *T. japonicus* have apparently increased and are widely-distributed across the landscape (see Chapter 3). Thus, these results may or may not be applicable to other regions where *T. japonicus* densities are lower.

While mean weekly captures were not significantly different, significant differences in captures among sites were observed in both years, with a significant interaction between sampling site and trap location in 2020. Interestingly, in 2020 the two sites at which a significant effect of YSC location was observed also were the sites with greatest *T. japonicus* captures. In both cases, captures were significantly greater in the lower canopy, although such an effect was not observed at these same locations the following year. Notably, individual traps in 2020 with highest captures of *T. japonicus* (>20 in one week) may have been deployed in close proximity to a parasitized egg mass. Thus, site-specific factors may be more important in influencing captures of *T. japonicus* than trap location within the tree canopy. *Trissolcus* species have been most commonly found in wooded, semi-natural, and ornamental habitats (Cornelius et al. 2016, Abram et al. 2017). Although our results were based exclusively on sampling from *A. altissima*, Quinn et al. (2021) reported that sampling *T. japonicus* using YSC in several common tree hosts of *H. halys* was not affected by tree species. Based on these findings, it is expected that trap location within the canopy should not influence captures of *T. japonicus* in other wild hosts of *H. halys*. Moreover, the attraction of female *T. japonicus* to *H. halys* volatile cues observed in laboratory assays may show promise for the integration of olfactory stimuli with the visual stimuli of YSC to further enhance the sensitivity of this sampling tool, a subject which is examined in Chapter 4. Ultimately, deploying YSC in the lower canopy of *A. altissima,* and likely other *H. halys* host trees is expected to improve the efficiency and convenience of efforts to track the presence, distribution and potential spread of adventive *T. japonicus*.

2.6 References

Abram, P. K., K. A. Hoelmer, A. Acebes-Doria, H. Andrews, E. H. Beers, J. C. Bergh, R. Bessin, D. Biddinger, P. Botch, M. L. Buffington, M. L. Cornelius, E. Costi, E. S. Delfosse, C. Dieckhoff, R. Dobson, Z. Donais, M. Grieshop, G. Hamilton, T. Haye, C. Hedstrom, M. V. Herlihy, M. S. Hoddle, C. R. R. Hooks, P. Jentsch, N. K. Joshi, T. P. Kuhar, J. Lara, J. C. Lee, A. Legrand, T. C. Leskey, D. Lowenstein, L. Maistrello, C. R. Mathews, J. M. Milnes, W. R. Morrison, A. L. Nielsen, E. C. Ogburn, C. H. Pickett, K. Poley, J. Pote, J. Radl, P. M. Shrewsbury, E. Talamas, L. Tavella, J. F. Walgenbach, R. Waterworth, D. C. Weber, C. Welty, and N. G. Wiman. 2017. Indigenous arthropod natural enemies of the invasive brown marmorated stink bug in North

America and Europe. J. Pest Sci. 90: 1009-1020.

Abram, P. K., E. J. Talamas, S. Acheampong, P. G. Mason, and T. D. Gariepy. **2019**. First detection of the samurai wasp, *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae), in Canada. J. Hymenopt. Res. 68: 29–36.

Allan, S. A., J. George, L. L. Stelinski, and S. L. Lapointe. **2020**. Attributes of yellow traps affecting attraction of *Diaphorina citri* (Hemiptera: Liviidae). Insects 11: 452.

Avila, G. A., and J. G. Charles. **2018**. Modelling the potential geographic distribution of *Trissolcus japonicus*: a biological control agent of the brown marmorated stink bug, *Halyomorpha halys*. BioControl 63: 505–518.

Bakken, A. J., S. C. Schoof, M. Bickerton, K. L. Kamminga, J. C. Jenrette, S. Malone, M. A. Abney, D. A. Herbert, D. Reisig, T. P. Kuhar, and J. F. Walgenbach. **2015**. Occurrence of brown marmorated stink bug (Hemiptera: Pentatomidae) on wild hosts in nonmanaged woodlands and soybean fields in North Carolina and Virginia. Environ. Entomol. 44: 1011–1021.

Bergh, J. C., and N. F. Quinn. **2018**. Can the dispersal behavior of *Halyomorpha halys* (Hemiptera: Pentatomidae) inform the use of insecticide-treated netting to mitigate homeowner issues from its fall invasion? Environ. Entomol. 47: 1501–1508.

Boyle, S. M., D. C. Weber, J. Hough-Goldstein, and K. A. Hoelmer. **2020**. Host kairomones influence searching behavior of *Trissolcus japonicus* (Hymenoptera: Scelionidae), a parasitoid of *Halyomorpha halys* (Heteroptera: Pentatomidae). Environ. Entomol. 49: 15–20.

Cornelius, M. L., C. Dieckhoff, B. T. Vinyard, and K. A. Hoelmer. **2016**. Parasitism and predation on sentinel egg masses of the brown marmorated stink bug (Hemiptera: Pentatomidae) in three vegetable crops: importance of dissections for evaluating the impact of native parasitoids on an exotic pest. Environ. Entomol. 45: 1536–1542.

Dieckhoff, C., K. M. Tatman, and K. A. Hoelmer. 2017. Natural biological control of *Halyomorpha halys* by native egg parasitoids: a multi-year survey in northern Delaware. J. Pest Sci. 90: 1143–1158.

Döring, T. F., and L. Chittka. **2007**. Visual ecology of aphids—a critical review on the role of colours in host finding. Arthropod-Plant Interact.1: 3–16.

Ferreira Santos de Aquino, M., A. M. Dias, M. Borges, M. C. B. Moraes, and R. A. Laumann. **2012**. Influence of visual cues on host-searching and learning behaviour of the egg parasitoids *Telenomus podisi* and *Trissolcus basalis*. Entomol. Exp. Appl. 145: 162–174.

Formella, A., S. J. Dorman, S. V Taylor, and T. P. Kuhar. **2020**. Effects of aggregation lure and tree species on *Halyomorpha halys* (Hemiptera: Pentatomidae) seasonal oviposition. J. Econ. Entomol. 113: 203–210.

Hancock, T. J., D. H. Lee, J. C. Bergh, W. R. Morrison, and T. C. Leskey. **2019**. Presence of the invasive brown marmorated stink bug *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) on home exteriors during the autumn dispersal period: results generated by citizen scientists. Agric. For. Entomol. 21: 99–108.

Hedstrom, C., D. Lowenstein, H. Andrews, B. Bai, and N. Wiman. **2017**. Pentatomid host suitability and the discovery of introduced populations of *Trissolcus japonicus* in Oregon. J. Pest Sci. 90: 1169–1179.

Hedstrom, C. S., P. W. Shearer, J. C. Miller, and V. M. Walton. **2014**. The effects of kernel feeding by *Halyomorpha halys* (Hemiptera: Pentatomidae) on commercial hazelnuts. J. Econ. Entomol. 107: 1858–1865.

Herlihy, M. V., E. J. Talamas, and D. C. Weber. **2016**. Attack and success of native and exotic parasitoids on eggs of *Halyomorpha halys* in three Maryland habitats. PLoS One 11: e0150275.

Hoebeke, E., and M. Carter. **2003**. *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. Proc. Entomol. Soc. Wash. 105: 225–237.

Holthouse, M. C., Z. R. Schumm, E. J. Talamas, L. R. Spears, and D. G. Alston. **2020**. Surveys in northern Utah for egg parasitoids of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) detect *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae).

Biodivers. Data J. 8: 53363.

Holthouse, M. C., L. R. Spears, and D. G. Alston. **2021**. Comparison of yellow and blue sticky cards for detection and monitoring parasitoid wasps of the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae). J. Insect Sci. 21: 1–1.

Inkley, D. B. 2012. Characteristics of home invasion by the brown marmorated stink bug (Hemiptera: Pentatomidae). J. Entomol. Sci. 47: 125–130.

Jones, A. L., D. E. Jennings, C. R. R. Hooks, and P. M. Shrewsbury. **2014**. Sentinel eggs underestimate rates of parasitism of the exotic brown marmorated stink bug, *Halyomorpha halys*. Biol. Control 78: 61–66.

Kuhar, T. P., and K. Kamminga. **2017**. Review of the chemical control research on *Halyomorpha halys* in the USA. J. Pest Sci. 90: 1021–1031.

Leskey, T. C., and A. L. Nielsen. **2018**. Impact of the invasive brown marmorated stink bug in North America and Europe: history, biology, ecology, and management. Annu. Rev. Entomol. 63: 599–618.

Leskey, T. C., B. D. Short, B. R. Butler, and S. E. Wright. **2012**. Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in Mid-Atlantic tree fruit orchards in the United States: case studies of commercial management. Psyche 2012: 1–14.

Malek, R., J. M. Kaser, G. Anfora, M. Ciolli, A. Khrimian, D. C. Weber, and K. A. Hoelmer. **2021**. *Trissolcus japonicus* foraging behavior: implications for host preference and classical biological control. Biol. Control 161: 104700.

Moore, L., P. Tirello, S. D, M. D. Toews, C. Duso, and A. Pozzebon. **2019**. Characterizing damage potential of the brown marmorated stink bug in cherry orchards in Italy. Entomol. Gen. 39: 271–283.

Morrison, W. R., B. R. Blaauw, A. L. Nielsen, E. Talamas, and T. C. Leskey. **2018**. Predation and parasitism by native and exotic natural enemies of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) eggs augmented with semiochemicals and differing host stimuli. Biol. Control 121: 140–150.

Morrison, W. R., C. R. Mathews, and T. C. Leskey. **2016**. Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. Biol. Control 97: 120–130.

Ogburn, E. C., R. Bessin, C. Dieckhoff, R. Dobson, M. Grieshop, K. A. Hoelmer, C. Mathews, J. Moore, A. L. Nielsen, K. Poley, J. M. Pote, M. Rogers, C. Welty, and J. F. Walgenbach. **2016**. Natural enemy impact on eggs of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in organic agroecosystems: a regional assessment. Biol. Control 101: 39–51.

Peterson, H. M., E. Talamas, and G. Krawczyk. **2021**. Survey for adventive populations of the samurai wasp, *Trissolcus japonicus* (Hymenoptera: Scelionidae) in Pennsylvania at commercial fruit orchards and the surrounding forest. Insects 12: 258.

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

Quinn, N. F., E. J. Talamas, A. L. Acebes-Doria, T. C. Leskey, and J. C. Bergh. 2019b. Vertical sampling in tree canopies for *Halyomorpha halys* (Hemiptera: Pentatomidae) life stages and its egg parasitoid, *Trissolcus japonicus* (Hymenoptera: Scelionidae). Environ. Entomol. 48: 173–180.

Quinn, N. F., E. J. Talamas, T. C. Leskey, and J. C. Bergh. **2021**. Seasonal captures of *Trissolcus japonicus* (Ashmead) (hymenoptera: Scelionidae) and the effects of habitat type and tree species on detection frequency. Insects 12: 1–12.

Shimoda, M., and K. I. Honda. **2013**. Insect reactions to light and its applications to pest management. Appl. Entomol. Zool. 48: 413–421.

Stahl, J., F. Tortorici, M. Pontini, M. C. Bon, K. Hoelmer, C. Marazzi, L. Tavella, and T. Haye. **2019**. First discovery of adventive populations of *Trissolcus japonicus* in Europe. J. Pest Sci. 92: 371–379.

Talamas, E. J., M. V. Herlihy, C. Dieckhoff, K. A. Hoelmer, M. L. Buffington, M. C. Bon, and D. C. Weber. **2015a**. *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae) emerges in North America. J. Hymenopt. Res. 43: 119–128.

Talamas, E. J., N. F. Johnson, and M. Buffington. **2015b**. Key to Nearctic species of *Trissolcus* Ashmead (Hymenoptera, Scelionidae), natural enemies of native and invasive stink bugs (Hemiptera, Pentatomidae). J. Hymenopt. Res. 43: 45–110.

Tillman, G., M. Toews, B. Blaauw, A. Sial, T. Cottrell, E. Talamas, D. Buntin, S. Joseph, R. Balusu, H. Fadamiro, S. Lahiri, and D. Patel. **2020**. Parasitism and predation of sentinel eggs of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in the southeastern US. Biol. Control 145: 104247.

Wiman, N. G., J. E. Parker, C. Rodriguez-Saona, and V. M. Walton. **2015**. Characterizing damage of brown marmorated stink bug (Hemiptera: Pentatomidae) in blueberries. J. Econ. Entomol. 108: 1156–1163.

Yang, S.-Y., Y.-Z. Zhong, J.-P. Zhang, X.-P. Wang, and F. Zhang. **2016**. A comparative scanning electron microscopy study on antennal sensilla of *Trissolcus japonicus* and *Trissolcus plautiae*, egg parasitoids of stink bugs (Pentatomidae). Ann. Entomol. Soc. Am. 109: 112–120.

Yang, Z.-Q., Y.-X. Yao, L.-F. Qiu, and Z.-X. Li. **2009**. A new species of *Trissolcus* (Hymenoptera: Scelionidae) parasitizing eggs of *Halyomorpha halys* (Heteroptera: Pentatomidae) in China with comments on its biology. Ann. Entomol. Soc. Am. 102: 39–47.

Zhang, J., F. Zhang, T. Gariepy, P. Mason, D. Gillespie, E. Talamas, and T. Haye. **2017**. Seasonal parasitism and host specificity of *Trissolcus japonicus* in northern China. J. Pest Sci. 90: 1127–1141.

Zhong, Y. Z., J. P. Zhang, L. L. Ren, R. Tang, H. X. Zhan, G. H. Chen, and F. Zhang. **2017**. Behavioral responses of the egg parasitoid *Trissolcus japonicus* to volatiles from adults of its stink bug host, *Halyomorpha halys*. J. Pest Sci. 90: 1097–1105.

2.7 Figures and Tables

Fig. 2.1. Backfolding yellow sticky cards affixed at the top of bamboo poles deployed in pairs from branches in the lower and mid-canopy of individual female *A. altissima* in Frederick County, VA, 2020 and 2021.

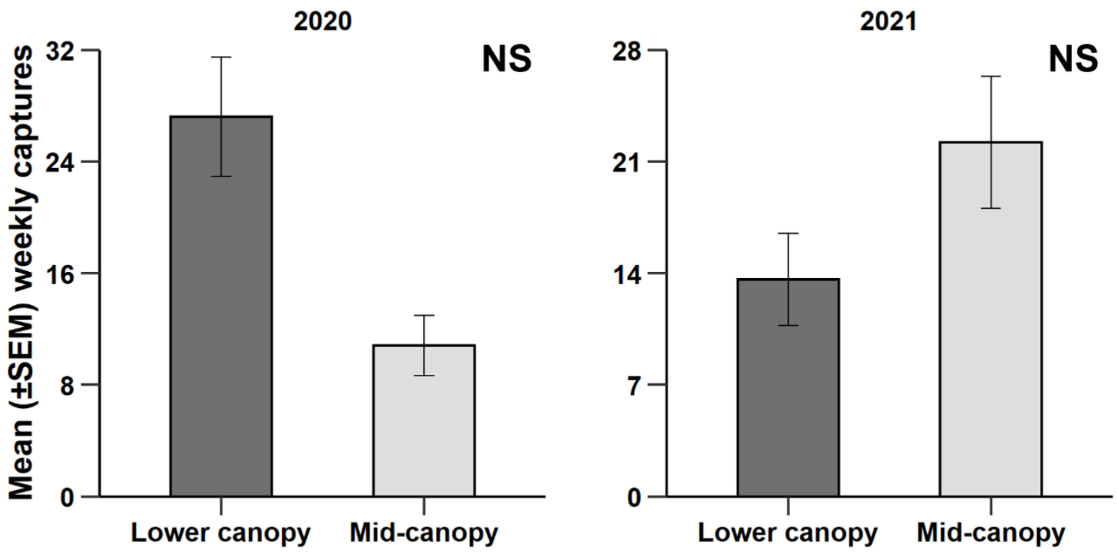

Fig. 2.2 Mean (± SEM) weekly captures of *T. japonicus* in YSC deployed in the mid- and lower canopy of individual *A. altissima* in 2020 and 2021. Traps were deployed and replaced weekly for five weeks from 16 July to 20 August, 2020 and from 6 July to 10 August, 2021.

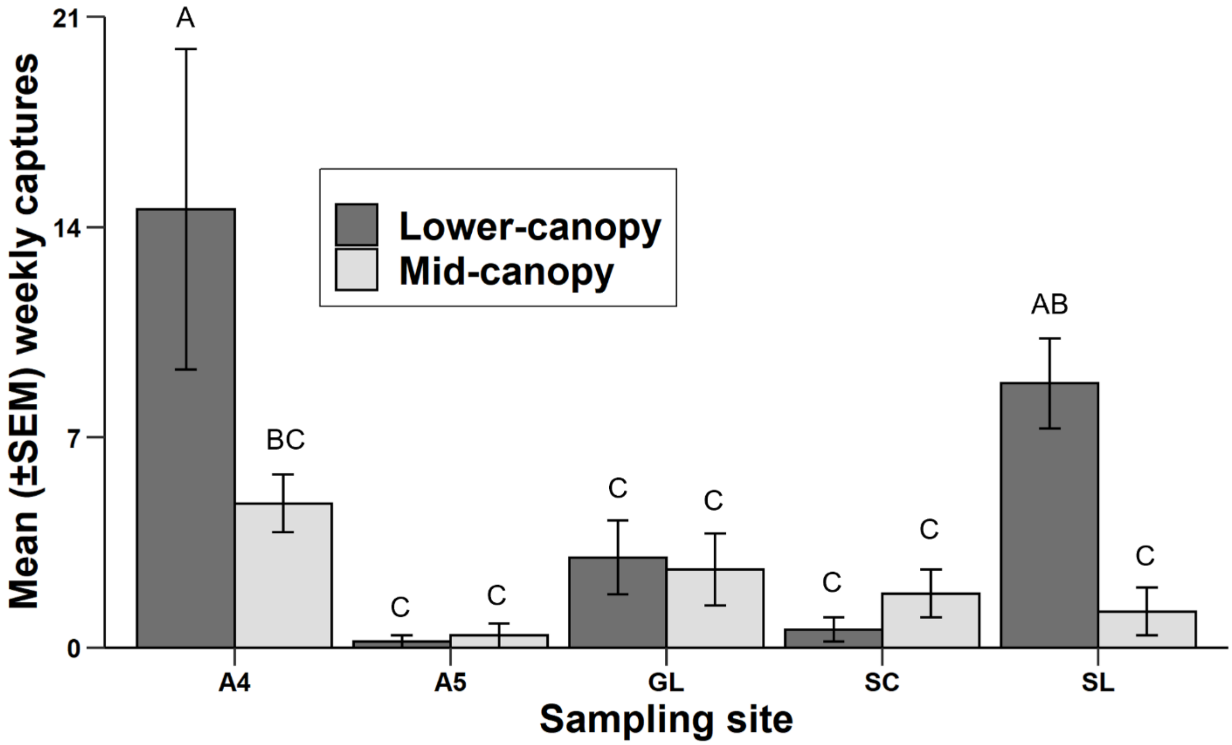

Fig. 2.3. Mean (± SEM) weekly captures of *T. japonicus* in YSC across sampling sites in 2020. Tukey-Kramer grouping for least square means. Different letters indicate statically significant differences.

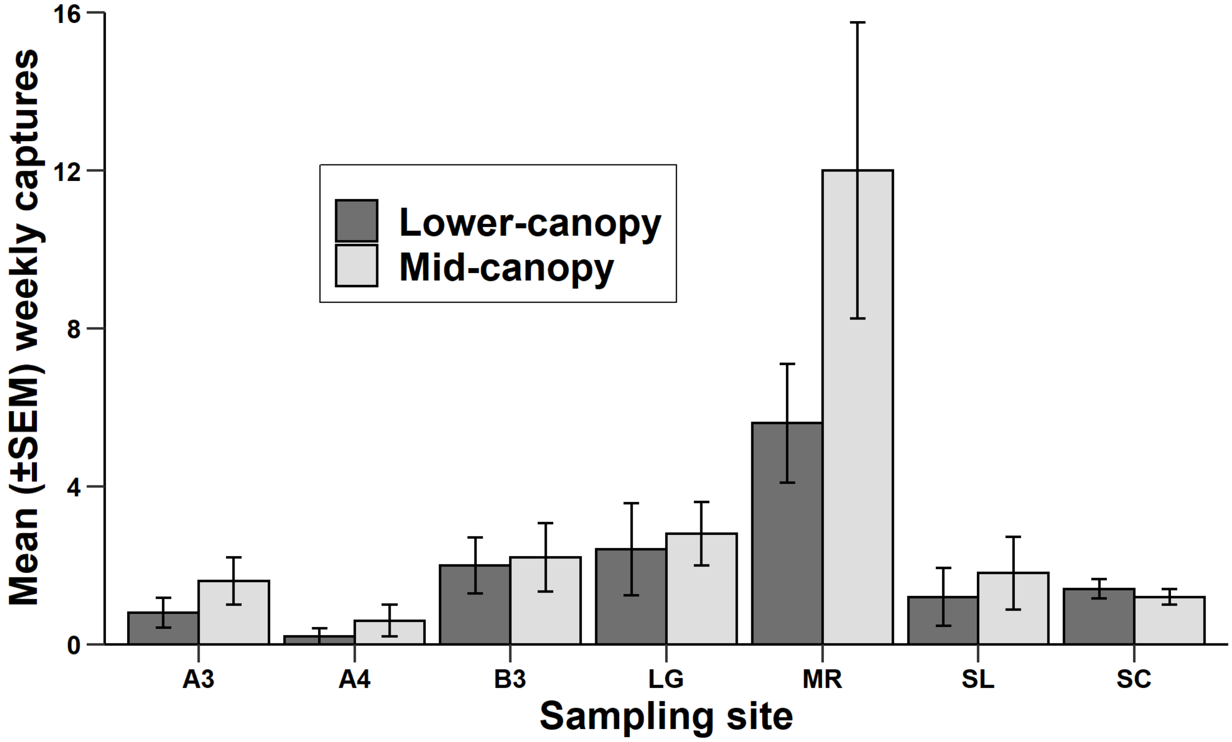

Fig. 2.4. Mean (± SEM) weekly captures of *T. japonicus* in YSC across sampling sites in 2021. There was not a significant interaction between trap placement and site location.

Table 2.1. Scelionid parasitoids[1] captured in YSC deployed in the lower and mid-canopy *of A. altissima* in Frederick County, VA from 16 July to 20 August, 2020 and 6 July to 10 August, 2021.

Treatment	*Trissolcus*					*Telenomus*				*Hadronotus*
	japonicus	*thyantae*	*hullensis*	*euschisti*	*brochymenae*	*podisi*	*persimilis*	*cristatus*	spp.	spp.
2020										
Lower canopy	136	7	1	28	9	59	0	0	5	6
Mid-canopy	54	1	1	9	2	36	0	0	1	4
2021										
Lower canopy	68	6	2	13	8	24	2	2	1	27
Mid-canopy	111	2	0	15	2	17	1	1	1	14

[1] All parasitoid species, with the exception of *Telenomus cristatus*, have been documented attacking *H. halys* egg masses in the field (Cornelius et al. 2016, Ogburn et al. 2016, Abram et al. 2017, Dieckhoff et al. 2017).

Chapter 3

Season-long monitoring of *Trissolcus japonicus* and *Halyomorpha halys*: The spatiotemporal relationship between a parasitoid and its host

3.1 Abstract

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an invasive agricultural and nuisance pest from Asia that has become established in parts of North America and Europe. In Asia, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) is considered a key natural enemy of *H. halys*, and adventive populations of this important egg parasitoid have been detected in the US since 2014. To determine the spatiotemporal relationships between the presence and relative abundance of adventive *T. japonicus* and *H. halys*, season-long sampling of both species was conducted in Frederick County, VA in 2020 and 2021, using 10 and 20 field sites in the respective years. Yellow sticky cards deployed in female tree of heaven, *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae) for *T. japonicus* and ground-deployed, pheromone-baited sticky traps for *H. halys* were assessed weekly for captures of each species between May and mid-August (2020) or May and September (2021). In both years, *T. japonicus* comprised the majority of Scelionidae captured and was detected at all sampling sites, with varying numbers among locations. In 2020, although weekly captures of *T. japonicus* were significantly correlated with captures of all *H. halys* life stages combined, no significant relationship was found between *T. japonicus* and individual *H. halys* life stages. In 2021, with twice the number of sampling sites, significant positive correlations were found between *T. japonicus* and *H. halys* adults, second instar nymphs, and all life stages combined. Seasonal captures of *T. japonicus* aligned with the phenology of *T. japonicus* previously reported from this area. The implications of these results for tracking adventive *T. japonicus* populations are discussed.

3.2 Introduction

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is a highly polyphagous herbivore native to northeast Asia that has become an invasive agricultural pest throughout much of North America and Europe (Leskey and Nielsen 2018). *Halyomorpha halys* has caused extensive damage to apple and peach orchards in the Mid-Atlantic region of the

US, resulting in losses of well over $37 million during its severe, initial outbreak in 2010 (Leskey et al. 2012). Heavy reliance on pyrethroids and other broad-spectrum insecticides for management of *H. halys* has disrupted the ecology of agroecosystems and led to secondary pest outbreaks in some commercial operations (Leskey et al. 2012). The need for environmentally-sound, long-term management of *H. halys* has led to investigations into the natural enemies of *H. halys* (Morrison et al. 2016, 2018, Dieckhoff et al. 2017), toward the possibility of promoting its biological control by native agents and/or the potential for classical biological control via non-native species. In Asia, *H. halys* is attacked by egg parasitoids from the families Scelionidae, Eupelmidae, and Encyrtidae (Lee et al. 2013). Of these, *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) (Ashmead) has been identified as the predominant parasitoid of *H. halys* in its native range (Yang et al. 2009).

Trissolcus japonicus, also known as the samurai wasp, is a solitary egg parasitoid with reported parasitism rates of *H. halys* eggs ranging from 50-80% in Asia (Yang et al. 2009, Zhang et al. 2017). Initially studied in various quarantine facilities in the United States and Europe, adventive populations of *T. japonicus* were detected in 2014 in Beltsville, Maryland (Talamas et al. 2015a, Herlihy et al. 2016). Since 2014, *T. japonicus* has been detected in 15 US states and the District of Columbia (www.stopbmsb.org), and in Canada (Abram et al. 2019) and Europe (Dieckhoff et al. 2021). Genetic analyses revealed that the quarantined population from Beijing was genetically distinct from all adventive populations detected, indicating that its populations in the United States were from multiple, independent introductions (Bon et al. 2017). The presence of *T. japonicus* is anticipated to affect *H. halys* populations in invaded regions to some degree, although neither its impact nor those of other egg parasitoids on *H. halys* population suppression in the native or introduced ranges have been documented based on empirical evidence (Abram et al. 2020). The establishment of adventive *T. japonicus* populations in North America presents an important opportunity to address the spatial and temporal relationships between the presence and relative abundance of *T. japonicus* and *H. halys*.

Spatiotemporal synchrony often drives interactions between natural enemies and pests (Welch and Harwood 2014). Consequently, the degree to which a parasitoid and its host(s) experience spatial and temporal overlap can influence metapopulation dynamics through impacting parasitoid population size and movement among host habitat patches (Van Nouhuys and Lei

2004). Phenological modeling predicts bivoltinism for *H. halys* throughout most of the United States (Nielsen et al. 2016), although environmental and climatic factors can influence the size and structure of these populations (Nielsen et al. 2017). Populations of *H. halys* tend to build from late spring toward a peak in late summer and early fall (Bakken et al. 2015, Leskey et al. 2015, Acebes-Doria et al. 2020). In Asia, *T. japonicus* is thought to complete several more generations than its co-evolved host, *H. halys* (Qiu et al. 2007), and season-long sentinel egg surveys there showed that parasitism by *T. japonicus* peaked in mid- to late summer (Zhang et al. 2017). In Frederick County, VA, *T. japonicus* has been detected annually since 2015, and the temporal pattern of its seasonal captures in yellow sticky cards (Quinn et al. 2021) aligned with data from China. However, the relationship between captures and densities of *T. japonicus* and *H. halys* has not been examined. Here, the results from weekly sampling of *H. halys* and *T. japonicus* at field sites in Frederick County, VA during two consecutive seasons were used to examine spatiotemporal relationships between the presence and relative abundance of adventive *T. japonicus* and its host, *H. halys*.

3.3 Methods

Sampling for *H. halys* and *T. japonicus* was conducted in Frederick County, VA from 4 May to 17 August, 2020 and 6 May to 23 September, 2021. Following previous studies (Quinn et al. 2019a,b, 2021), mature, female tree of heaven, *Ailanthus altissima,* (Mill.) Swingle (Sapindales: Simaroubaceae) was used as the standard host for sampling *T. japonicus*. In 2020 and 2021, respectively, ten and twenty *A. altissima* were selected from wooded windbreaks, isolated wooded patches, and the edge of unmanaged woodlands adjacent to commercial fruit orchards and experimental orchards at Virginia Tech's Alson H. Smith Research and Extension Center near Winchester, VA (Fig. 3.1). In 2021, the ten trees used in 2020 were reused.

Following Quinn et al. (2019b, 2021), *T. japonicus* was sampled using one backfolding yellow sticky card (YSC) (46 × 28 cm; AlphaScents, Inc., West Linn, OR) per site, attached at the top of a 4.6-m bamboo pole suspended from a mid-canopy branch via a hook attached to the pole just below the YSC. Cards were replaced weekly and inspected for known scelionid parasitoids of *H. halys* using a dissecting microscope at 10x magnification. All specimens of interest were removed *in situ* on a small piece of YSC that was affixed to a small Petri dish with double-sided tape, and identified following Talamas et al. (2015b). The number of *T. japonicus* captured in each trap was recorded and their sex was determined using antennal morphology (Yang et al. 2016).

Following Acebes-Doria et al. (2018), *H. halys* was sampled using one clear, double-sided sticky trap (Trécé, Inc., Adair, OK) per site, deployed at the edge of the wooded area. Traps were secured to the top of a 1.5-m wooden stake using a binder clip and baited with a BMSB Dual Lure (Trécé, Inc., Adair, OK) containing 5 mg of *H. halys* aggregation pheromone and 50 mg of methyl (2*E*,4*E*,6*Z*)-decatrienoate, an *H. halys* pheromone synergist (Weber et al. 2014). Stakes were driven into the ground at approximately 10 m from the tree in which the YSC was deployed. Captures of *H. halys* adults and nymphs were recorded weekly. Identification of *H. halys* instars were based on the relative size of nymphs and the morphological characteristics of each instar, following Hoebeke and Carter (2003). At each inspection, all traps were either cleaned of insects and debris or replaced, as needed. The pheromone lures were replaced at 12-week intervals, according to manufacturer recommendations.

Data analysis

In 2020 and 2021, respectively, data from 6 and 18 sampling weeks across all sites and weeks were excluded from analyses because of damage from miscellaneous sources (e.g. wildlife, weather) to the YSC or the *H. halys* trap at a given site. For correlation analyses of *H. halys* and *T. japonicus* captures, data were constrained and informed by the seasonal phenology of both species. In this area, seasonal captures of *H. halys* adults consistently show a pronounced peak from late August through September (Acebes-Doria et al. 2020). Moreover, seasonal captures of *T. japonicus* have shown a consistent and marked decline beginning about mid-August (Quinn et al. 2021), coinciding with the cessation of *H. halys* oviposition (Nielsen and Hamilton 2009). Although not confirmed, this may also be associated with the movement of *T. japonicus* to overwintering sites. Consequently, we concluded that comparing *H. halys* and *T. japonicus* sampling data during the period when their respective captures are naturally increasing and decreasing was not biologically relevant. Therefore, correlation analyses included captures of *T. japonicus* and *H. halys* that occurred by 17 August, the final sampling date in 2020. In 2021, when sampling continued into September, 94.1% of *T. japonicus* captures occurred by 19 August. Similarly, analyses of the relationship between *T. japonicus* and *H. halys* nymphs included only data from when second instars were first detected in traps in June, through mid-

August in 2020 and September in 2021. To accommodate instances of zero captures, weekly captures of *T. japonicus* and *H. halys* at each site were transformed using X + 1, then log-transformed to meet the assumptions of normality. Pearson's correlation was used for comparisons between *T. japonicus* and *H. halys* adults, all nymphs, second instars, and total captures (all life stages).

3.4 Results

In 2020 and 2021, respectively, 180 and 389 *T. japonicus* were captured on YSC across all sites, and comprised 50.0% and 60.1%, of total Scelionidae captures in the respective years (Table 3.1). Captures of *T. japonicus* were female-biased in 2020 (89.4% female) and 2021 (86% female). First captures of *T. japonicus* were recorded on 18 May, 2020 and 20 May, 2021, while last captures occurred on 17 August, 2020 (final sampling date) and 16 September, 2021 (Fig. 3.2). In both years, *T. japonicus* was captured at all sites, with 18.0 ± 4.2 (range = 5 - 47) and 18.5 ± 3.4 (range = 5 - 58) per site in 2020 and 2021, respectively.

Adult *H. halys* were present throughout each season and total seasonal captures were 250 and 2323 in 2020 and 2021, respectively (Fig. 3.2). First captures of *H. halys* nymphs each year were on 29 June, 2020 and 16 June, 2021, and total seasonal nymph captures were 596 and 1792 in 2020 and 2021, respectively. In 2020, mean total captures of *H. halys* adults and nymphs, respectively, were 25. 0 ± 5.7 and 59.6 ± 15.7 per site. In 2021, with twice the number of sites, total captures of *H. halys* adults and nymphs averaged 116.2 ± 17.2 and 89.6 ± 15.0 per site, respectively. Like *T. japonicus, H. halys* was detected at every site at which traps were deployed, and captures of all *H. halys* life stages were 84.6 ± 19.5 (range = 16 - 206) and 195.9 ± 24.5 (range = 53 - 425) per site in 2020 and 2021, respectively. The variability of *H. halys* and *T. japonicus* captures among sites each year lent strength to the correlation analyses of between them.

In 2020, there was a significant positive correlation between weekly captures of *T. japonicus* and all *H. halys* life stages combined (Table 3.2, Fig. 3.3A), but not between *T. japonicus* and *H. halys* adults, all instars combined, or second instars (Table 3.2, Fig. 3.3B-D). In 2021, there was a significant positive correlation between captures of *T. japonicus* and adult *H. halys*, second instars, and all *H. halys* life stages combined (Table 3.2, Fig. 3.4A-B, D), but not between captures of *T. japonicus* and *H. halys* nymphs (Table 3.2, Fig. 3.4C).

3.5 Discussion

Here, we report the first documentation of a significant relationship between the relative abundance of adventive *T. japonicus* and *H. halys* in North America. In both 2020 and 2021, captures of *T. japonicus* were positively correlated with captures of all *H. halys* life stages combined, and in 2021, there were significant correlations between *T. japonicus* and *H. halys* adults, second instars, and between *T. japonicus* and all *H. halys* life stages.

The seasonal phenology of *T. japonicus* aligned closely with that documented previously in the Mid-Atlantic region by Quinn et al. (2021); captures began in mid-May, followed by an initial, comparatively small peak in early June and by larger peaks in July and August, after which numbers declined in late August. These trends aligned well with patterns of seasonal parasitism of *H. halys* sentinel eggs masses observed in China and Japan (Zhang et al. 2017, Kamiyama et al. 2021). Results from the present study indicated a close spatial and temporal synchrony between *H. halys* and adventive *T. japonicus*. Moreover, the correlation between captures of *H. halys* and *T. japonicus* is indicative of a positive density-dependent relationship between this parasitoid and its host. Such density-dependent relationships have also been documented for the braconid parasitoid, *Biosteres arisanus* (Sonan), and the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Vargas et al. 1993). Not only was *T. japonicus* detected at sites where *H. halys* was present (i.e., all sites), but seasonal captures of *T. japonicus* corresponded with key phenological events observed in *H. halys*. Initial detections of *T. japonicus* in May aligned with the peak emergence of adult *H. halys* from overwintering sites in Frederick County, VA (Bergh et al. 2017). This first initial peak in abundance indicated that *T. japonicus* had emerged from overwintering sites and was present at the onset of *H. halys* oviposition in early June (Nielsen and Hamilton 2009). Subsequent peaks in *T. japonicus* abundance later in the season lagged behind predicted periods of peak oviposition by *H. halys* (Nielsen et al. 2016), after which a marked declined in captures was observed.

In 2021, captures of second instar *H. halys* correlated significantly with captures of *T. japonicus*. Second instars of *H. halys* are the earliest detectable life stage in pheromone traps, as the first instars remain on the egg mass to acquire essential gut microsymbionts (Taylor et al. 2014) until molting to the second instar, which disperse and begin feeding on plants. Certainly, the developmental duration of *H. halys* eggs, which averages 6.10 d at constant 25 ^{o}C (Nielsen et al.

2008) and the duration of first instar development, which averages 4.82 d at 25 ^{o}C (Nielsen et al. 2008), results in a lag between oviposition and the detection of second instars. However, given that second instars are the life stage most indicative of the recent presence of egg masses, these data suggest that the phenology of adventive *T. japonicus* is well aligned with peak periods of *H. halys* oviposition in Virginia (Formella et al. 2020). *Trissolcus japonicus* that emerged from experimental overwintering sites survived for 27.8 – 59.6 d showed a longevity of up to 220 d (Lowenstein et al. 2019). This suggests that female *T. japonicus* can persist during the period between emerging from overwintering sites and the onset of *H. halys* oviposition, although the longevity of subsequent generations in the field is unknown. Recent surveys in Kyoto, Japan reported parasitism of an *H. halys* sentinel egg mass as early as March, although subsequent activity was not detected until mid-May (Kamiyama et al. 2021). In China, sentinel egg deployments in mixed fruit orchards (Zhang et al. 2017) and kiwifruit orchards (Avila et al. 2021) revealed peak parasitism of sentinel *H. halys* eggs by *T. japonicu*s in July and August, also aligning with our results showing peak detections during that period.

Notably, that *T. japonicus* was detected at all sampling sites used in Frederick County, VA, indicates that it continues to persist and has become widely distributed in this area. Moreover, and importantly, *T. japonicus* appears to have increased in abundance in this area. Previously, Quinn et al. (2021) also used YSC to track the seasonal abundance of *T. japonicus* at 15 sites in Frederick County, VA, some of which were used in the present study, and reported 101 and 104 *T. japonicus* captured in 2018 and 2019, respectively. In comparison, 180 and 389 *T. japonicus* were captured at 10 and 20 sampling sites in 2020 and 2021, respectively. While the increase in *T. japonicus* captures between these studies suggests an increase in its abundance and/or distribution, the proportion of *T. japonicus* relative to the other Scelionidae species captured has also increased. Across the four years encompassed by the two different studies, *T. japonicus* was the most abundant Scelionidae species captured, ranging from 40-60% of all captures. Similarly, the species of other Scelionidae captured was also very similar among years, with *T. euschisti* (Ashmead) and *Telenomus podisi* (Ashmead) being the most common native species detected.

My studies used *A. altissima* as a standardized host tree for sampling *T. japonicus,* and traps were deployed in mid-canopy under the assumption, following Quinn et al. (2019a,b), that the likelihood of detecting *T. japonicus* would be maximized. Recent work on comparing captures of

T. japonicus among wild hosts (Quinn et al. 2021) and between the mid- and lower canopy (Chapter 2) suggest greater flexibility in sampling and surveillance of adventive *T. japonicus*. Regardless, sampling in the mid-canopy *of A. altissima* has proven very effective, supported by the fact that the number of *T. japonicus* specimens captured far surpassed the numbers reported from comparable surveys using YSC in Utah (Holthouse et al. 2021), Pennsylvania (Peterson et al. 2021), and Virginia (Quinn et al. 2021). While the impact of *T. japonicus* on *H. halys* populations remains unclear (Abram et al. 2020), the current study offers insights into the seasonal presence and abundance of adventive *T. japonicus* in relation to those of *H. halys*, and are expected to inform the interpretation of results from releases and redistributions of *T. japonicus* in the United States.

3.6 References

Abram, P. K., N. J. Mills, and E. H. Beers. **2020**. Review: classical biological control of invasive stink bugs with egg parasitoids – what does success look like? Pest Manag. Sci. 76: 1980–1992.

Abram, P. K., E. J. Talamas, S. Acheampong, P. G. Mason, and T. D. Gariepy. **2019**. First detection of the samurai wasp, *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae), in Canada. J. Hymenopt. Res. 68: 29–36.

Acebes-Doria, A. L., A. M. Agnello, D. G. Alston, H. Andrews, E. H. Beers, J. C. Bergh, R. Bessin, B. R. Blaauw, G. D. Buntin, E. C. Burkness, S. Chen, T. E. Cottrell, K. M. Daane, L. E. Fann, S. J. Fleischer, C. Guédot, L. J. Gut, G. C. Hamilton, R. Hilton, K. A. Hoelmer, W. D. Hutchison, P. Jentsch, G. Krawczyk, T. P. Kuhar, J. C. Lee, J. M. Milnes, A. L. Nielsen, D. K. Patel, B. D. Short, A. A. Sial, L. R. Spears, K. Tatman, M. D. Toews, J. D. Walgenbach, C. Welty, N. G. Wiman, J. Van Zoeren, and T. C. Leskey. **2020**. Season-long monitoring of the brown marmorated stink bug (Hemiptera: Pentatomidae) throughout the United States using commercially available traps and lures. J. Econ. Entomol. 113: 159–171.

Acebes-Doria, A., W. Morrison, B. Short, K. Rice, H. Bush, T. Kuhar, C. Duthie, and T. Leskey. 2018. Monitoring and biosurveillance tools for the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). Insects 9: 82.

Avila, G. A., J. Chen, W. Li, M. Alavi, Q. Mi, M. Sandanayaka, F. Zhang, and J. Zhang. **2021**. Seasonal abundance and diversity of egg parasitoids of *Halyomorpha halys* in kiwifruit orchards in China. Insects 12: 428.

Bakken, A. J., S. C. Schoof, M. Bickerton, K. L. Kamminga, J. C. Jenrette, S. Malone, M. A. Abney, D. A. Herbert, D. Reisig, T. P. Kuhar, and J. F. Walgenbach. **2015**. Occurrence of brown marmorated stink bug (Hemiptera: Pentatomidae) on wild hosts in nonmanaged woodlands and soybean fields in North Carolina and Virginia. Environ. Entomol. 44: 1011–1021.

Bergh, J. C., W. R. Morrison, S. V. Joseph, and T. C. Leskey. **2017**. Characterizing spring emergence of adult *Halyomorpha halys* using experimental overwintering shelters and commercial pheromone traps. Entomol. Exp. Appl. 162: 336–345.

Bon, M.-C., K. Hoelmer, E. J. Talamas, M. L. Buffington, F. Guermache, and D. C. Weber. 2017. Genetic diversity and origins of *Halyomorpha halys* in the U.S. and of its potential biological control agent unexpectedly recovered from the wild in the United States, pp. 240–242. In: PG, M., DR, G., C, V. (eds.), Proc. 5th Int. Symp. Biol. Control Arthropods. CAB International, Wallingford, United Kingdom., Langkawi, Malaysia.

Dieckhoff, C., K. M. Tatman, and K. A. Hoelmer. **2017**. Natural biological control of *Halyomorpha halys* by native egg parasitoids: a multi-year survey in northern Delaware. J. Pest Sci. 90: 1143–1158.

Dieckhoff, C., S. Wenz, M. Renninger, A. Reißig, H. Rauleder, C. P. W. Zebitz, J. Reetz, and O. Zimmermann. **2021**. Add Germany to the list—adventive population of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) emerges in Germany. Insects 12: 414.

Formella, A., S. J. Dorman, S V Taylor, and T. P. Kuhar. 2020. Effects of aggregation lure and tree species on *Halyomorpha halys* (Hemiptera: Pentatomidae) seasonal oviposition. J. Econ. Entomol. 113: 203–210.

Herlihy, M. V., E. J. Talamas, and D. C. Weber. **2016**. Attack and success of native and exotic parasitoids on eggs of *Halyomorpha halys* in three Maryland Habitats. PLoS One 11: e0150275.

Hoebeke E.R., and M. E. Carter. 2003. *Halyomorpha halys* (Stål) (Heteroptera: Pentatomidae): a polyphagous plant pest from Asia newly detected in North America. Proc. Entomol. Soc. Wash. 105: 225–237.

Holthouse, M. C., L. R. Spears, and D. G. Alston. **2021**. Comparison of yellow and blue sticky cards for detection and monitoring parasitoid wasps of the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae). J. Insect Sci. 21: 1–1.

Kamiyama, M. T., K. Matsuura, T. Hata, · Tsuyoshi Yoshimura, · Chin-Cheng, and S. Yang. 2021. Seasonal parasitism of native egg parasitoids of brown marmorated stink bug (*Halyomorpha halys*) in Japan. J. Pest Sci. 2021. 1: 1–13.

Lee, D. H., B. D. Short, S. V. Joseph, J. C. Bergh, and T. C. Leskey. 2013. Review of the biology, ecology, and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. Environ. Entomol. 42: 627-641.

Leskey, T. C., A. Agnello, J. C. Bergh, G. P. Dively, G. C. Hamilton, P. Jentsch, A. Khrimian, G. Krawczyk, T. P. Kuhar, D. H. Lee, W. R. Morrison, D. F. Polk, C. Rodriguez-Saona, P. W. Shearer, B. D. Short, P. M. Shrewsbury, J. F. Walgenbach, D. C. Weber, C. Welty, J. Whalen, N. Wiman, and F. Zaman. **2015**. Attraction of the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae) to traps baited with semiochemical stimuli across the United States. J. Econ. Entomol. 44: 746–756.

Leskey, T. C., and A. L. Nielsen. **2018**. Impact of the invasive brown marmorated stink bug in North America and Europe: history, biology, ecology, and management. Annu. Rev. Entomol. 63: 599–618.

Leskey, T. C., B. D. Short, B. R. Butler, and S. E. Wright. **2012**. Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in mid-Atlantic tree fruit orchards in the United States: case studies of commercial management. Psyche 2012: 1–14.

Lowenstein, D. M., H. Andrews, R. J. Hilton, C. Kaiser, and N. G. Wiman. 2019. Establishment in an introduced range: dispersal capacity and winter survival of *Trissolcus japonicus*, an adventive egg parasitoid. Insects 10: 443.

Morrison, W. R., B. R. Blaauw, A. L. Nielsen, E. Talamas, and T. C. Leskey. **2018**.

Predation and parasitism by native and exotic natural enemies of *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) eggs augmented with semiochemicals and differing host stimuli. Biol. Control 121: 140–150.

Morrison, W. R., C. R. Mathews, and T. C. Leskey. **2016**. Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. Biol. Control 97: 120–130.

Nielsen, A. L., S. Chen, and S. J. Fleischer. **2016**. Coupling developmental physiology, photoperiod, and temperature to model phenology and dynamics of an invasive heteropteran, *Halyomorpha halys*. Front. Physiol. 7: 165.

Nielsen, A. L., S. Fleischer, G. C. Hamilton, T. Hancock, G. Krawczyk, J. C. Lee, E. Ogburn, J. M. Pote, A. Raudenbush, A. Rucker, M. Saunders, V. P. Skillman, J. Sullivan, J. Timer, J. Walgenbach, N. G. Wiman, and T. C. Leskey. **2017**. Phenology of brown marmorated stink bug described using female reproductive development. Ecol. Evol. 7: 6680–6690.

Nielsen, A. L., and G. C. Hamilton. **2009**. Life history of the invasive species *Halyomorpha halys* (Hemiptera: Pentatomidae) in northeastern United States. Ann. Entomol. Soc. Am. 102: 608–616.

Nielsen, A. L., G. C. Hamilton, and D. Matadha. 2008. Developmental rate estimation and life table analysis for *Halyomorpha halys* (Hemiptera: Pentatomidae). Environ. Entomol. 37: 348–355.

Peterson, H. M., E. Talamas, and G. Krawczyk. **2021**. Survey for adventive populations of the samurai wasp, *Trissolcus japonicus* (Hymenoptera: Scelionidae) in Pennsylvania at commercial fruit orchards and the surrounding forest. Insects 12: 258.

Qiu, L.-F., Z.-Q. Yang, and W. Q. Tao. **2007**. Biology and population dynamics of *Trissolcus halyomorphae*. Sci. Silvae Sin. 43: 62–65.

Quinn, N. F., E. J. Talamas, A. L. Acebes-Doria, T. C. Leskey, and J. C. Bergh. **2019a**. Vertical sampling in tree canopies for *Halyomorpha halys* (Hemiptera: Pentatomidae) life stages and its egg parasitoid, *Trissolcus japonicus* (Hymenoptera: Scelionidae). Environ.

Entomol. 48: 173–180.

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

Quinn, N. F., E. J. Talamas, T. C. Leskey, and J. C. Bergh. **2021**. Seasonal captures of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) and the effects of habitat type and tree species on detection frequency. Insects 12: 1–12.

Talamas, E. J., M. V. Herlihy, C. Dieckhoff, K. A. Hoelmer, M. L. Buffington, M. C. Bon, and D. C. Weber. **2015a**. *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae) emerges in North America. J. Hymenopt. Res. 43: 119–128.

Talamas, E. J., N. F. Johnson, and M. Buffington. **2015b**. Key to Nearctic species of *Trissolcus* Ashmead (Hymenoptera, Scelionidae), natural enemies of native and invasive stink bugs (Hemiptera, Pentatomidae). J. Hymenopt. Res. 43: 45–110.

Taylor, C. M., P. L. Coffey, B. D. DeLay, and G. P. Dively. **2014**. The importance of gut symbionts in the development of the brown marmorated stink bug, *Halyomorpha halys* (Stål). PLoS One 9: e90312.

Van Nouhuys, S., and G. Lei. 2004. Parasitoid–host metapopulation dynamics: the causes and consequences of phenological asynchrony. J. Anim. Ecol. 73: 526–535.

Vargas, R. I., J. D. Stark, G. K. Uchida, and M. Purcell. 1993. Opiine parasitoids (Hymenoptera: Braconidae) of oriental fruit fly (Diptera: Tephritidae) on Kauai Island, Hawaii: islandwide relative abundance and parasitism rates in wild and orchard guava habitats. Environ. Entomol. 22: 246–253.

Weber, D. C., T. C. Leskey, G. C. Walsh, and A. Khrimian. **2014**. Synergy of aggregation pheromone with methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate in attraction of *Halyomorpha halys* (Hemiptera: Pentatomidae). J. Econ. Entomol. 107: 1061–1068.

Welch, K. D., and J. D. Harwood. **2014**. Temporal dynamics of natural enemy–pest interactions in a changing environment. Biol. Control 75: 18–27.

Yang, S.-Y., Y.-Z. Zhong, J.-P. Zhang, X.-P. Wang, and F. Zhang. **2016**. A comparative scanning electron microscopy study on antennal sensilla of *Trissolcus japonicus* and *Trissolcus plautiae*, egg parasitoids of stink bugs (Pentatomidae). Ann. Entomol. Soc. Am. 109: 112–120.

Yang, Z.-Q., Y.-X. Yao, L.-F. Qiu, and Z.-X. Li. **2009**. A new species of *Trissolcus* (Hymenoptera: Scelionidae) parasitizing eggs of *Halyomorpha halys* (Heteroptera: Pentatomidae) in China with comments on its biology. Ann. Entomol. Soc. Am. 102: 39–47.

Zhang, J., F. Zhang, T. Gariepy, P. Mason, D. Gillespie, E. Talamas, and T. Haye. **2017**. Seasonal parasitism and host specificity of *Trissolcus japonicus* in northern China. J. Pest Sci. 90: 1127–1141.

3.7 Figures and Tables

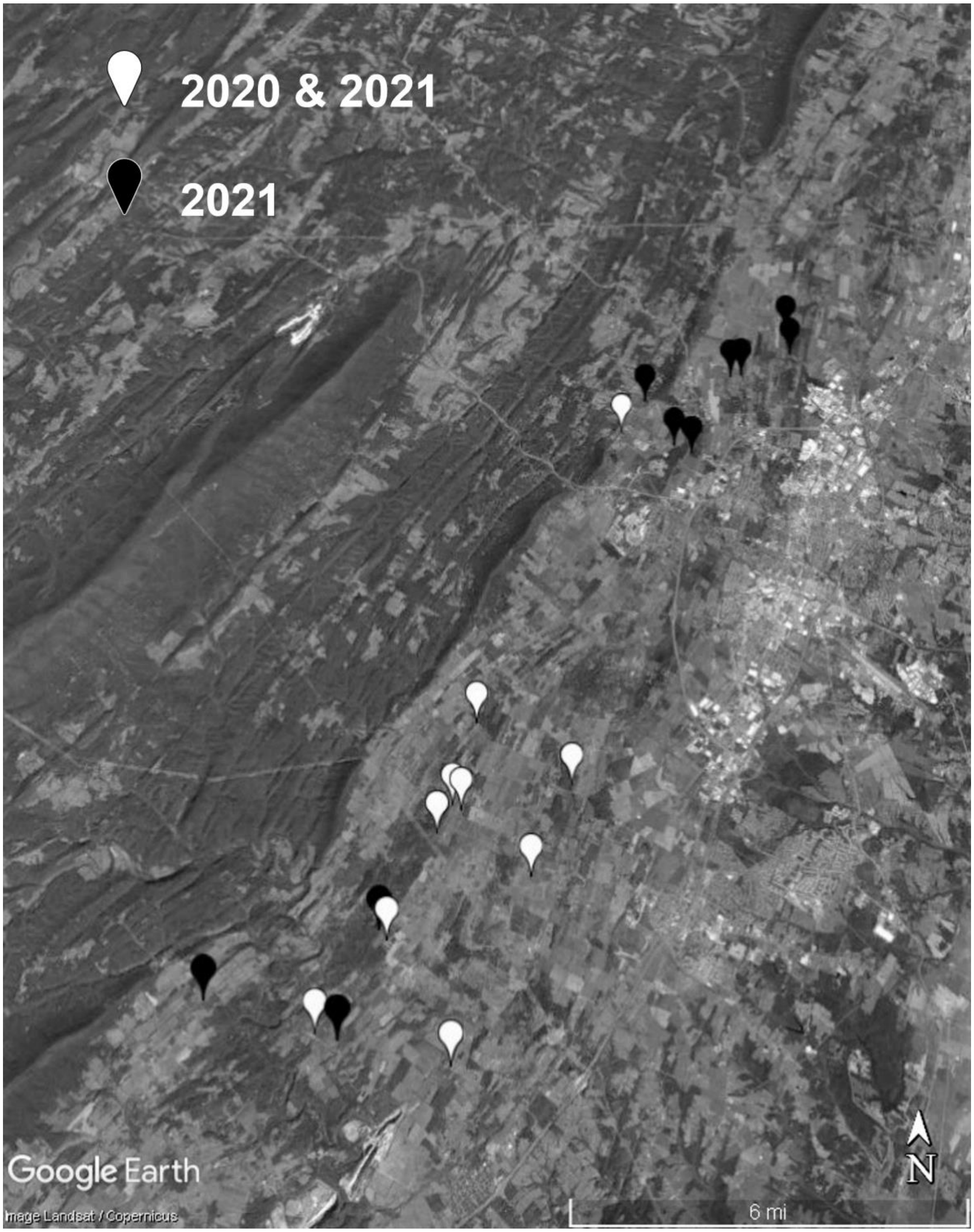

Fig. 3.1. Sampling sites for *T. japonicus* and *H. halys* in Frederick County, Virginia in 2020 and 2021. White and black markers, respectively, denote the location of sites used in both 2020 and 2021, and in 2021 only.

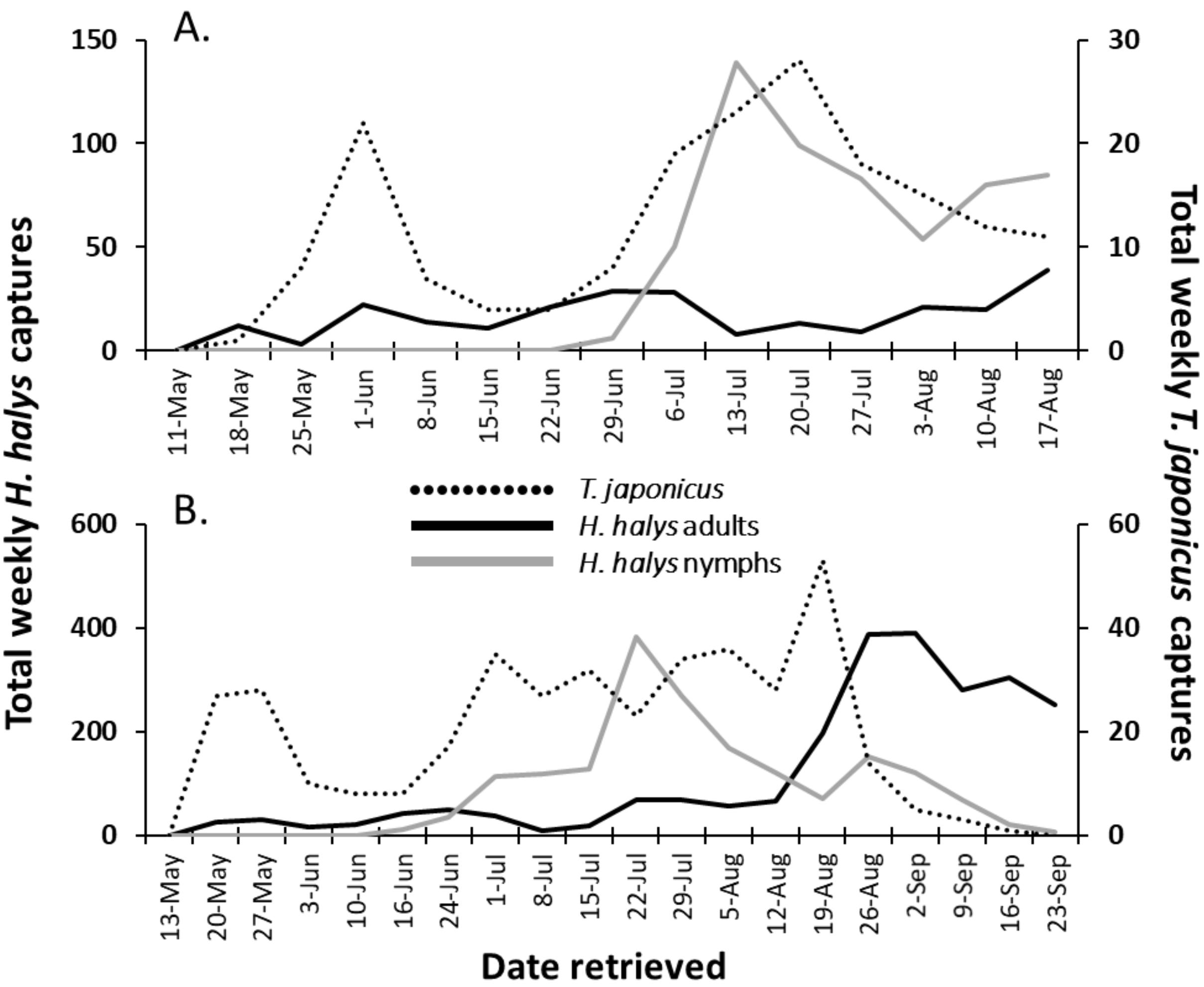

Fig. 3.2. Seasonal weekly captures of *T. japonicus*, *H. halys* adults, and *H. halys* nymphs in A) 2020 and B) 2021.

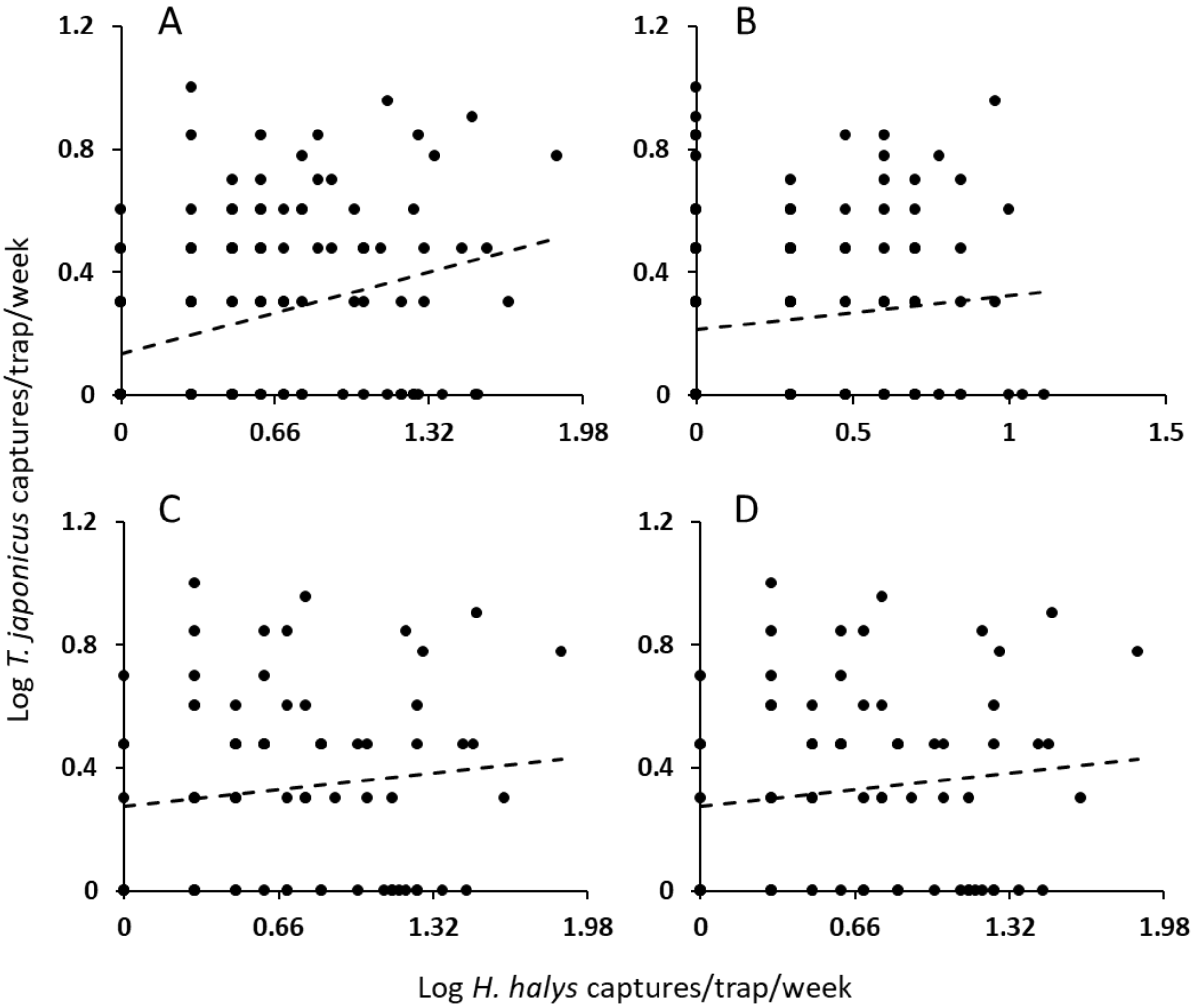

Fig. 3.3. Scatter plots of weekly captures of *T. japonicus* and A) all *H. halys* life stages, B) *H. halys* adults, C) all *H. halys* instars, and D) second instar *H. halys* in pairs of traps deployed at 10 sites in Frederick County, Virginia, 2020. Captures of *T. japonicus* were via yellow sticky cards deployed in the mid-canopy of mature, female *A. altimissima*, while *H. halys* captures were via ground-deployed, pheromone-baited sticky cards placed at 10 m from the tree in which *T. japonicus* was sampled.

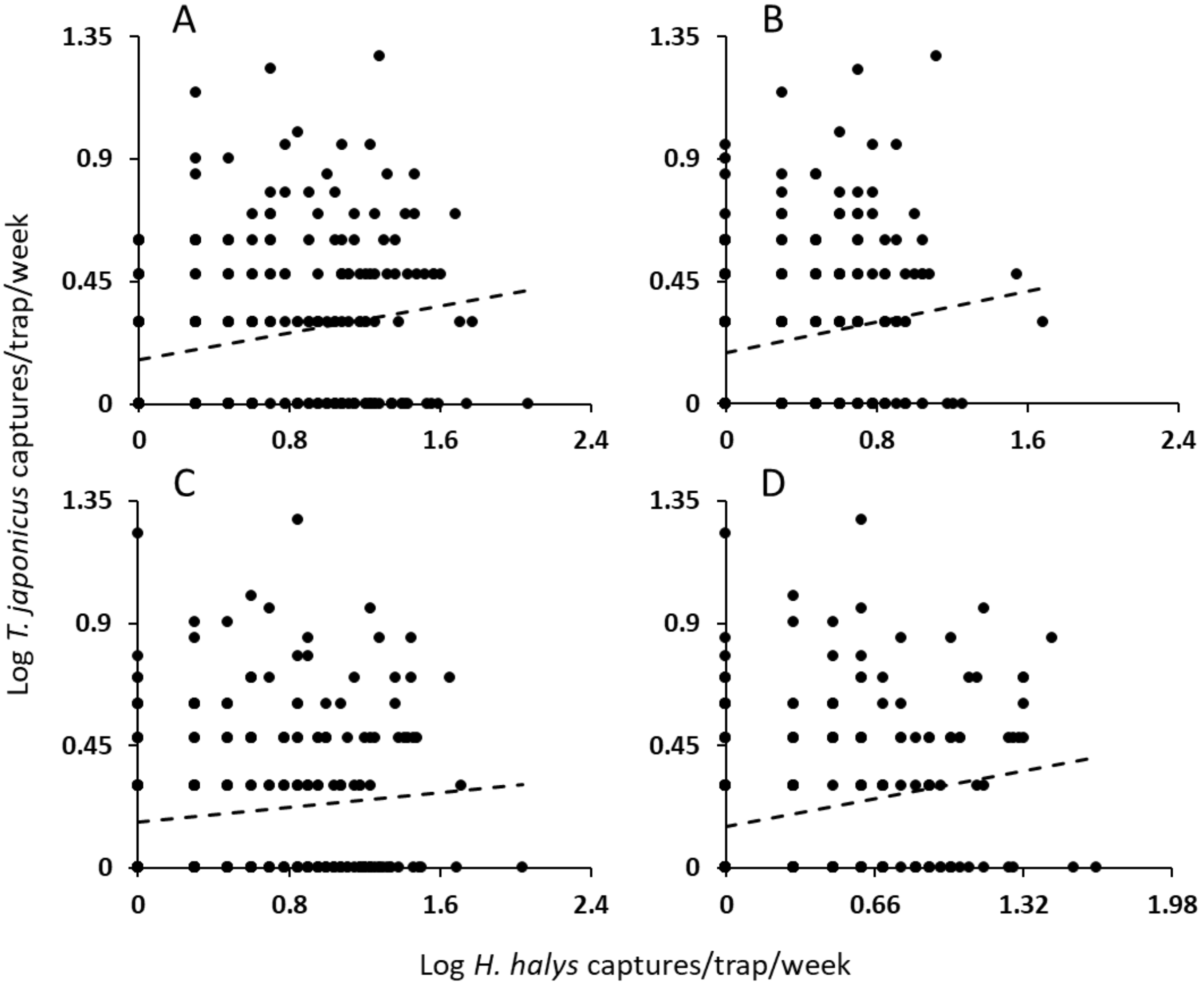

Fig. 3.4. Scatter plots of weekly captures of *T. japonicus* and A) all *H. halys* life stages, B) *H. halys* adults, C) all *H. halys* instars, and D) second instar *H. halys* in pairs of traps deployed at 10 sites in Frederick County, Virginia, 2021. Captures of *T. japonicus* were via yellow sticky cards deployed in the mid-canopy of mature, female *A. altimissima*, while *H. halys* captures were via ground-deployed, pheromone-baited sticky cards placed at 10 m from the tree in which *T. japonicus* was sampled.

Table 3.1. Counts of scelionid parasitoids from yellow sticky cards deployed the mid-canopy of *A. altiissima* in Frederick County, VA from 4 May to 17 August, 2020 and 6 May to 23 September, 2021.

Genus	**Species**	**2020**	**2021**
Trissolcus	*japonicus*	180	389
	brochymenae	8	30
	euschisti	61	51
	hullensis	1	0
	thyantae	3	8
	edessae	0	1
Telenomus	*podisi*	80	117
	cristatus	1	4
	persimilis	5	12
	spp.	15	12
Gryon	*pennsylvanicum*	3	20
	spp.	3	3

Table 3.2. Pearson's correlation coefficients for weekly, season-long captures of *T. japonicus* in yellow sticky cards and captures of *H. halys* life stages in and pheromone-baited sticky traps deployed at 10 and 20 sites in 2020 and 2021, respectively, in Frederick County, Virginia.

Log captures	**2020**			**2021**		
	r	*P*	*df*	*r*	*P*	*df*
H. halys adults[1]	0.12214	0.1447	142	0.17417	0.0033	281
All *H. halys* life stages[1]	0.33148	<0.0001	142	0.21331	0.0003	281
All *H. halys* nymphs[2]	0.12461	0.2835	74	0.11331	0.0556	284
H. halys second instars[2]	0.18754	0.1047	74	0.22394	0.0001	284

[1] Correlations calculated from log-transformed captures of *H. halys* and *T. japonicus* between 4 May and 17 August, 2020, and between 6 May and 19 August, 2021.

[2] Correlations calculated from log-transformed captures of *H. halys* and *T. japonicus*, starting from first detection of second instars on 29 June, 2020 and 16 June, 2021 through the end of sampling on 17 August 2020 and 23 September 2021.

Chapter 4

Evaluating the utility of host-associated olfactory stimuli to enhance surveillance of adventive populations of *Trissolcus japonicus*

4.1 Abstract

Trissolcus japonicus (Ashmead) (Hymenoptera: Scelionidae) is the predominant egg parasitoid of brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) in Asia. Detections of adventive *T. japonicus* populations in North America and Europe, where invasive *H. halys* populations have impacted the production of various specialty crops, have prompted research toward the optimization of sampling methods to track the distribution, relative density, and spread of this promising biological control agent. Female *T. japonicus* exhibit positive behavioral responses to several olfactory stimuli associated with *H. halys* under laboratory conditions, but such stimuli have not been evaluated for their potential utility to enhance surveillance of adventive populations. In northwestern Virginia, where *T. japonicus* is well established, the effect of baiting tree of heaven, *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae), with the *H. halys* aggregation pheromone on the abundance of *H. halys* egg masses and captures of *T. japonicus* in yellow sticky cards (YSC) was examined. In addition, the effect of baiting YSC with *H. halys* egg masses or *n*-tridecane on *T. japonicus* captures was assessed. Destructive sampling of pheromone-baited and non-baited trees revealed no significant differences in *H. halys* egg mass abundance. Similarly, YSC deployed in pheromone-baited and non-baited trees showed no significant differences in *T. japonicus* captures. Moreover, YSC augmented with *H. halys* egg masses or *n*-tridecane showed no increase in *T. japonicus* captures compared with non-baited controls. The implications for surveillance of adventive *T. japonicus* are discussed.

4.2 Introduction

In 2010, an outbreak of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in the Mid-Atlantic region of the United States caused more than $37 million in losses to apple orchards and additional, unquantified impacts to other specialty crops (Leskey et al. 2012). Native to Asia, *H. halys* is a highly polyphagous pest with over 170 known wild and cultivated hosts, including economically important crops such as tree fruits and some row and field crops. *Halyomorpha halys* has established or been detected in 47 US states (www.stopbmsb.org) and several other countries in the Northern and Southern Hemispheres (Leskey and Nielsen 2018). In the United States, its management in affected crops has relied primarily on applications of broad-spectrum insecticides, which in tree fruit systems have disrupted IPM practices and led to secondary pests outbreaks (Leskey et al. 2012). Thus, the natural enemies of *H. halys* and their effects against it have received considerable attention (Morrison et al. 2016, Abram et al. 2017, Dieckhoff et al. 2017).

In its native Asian range, a guild of hymenopteran parasitoids attacks *H. halys* eggs (Zhang et al. 2017), although their impact on *H. halys* populations has not been quantified empirically (Abram et al. 2020). In North America, a suite of generalist predators and native parasitoids that attack various *H. halys* life stages (Cornelius et al. 2016, Morrison et al. 2016, Ogburn et al. 2016) have not yet provided sufficient suppression of invasive *H. halys* populations (Abram et al. 2017).

Trissolcus japonicus (Ashmead) (Hymenoptera: Scelionidae) is a solitary endoparasitoid of pentatomid eggs and the primary parasitoid of *H. halys* in Asia (Zhang et al. 2017). In 2014, an adventive population of *T. japonicus* was detected in Beltsville, MD (Talamas et al. 2015a, Herlihy et al. 2016) and subsequent detections have occurred elsewhere in the United States (Leskey and Nielsen 2018, www.stopbmsb.org), Canada (Abram et al. 2019), Switzerland (Stahl et al. 2019), Italy (Peverieri et al. 2018), and Germany (Dieckhoff et al. 2021). In the United States, adventive *T. japonicus* has been detected in urban and semi-urban settings (Milnes et al. 2016, Hedstrom et al. 2017), unmanaged woodlands (Quinn et al. 2021), and agricultural systems (Kaser et al. 2019, Peterson et al. 2021). CLIMEX models predict the continued spread of *T. japonicus* to many regions where *H. halys* has or is likely to become established (Avila and Charles 2018). Thus, the development of sensitive and efficient sampling protocols will be critical for tracking the distribution, spread, and growth of adventive *T. japonicus* populations. To date, new state records of adventive *T. japonicus* have been established via the use of sentinel *H. halys* egg masses or yellow sticky cards (YSC). YSC are effective for *T. japonicus* surveillance (Quinn et al., 2019a, 2021), yielding >500 captures during a single season from recent work in Frederick County, VA (Dyer, unpublished data).

In laboratory bioassays, *T. japonicus* exhibits positive behavioral responses to stimuli produced by *H. halys*, including tarsal residues from adults (Boyle et al. 2020, Malek et al. 2021), and the defensive secretion, *n*-tridecane (Zhong et al. 2017, Malek et al. 2021). While the visual stimulus associated with YSC certainly elicits attraction to these traps, integrating the visual stimulus with olfactory stimuli from *H. halys* may further enhance the sensitivity of YSC and their effectiveness for *T. japonicus* surveillance. Here, we report studies conducted in northwestern Virginia, where *T. japonicus* is well-established (Quinn et al. 2021, Dyer unpublished), that evaluated the effect of baiting trees with a commercial *H. halys* aggregation pheromone lure containing a blend of (3S,6S,7R,10S)-10,11-epoxy-1-bis- abolen-3-ol and (3R,6S,7R,10S)-10,11-epoxy-1-bisabolen- 3-ol (Khrimian et al. 2014), and a pheromone synergist, methyl (E,E,Z)-2,4,6-decatrienoate (MDT) (Weber et al. 2014) on *H. halys* egg mass abundance and *T. japonicus* captures, and the effect of baiting YSC with fresh *H. halys* egg masses or synthetic *n*-tridecane on *T. japonicus* captures.

4.3 Methods

Sampling Trees

Across all studies, tree of heaven, *Ailanthus altissima* (Mill.) Swingle (Sapindales: Simaroubaceae), was the standard host used for sampling. Being among the most abundant and common wild host of *H. halys* in this region (Acebes-Doria et al. 2016), its fruiting structures support *H. halys* development (Acebes-Doria et al. 2017), and previous research in this area yielded *T. japonicus* detections from it over several consecutive years (Quinn et al. 2019b, 2021). *Ailanthus altissima* is a dioecious species; in 2019, trees of both sexes were used, while in 2020, only mature female trees bearing seeds were used. Additionally, trees were selected based on foliage and branching being present in the lower, middle, and upper portions of the tree.

H. halys Egg Mass Collections from Pheromone-Baited and Non-Baited Trees

The BMSB Dual Lure (Trécé, Inc., Adair, OK) is commonly used to monitor *H. halys* (Acebes-Doria et al. 2018, 2020, Leskey et al. 2021). This lure contains 5 mg of the *H. halys* aggregation pheromone ((3S,6S,7R,10S)-10,11-epoxy-1-bis- abolen-3-ol and (3R,6S,7R,10S)-10,11-epoxy-1-bisabolen- 3-ol) (Khrimian et al. 2014), and 50 mg of methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate (MDT). When these compounds are used in combination, the number of responding *H. halys* adults and nymphs is synergized, compared with either alone (Weber et al. 2014, Leskey et al. 2015, Morrison et al. 2018). To assess the effect of baiting trees with this lure on the abundance of *H. halys* egg masses in the tree canopy, sampling was conducted in pairs of trees growing at the edge of contiguous forest, in tree lines, or as isolated clusters, all of which adjoined tree fruit orchards or diversified agricultural land. In 2019 and 2020, respectively, sampling sites were in Warren County, VA (10 sites) and Frederick County, VA and Jefferson County, WV (5 sites). In 2019, sampling in June and August coincided with predicted peaks of *H. halys* oviposition (Nielsen et al. 2016), with five tree pairs per sampling period. In 2020, sampling was conducted only in June, due mainly to disruption from the COVID pandemic. The mean (±SD) distance between the trees in each pair was 95.09 ± 37.64 m in 2019 and 287.40 ± 225.35 m in 2020.

Three weeks prior to sampling egg masses, one tree per pair was baited with a BMSB Dual Lure and the other was not baited. Following Quinn et al. (2021), the lure was attached at the top of a 4.6-m bamboo pole suspended from a mid-canopy branch via a wire hook near the top of the pole. Three weeks after lure deployment, baited and non-baited trees were felled, and their height recorded. In 2019 and 2020, respectively, the mean (±SD) height of the felled trees was 9.02 ± 2.04 m and 12.64 ± 1.92 m. All leaves from each tree were inspected for *H. halys* egg masses, which were collected and held in a controlled environment chamber at 25.0 °C and a 14 h photoperiod. Initially, the number of egg masses, number of eggs per mass, and their status (hatched, unhatched, predated) was recorded for each tree. Eggs classified as hatched were, 1) those showing an egg burster, indicating prior emergence of *H. halys* nymphs, and 2) those indicative of adult parasitoid emergence, based on a ragged-edged exit hole at the operculum. The fate of unhatched egg masses was monitored for up to five weeks, and these were ultimately classified as, 1) no emergence, 2) stink bug nymphs emerged, or 3) adult parasitoids emerged. Adult wasps that emerged were counted and identified to species.

Sampling T. japonicus in Pheromone-Baited and Non-Baited Trees

Female *T. japonicus* have demonstrated a behavioral response to *H. halys* tarsal prints left on leaf substrates in laboratory assays (Boyle et al. 2020, Malek et al. 2021). Thus, to assess the effect of baiting trees with the *H. halys* aggregation pheromone lure on *T. japonicus* captures, two trees separated by ≥50 m were selected from tree lines adjacent to fruit orchards in Frederick County, VA in 2019 (n = 7 sites) and 2020 (n = 5 sites). One tree per pair was baited with a BMSB Dual

Lure, as described above, and lures remained in the trees for 11 weeks, spanning the duration of the study. Per manufacturer guidance, these lures remain attractive to *H. halys* for at least 12 weeks in the field. Each year, sampling occurred for two, three-week intervals from late June to mid-July and from late July to mid-August, coinciding with peaks in *T. japonicus* abundance in this area (Quinn et al. 2021). For the first interval, sampling began 3 weeks after lure deployment. Following Quinn et al. (2021), one backfolding YSC (46 × 28 cm; AlphaScents, Inc., West Linn, OR) attached atop a bamboo pole was deployed in the mid-canopy of baited and non-baited trees. The YSC were collected and replaced weekly and inspected for *H. halys* parasitoids under a dissecting microscope at 10x magnification. As described below, parasitoid specimens of interest were removed from the YSC and prepared for species identification.

Augmenting YSC with H. halys Egg Masses

Adult *H. halys* were maintained in screened cages (12 x 12 x 12 in; BioQuip Products, Inc., Rancho Dominguez, CA) in a room at Virginia Tech's Agricultural Research and Extension Center, Winchester, VA, at 25 °C, 16 h photoperiod, and 55% RH. Cages were provisioned with water, an assortment of seeds, nuts, fruits, and vegetables, and small, potted green bean plants as an oviposition substrate. Egg masses (≤24-h-old) were collected daily from bean leaves. Leaf pieces with an intact egg mass consisting of ≥20 eggs were trimmed to a ca. 1.3 cm^2 square and used the same day.

For baited YSC, two leaf pieces with a fresh egg mass were placed along the midline of the adhesive surface on both sides of the card i.e., four egg masses per YSC (Figure 1a). To control for the possible influence of *H. halys* tarsal prints on *T. japonicus* host-searching behavior (Boyle et al. 2020), bean leaves from the *H. halys* colony were cut into 1.3 cm^2 pieces and held in a Petri dish with three adult female *H. halys* for 30 min, after which four leaf pieces were affixed to each non-baited YSC.

Pairs of baited and non-baited YSC were deployed periodically at sites in Frederick County, VA between mid-June and early August, 2019 and 2020. Between one and five pairs of YSC were deployed on a given day, according to the daily availability of fresh egg masses, with 12 and 13 pairs used in 2019 and 2020, respectively. Using the bamboo poles described previously, one baited and one non-baited YSC was placed in mid-canopy of the canopy of the same tree, and separated by about 1.5 m. Because the acceptability of *H. halys* egg masses to *T. japonicus* declines after about three days (Qiu et al. 2007), deployment intervals were limited to 72 hr. The position of YSC treatments in each tree was randomly assigned initially and alternated at each redeployment. Parasitoid specimens of interest were removed and held for later identification. Egg masses on the YSC also were removed upon retrieval and held at 25^{o} C to monitor the emergence of *H. halys* nymphs, which confirmed their viability.

Augmenting YSC with n-tridecane

Five pairs of trees at sites in Frederick County, VA were used for sampling *T. japonicus* in 2020, with trees in each pair separated by ≥50 m. YSC were baited with *n*-tridecane by dispensing 5 mL of compound into 15 mL, UVC-resistant polyethylene vials (DWK Life Sciences, LLC, Millville, NJ) and attaching the vial to a 4.5 m bamboo pole at 3 cm below the base of the YSC attached at the top (Figure 4.1b). Gravimetric analysis of vials containing *n*-tridecane was conducted prior to and throughout deployment, yielding a mean (±SD) release rate of 0.236 ± 0.062 ml per vial per week. In each pair of trees, one baited and one non-baited YSC was deployed in the mid-canopy for seven days over five weeks between early July and mid-August. Baited and non-baited YSC were randomly assigned to trees in each pair initially and alternated at each redeployment. The YSC were replaced weekly and inspected for *H. halys* parasitoids.

Scelionid parasitoids captured on a YSC or that emerged from an egg mass, respectively, were removed *in situ* on a small piece of the card or placed on a piece of YSC. These were affixed to a Petri dish using double-sided sticky tape and identified following Talamas et al. (2015b). Sex determination of *T. japonicus* specimens was based on antennal morphology (Yang et al. 2016).

Statistical Analysis

Data analyses used SAS® Studio 3.8 (SAS Institute Inc., Cary, NC, USA) and all comparisons were considered significant at $\alpha = 0.05$. The distributions of *T. japonicus* captures and *H. halys* egg masses from pheromone-baited and non-baited trees were assessed for normality using the Shapiro-Wilk test. Because the counts of *H. halys* eggs masses from baited and non-baited trees were not normally distributed, a non-parametric Wilcoxon signed-rank test was used to compare *H. halys* egg mass counts. Data from the early and late season sampling in 2019 were analyzed separately, to account for the cumulative effect of hatched egg masses deposited prior to or during the first sampling period on those found during the subsequent sampling interval. To compare *T. japonicus* captures between pheromone-baited and non-baited trees, captures across the six weeks of sampling in each year were analyzed using a Paired t-test. At one site in 2019, there were no *T. japonicus* captures in baited or non-baited trees, and this site was excluded from analyses. Captures of *T. japonicus* were compared between egg-baited and non-baited YSC using the Wilcoxon signed-rank test, while captures of *T. japonicus* in *n*-tridecane baited and non-baited cards were pooled across sampling dates and compared using a Paired t-test.

4.4 Results

H. halys Egg Mass Collections from Pheromone-Baited and Non-Baited Trees

In 2019, 16 and 28 *H. halys* egg masses were collected from felled trees in June and August, respectively, and the mean (±SEM) number of eggs per mass was 24.59 ± 0.89. In June 2020, 51 *H. halys* egg masses were collected, with a mean of 26.86 ± 0.44 eggs per mass. There was no significant effect of baiting trees with an *H. halys* aggregation pheromone lure on the number of *H. halys* egg masses found in June 2019 ($P = 0.2500$, $S = 4$), August 2019 ($P = 1.0000$, $S = 0$) or June 2020 ($P = 0.6250$, $S = -2.5$) (Fig. 4.2). Table 4.1 summarizes the status and fate of all egg masses collected from baited and non-baited trees. Of the egg masses that were unhatched at the time of collection, 12.5, 14.2, and 23.5% yielded parasitoids in June 2019, August 2019, and June 2020, respectively (Table 4.1). Egg masses which indicated parasitoids had once previously emerged were never found. While no *T. japonicus* emerged from egg masses collected in June 2019, *T. japonicus* comprised 96.6% and 56.4% of all adult parasitoids that emerged in August 2019 and June 2020, respectively (Table 4.2). In addition, three female wasps guarding egg masses were collected in 2020, one of which was *Trissolcus brochymenae* (Ashmead) and the others *T. japonicus*.

T. japonicus Captures in Pheromone-Baited and Non-Baited Trees

In 2019 and 2020, respectively, 119 and 146 wasps from a total of 6 Scelionidae species were captured (Table 4.3). Captures of *T. japonicus* predominated each year (Table 4.3), but there was not a significant effect of baiting trees with the *H. halys* aggregation pheromone on its captures in 2019 ($t = 0.91$, df = 5, $P = 0.4056$) or 2020 ($t = -1.69$, df = 4, $P = 0.1660$) (Fig. 4.3). Captures of *T. japonicus* were female-biased in 2019 (93.7% female) and 2020 (92.9% female).

Augmenting YSC with H. halys Egg Masses and n-Tridecane

In 2019 and 2020, respectively, 91.7% and 100% of the fresh egg masses deployed with YSC yielded *H. halys* nymphs, confirming their viability. In 2019, one egg mass yielded *T. japonicus* adults. Total Scelionidae captures in YSC were 46 and 99 in 2019 and 2020, respectively (Table 4.4). In total, 36 (77.8% female) and 22 (86.4% female) *T. japonicus* were captured in 2020 and 2021, respectively, and there was not a significant difference in captures between egg-baited and non-baited YSC in 2019 ($P = 0.6797$, $S = 6$) or 2020 ($P = 0.6768$, $S = -5.5$) (Fig. 4.4).

In 2020, 66 *T. japonicus* (95.5% female) were captured in YSC baited with *n*-tridecane and non-baited cards across five weeks of sampling, and there was not a significant difference ($t = 0.19$, df = 4, $P = 0.8574$) in mean weekly captures between baited (6.8 ± 3.40 SE) and non-baited (6.4 ± 1.44 SE) YSC. Of the 196 Scelionidae specimens captured, 33.7% were *T. japonicus*, with the remainder being *Telenomus podisi* (43.9%), *T. euschisti* (15.8%), *T. brochymenae* (2.6%), *T. thyantae* (2.6%), 2 other *Telenomus* spp. (1%), and *Gryon pennsylvanicum* (0.5%).

4.5 Discussion

It is well established that commercial lures combining the *H. halys* aggregation pheromone and the MDT synergist, consistently result in increased densities of *H. halys* adults and nymphs in the vicinity of the lure (Weber et al. 2014, Leskey et al. 2015, Morrison et al. 2017, Formella et al. 2020). Furthermore, when a lure is placed in the canopy of a suitable plant host, aggregating adults are retained on the plant for a considerable period of time (Morrison et al. 2018). Given this behavioral response, it is reasonable to hypothesize that higher densities of *H. halys* in the canopy of a pheromone-baited tree may result in more deposition of egg masses than on a non-baited tree. A potential corollary to this is that increased density of egg masses may enhance the attraction and/or retention of *T. japonicus*

In semi-urban settings in Blacksburg, VA, where *T. japonicus* has not yet been detected, Formella et al. (2020) examined the response of *H. halys* to its aggregation pheromone lure deployed in the lower canopy of ornamental trees using ground-based visual surveys. Results revealed a significantly greater abundance of *H. halys* adults and nymphs in baited than non-baited trees, but no significant difference in the number of egg masses observed. Because Quinn et al. (2019b) showed consistently and significantly higher captures of *H. halys* in the mid- and upper canopy compared with the lower canopy and highest number of egg masses in the upper portions of *A. altissima*, I conducted modified a version of the Formella et al. (2020) study, using a wild tree host and exhaustive inspection of all leaves. However, results obtained from paired baited and non-baited mature, *A. altissima* trees that were felled yielded no significant differences in egg mass abundance, concurring with the results of Formella et al. (2020) and confirming that higher densities of *H. halys* did not result in higher numbers of egg masses.

While the plume reach and total area over which traps baited with commercial pheromone lures attract and reliably capture *H. halys* has been quantified for both adults and nymphs (Kirkpatrick et al. 2019), the influence of this aggregation behavior on mating and reproduction and the ultimate ecological role of the pheromone is poorly understood. Highly polyphagous insect herbivores such as *H. halys* experience less selective pressure in choosing host plants and may be driven by other factors, such as the presence of conspecifics or natural enemies, when selecting oviposition sites (Martínez et al. 2013). For example, female harlequin bugs, *Murgantia histrionica* (Hahn), oviposit on several plant hosts to increase the survivorship of their offspring. (English-Loeb and Collier 1987). For some Pentatomidae, oviposition is not limited to the immediate host plant on which mating and feeding occur. *Nezara viridula* females, for example, have been observed dispersing from mating sites to oviposit elsewhere on undamaged hosts (Kiritani et al. 1965). Importantly, a lack of increased oviposition implies a lack of increased release of oviposition-induced plant volatiles and/or cues associated with the egg mass. Under laboratory conditions, *T. japonicus* has demonstrated behavioral preference toward host plants with *H. halys* herbivory and oviposition (Bertoldi et al. 2019), suggesting that indirect plant defense mechanisms may also serve a role in the host location process of female *T. japonicus*.

Additionally, tarsal residues from adult stink bugs have been well documented to elicit host-searching behavior in Scelionidae (Colazza et al. 2007, Conti et al. 2004, Peri et al. 2016, Salerno et al. 2006). Indeed, laboratory assays in which *T. japonicus* was exposed to tarsal residues (i.e., “footprints”) left by *H. halys* adults and nymphs on various substrates showed that females exhibited increased residence time, slower walking velocity, and greater angular velocity compared with controls (Boyle et al. 2020, Malek et al. 2021). Furthermore, *T. japonicus* can discriminate between the sexes based on these tarsal residues, residing longer on patches contaminated by female *H. halys* (Arif et al. 2021). Thus, it was also reasonable to speculate that increased concentrations of attractive and/or retentive *H. halys* footprint may promote increased foraging activity of *T. japonicus* and/or increased retention duration if more *H. halys* tarsal prints were present. However, my companion study that assessed the effect of pheromone-baiting on *T. japonicus* captures in YSC revealed no significant differences between baited and non-baited trees, suggesting that increased tarsal footprints from aggregated *H. halys* did not influence *T. japonicus* abundance in baited trees. Similarly, augmenting YSC with fresh *H. halys* egg masses or *n*-tridecane had no effect on *T. japonicus* captures. In combination, results from these studies indicate that use of the *H. halys* aggregation pheromone lure, augmenting YSCs with its egg masses, or the component of its defensive compound, *n*-tridecane, would not be expected to improve the effectiveness or sensitivity of surveillance tools for *T. japonicus*.

Recent work in controlled conditions has shown that the duration for which tarsal residues from *H. halys* can elicit searching behavior in female is at least 4 days (Malek et al. 2021), although such bioactivity may be affected by epicuticular waxes present on leaf substrate (Rostás et al. 2008). Moreover, it is not yet understood how environmental factors affect the duration of this bioactivity in the field. Contact kairomones such as those present on egg surfaces or in tarsal residues typically mediate short-range host location in parasitoids once foraging female has found suitable host habitat (Fatouros et al. 2008). Such cues can often serve as arrestants which prompt further increased searching effort in a specific area (Conti et al. 2003, Colazza et al. 2014). Consequently, chemical cues present in *H. halys* footprints or egg masses may be similarly limited in their range and thus do not attract female *T. japonicus* toward baited trees, but rather, elicit more concentrated searching, as observed at close range in laboratory assays (Boyle et al. 2020, Malek et al. 2021). Moreover, because the presence of the *H. halys* pheromone lure in tree canopies had no observable effect on *H. halys* oviposition compared with non-baited canopies, it follows that there would be no observed differences in the relative abundance of *T. japonicus* in baited and non-baited tree canopies.

Kairomones present on the chorion surface typically act locally and may elicit host recognition in female parasitoids (Conti and Colazza 2012). Failure of female parasitoids to recognize surface chemicals on host eggs can limit successful parasitism, particularly in instances of an evolutionary mismatch, as is the case with *H. halys* and many North American Scelionidae (Tognon et al. 2017). Adhesive secretions produced by ovipositing female stink bugs can serve as contact kairomones used in the host recognition process (Bin et al. 1993, Borges et al. 1999). For example, chemical extracts of the adhesive produced by *Euschistus heros* (Fabricius) during oviposition, when applied to glass beads, initiated host searching and acceptance behavior in *Telenomus podisi* (Borges et al. 1999). Similar responsiveness to adhesive material from egg masses has also been observed in *T. basalis* in relation to its host, *Nezara viridula* (Linnaeus) (Bin et al. 1993). Additionally, such adhesive material on eggs may be the source of attractive, though limited, volatiles, as shown in olfactometer assays with *T. brochymenae* using egg volatiles from *M. histrionica* (Conti et al. 2003). However, as noted by Borges et al. (1999), the chemistry of egg adhesive is of relatively low volatility compared with pheromones produced by adults. Thus, cues associated with host eggs may serve as contact kairomones that are commonly attributed to host recognition rather than long-range habitat or host location (Fatouros et al. 2008, Conti and Colazza 2012). In the present study, we found no significant difference in *T. japonicus* captures between traps with and without *H. halys* egg masses, supporting the localized nature of attraction to any egg kairomones that may be present.

While tridecane elicits positive behavioral and electrophysiological antennal responses from female *T. japonicus* under laboratory conditions (Zhong et al. 2017), we found no significant effect of tridecane on captures of *T. japonicus*. To our knowledge, this is the first study to test the responsiveness of adventive *T. japonicus* to this kairomone in the field. Tridecane is a defensive compound found in the metathoracic secretions of stink bugs and is produced by *H. halys* males, females, and nymphs (Kitamura et al. 1984, Harris et al. 2015). Moreover, tridecane may serve as a host-finding kairomone for some parasitoids. Laumann et al. (2009) found that *Telenomus podisi* (Ashmead) responded positively to tridecane and among other defensive compounds from *Euschistus heros*. In *T. japonicus,* low doses of tridecane attracted *T. japonicus* in Y-tube assays and reduced host searching time when applied to *H. halys* eggs (Zhong et al. 2017). However, responses in Y-tube assays only occurred at a single, minute dosage under simplified, laboratory conditions. In our study, we did not observe any significant effect of tridecane baiting on captures of *T. japonicus* under field conditions. Similarly, Fraga et al. (2017) found no significant increase in *H. halys* egg predation by *Orius insidiosus* when plants were baited with tridecane, despite a previously observed attraction to tridecane-baited plants. While tridecane does appear to elicit response under laboratory conditions, its ability to enhance *T. japonicus* detections in the field remains to be demonstrated.

Trissolcus japonicus has become well established in Frederick County, VA where it has been detected annually since its initial discovery in 2015 (Quinn et al. 2021). In 2020, >500 *T. japonicus* were captured using YSC across several studies (Dyer, unpublished data). Recent season-long sampling in Frederick County revealed that *T. japonicus* was present at all sites (n = 20) across several habitats (Chapter 3), yet, the effect of its widespread establishment on local *H. halys* populations remains unknown. The presence of adventive *T. japonicus* populations in VA and elsewhere in the United States creates opportunities to explore the potential to enhance the sensitivity of surveillance tools by augmenting them with host-associated semiochemical cues. While none of the stimuli tested in this study increased detections of *T. japonicus*, the integration of olfactory attractants with the visual stimuli provided by YSC still has the potential to enhance the sensitivity of current sampling tools. The role of herbivore-induced plant volatiles on the foraging behavior female *T. japonicus* in the field is an area which remains to be explored fully. However, early work examining the response of *T. japonicus* to HIPVs induced by *H. halys* in olfactometer assays (Bertoldi et al. 2019) show promising signs which suggest that such stimuli may be used in the field. Moreover, recent efforts have been made with regards to the visual attraction of sticky cards to reduce non-target captures by using blue-colored sticky cards as an alternative to broadly-attractant yellow (Holthouse et al. 2021). Ultimately, having sensitive surveillance tools to measure both *T. japonicus* and *H. halys* relative densities will be critical to quantifying this potential suppression.

References 4.6

Abram, P. K., K. A. Hoelmer, A. Acebes-Doria, H. Andrews, E. H. Beers, J. C. Bergh, R. Bessin, D. Biddinger, P. Botch, M. L. Buffington, M. L. Cornelius, E. Costi, E. S. Delfosse, C. Dieckhoff, R. Dobson, Z. Donais, M. Grieshop, G. Hamilton, T. Haye, C. Hedstrom, M. V. Herlihy, M. S. Hoddle, C. R. R. Hooks, P. Jentsch, N. K. Joshi, T. P. Kuhar, J. Lara, J. C. Lee, A. Legrand, T. C. Leskey, D. Lowenstein, L. Maistrello, C. R. Mathews, J. M. Milnes, W. R. Morrison, A. L. Nielsen, E. C. Ogburn, C. H. Pickett, K. Poley, J. Pote, J. Radl, P. M. Shrewsbury, E. Talamas, L. Tavella, J. F. Walgenbach, R. Waterworth, D. C. Weber, C. Welty, and N. G. Wiman. **2017**. Indigenous arthropod natural enemies of the invasive brown marmorated stink bug in North America and Europe. J. Pest Sci. 90: 1009-1020.

Abram, P. K., N. J. Mills, and E. H. Beers. **2020**. Review: classical biological control of invasive stink bugs with egg parasitoids – what does success look like? Pest Manag. Sci. 76: 1980–1992.

Abram, P. K., E. J. Talamas, S. Acheampong, P. G. Mason, and T. D. Gariepy. **2019**. First detection of the samurai wasp, *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae), in Canada. J. Hymenopt. Res. 68: 29–36.

Acebes-Doria, A. L., A. M. Agnello, D. G. Alston, H. Andrews, E. H. Beers, J. C. Bergh, R. Bessin, B. R. Blaauw, G. D. Buntin, E. C. Burkness, S. Chen, T. E. Cottrell, K. M. Daane, L. E. Fann, S. J. Fleischer, C. Guédot, L. J. Gut, G. C. Hamilton, R. Hilton, K. A. Hoelmer, W. D. Hutchison, P. Jentsch, G. Krawczyk, T. P. Kuhar, J. C. Lee, J. M. Milnes, A. L. Nielsen, D. K. Patel, B. D. Short, A. A. Sial, L. R. Spears, K. Tatman, M. D. Toews, J. D. Walgenbach, C. Welty, N. G. Wiman, J. Van Zoeren, and T. C. Leskey. 2020. Season-long monitoring of the brown marmorated stink bug (Hemiptera: Pentatomidae) throughout the United States using commercially available traps and lures. J. Econ. Entomol. 113: 159-171.

Acebes-Doria, A. L., T. C. Leskey, and J. C. Bergh. **2016**. Host plant effects on *Halyomorpha halys* (Hemiptera: Pentatomidae) nymphal development and survivorship. Environ. Entomol. 45: 663–670.

Acebes-Doria, A. L., T. C. Leskey, and J. C. Bergh. **2017**. Temporal and directional patterns of nymphal *Halyomorpha halys* (Hemiptera: Pentatomidae) movement on the trunk of selected wild and fruit tree hosts in the mid-Atlantic region. Environ. Entomol. 46: 258–267.

Acebes-Doria, A., W. Morrison, B. Short, K. Rice, H. Bush, T. Kuhar, C. Duthie, and T. Leskey. 2018. Monitoring and biosurveillance tools for the brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). Insects 9: 82.

Arif, M. A., T. Alınç, S. Guarino, S. Colazza, A. Cusumano, and E. Peri. **2021**. Only females oviposit: chemical discrimination of adult stink bug sex by the egg parasitoid *Trissolcus japonicus*. Front. Ecol. Evol. 9: 213.

Avila, G. A., and J. G. Charles. **2018**. Modelling the potential geographic distribution of *Trissolcus japonicus*: a biological control agent of the brown marmorated stink bug, *Halyomorpha halys*. BioControl 63: 505–518.

Bertoldi, V., G. Rondoni, J. Brodeur, and E. Conti. **2019**. An egg parasitoid efficiently exploits cues from a coevolved host but not those from a novel host. Front. Physiol. 10: 746.

Bin, F., S. B. Vinson, M. R. Strand, S. Colazza, and W. A. Jones. **1993**. Source of an egg kairomone for *Trissolcus basalis,* a parasitoid of *Nezara viridula*. Physiol. Entomol. 18: 7–15.

Borges, M., M. L. M. Costa, E. R. Sujii, M. D. G. Cavalcanti, G. F. Redígolo, I. S. Resck, and E. F. Vilela. **1999**. Semiochemical and physical stimuli involved in host recognition by *Telenomus podisi* (Hymenoptera: Scelionidae) toward *Euschistus heros* (Heteroptera: Pentatomidae). Physiol. Entomol. 24: 227–233.

Boyle, S. M., D. C. Weber, J. Hough-Goldstein, and K. A. Hoelmer. **2020**. Host kairomones influence searching behavior of *Trissolcus japonicus* (Hymenoptera: Scelionidae), a parasitoid of *Halyomorpha halys* (Heteroptera: Pentatomidae). Environ. Entomol. 49: 15–20.

Colazza, S., G. Aquila, C. De Pasquale, E. Peri, and J. G. Millar. **2007**. The egg parasitoid *Trissolcus basalis* uses n-nonadecane, a cuticular hydrocarbon from its stink bug host *Nezara viridula*, to discriminate between female and male hosts. J. Chem. Ecol. 33: 1405–1420.

Colazza, S., A. Cusumano, D. Lo Giudice, and E. Peri. 2014. Chemo-orientation responses in hymenopteran parasitoids induced by substrate-borne semiochemicals. BioControl 59: 1-17.

Conti, E., and S. Colazza. **2012**. Chemical ecology of egg parasitoids associated with true bugs. Psyche 2012: 1-11.

Conti, E., G. Salerno, F. Bin, and S. B. Vinson. **2004**. The role of host semiochemicals in parasitoid specificity: a case study with *Trissolcus brochymenae* and *Trissolcus simoni* on pentatomid bugs. Biol. Control 29: 435–444.

Conti, E., G. Salerno, F. Bin, H. J. Williams, and S. B. Vinson. **2003**. Chemical cues from

Murgantia histrionica eliciting host location and recognition in the egg parasitoid *Trissolcus brochymenae*. J. Chem. Ecol. 29: 115–130.

Cornelius, M. L., C. Dieckhoff, B. T. Vinyard, and K. A. Hoelmer. **2016**. Parasitism and predation on sentinel egg masses of the brown marmorated stink bug (Hemiptera: Pentatomidae) in three vegetable crops: importance of dissections for evaluating the impact of native parasitoids on an exotic pest. Environ. Entomol. 45: 1536–1542.

Dieckhoff, C., K. M. Tatman, and K. A. Hoelmer. **2017**. Natural biological control of *Halyomorpha halys* by native egg parasitoids: a multi-year survey in northern Delaware. J. Pest Sci. 90: 1143–1158.

Dieckhoff, C., S. Wenz, M. Renninger, A. Reißig, H. Rauleder, C. P. W. Zebitz, J. Reetz, and O. Zimmermann. **2021**. Add Germany to the list—adventive population of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) emerges in Germany. Insects 12: 414.

English-Loeb, G. M., and B. D. Collier. **1987**. Nonmigratory movement of adult harlequin bugs *Murgantia histrionica* (Hemiptera: Pentatomidae) as affected by sex, age and host plant quality. Am. Midl. Nat. 118: 189–197.

Fatouros, N. E., M. Dicke, R. Mumm, T. Meiners, and M. Hilker. **2008**. Foraging behavior of egg parasitoids exploiting chemical information. Behav. Ecol. 19: 677-689.

Formella, A., S. J. Dorman, S. V Taylor, and T. P. Kuhar. **2020**. Effects of aggregation lure and tree species on *Halyomorpha halys* (Hemiptera: Pentatomidae) seasonal oviposition. J. Econ. Entomol. 113: 203–210.

Fraga D. F., J. Parker J, A. C. Busoli, G. C. Hamilton, A. L. Nielsen, and C. Rodriguez-Saona. 2017. Behavioral responses of predacious minute pirate bugs to tridecane, a volatile emitted by the brown marmorated stink bug. J Pest Sci. 90: 1107-1118.

Harris, C., S. Abubeker, M. Yu, T. Leskey, and A. Zhang. **2015**. Semiochemical production and laboratory behavior response of the brown marmorated stink bug, *Halyomorpha Halys*. PLoS One 10: e0140876.

Hedstrom, C., D. Lowenstein, H. Andrews, B. Bai, and N. Wiman. **2017**. Pentatomid host suitability and the discovery of introduced populations of *Trissolcus japonicus* in Oregon. J.

Pest Sci. 90: 1169–1179.

Herlihy, M. V., E. J. Talamas, and D. C. Weber. **2016**. Attack and success of native and exotic parasitoids on eggs of *Halyomorpha halys* in three Maryland habitats. PLoS One 11: e0150275.

Holthouse, M. C., L. R. Spears, and D. G. Alston. **2021**. Comparison of yellow and blue sticky cards for detection and monitoring parasitoid wasps of the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae). J. Insect Sci. 21: 1–10.

Kaser, J. M., C. Akotsen-Mensah, E. J. Talamas, and A. L. Nielsen. **2019**. First report of *Trissolcus japonicus* parasitizing *Halyomorpha halys* in North American agriculture. Florida Entomol. 101: 680.

Khrimian, A., A. Zhang, D. C. Weber, H. Y. Ho, J. R. Aldrich, K. E. Vermillion, M. A. Siegler, S. Shirali, F. Guzman, and T. C. Leskey. **2014**. Discovery of the aggregation pheromone of the brown marmorated stink bug (*Halyomorpha halys*) through the creation of stereoisomeric libraries of 1-bisabolen-3-ols. J. Nat. Prod. 77: 1708–1717.

Kiritani, K., N. Hokyo, K. Kimura, and F. Nakasuji. **1965**. Imaginal dispersal of the southern green stink bug, *Nezara viridula* L., in relation to feeding and oviposition. Japanese J. Appl. Entomol. Zool. 9: 291–297.

Kirkpatrick, D. M., A. L. Acebes-Doria, K. B. Rice, B. D. Short, C. G. Adams, L. J. Gut, T. C. Leskey, and L. Stelinski. **2019**. Estimating monitoring trap plume reach and trapping area for nymphal and adult *Halyomorpha halys* (Hemiptera: Pentatomidae) in crop and non-crop habitats. Environ. Entomol. 48: 1104–1112.

Kitamura, C., S. Wakamura, and S. Takahashi. **1984**. Identification and functions of ventral glands secretion of some Heteroptera. Appl. Entomol. Zool. 19: 33–41.

Laumann, R., M. Aquino, M. Moraes, M. Pareja, and M. Borges. 2009. Response of the egg parasitoids *Trissolcus basalis* and *Telenomus podisi* to compounds from defensive secretions of stink bugs. J. Chem. Ecol. 35: 8-19.

Leskey, T. C., A. Agnello, J. C. Bergh, G. P. Dively, G. C. Hamilton, P. Jentsch, A. Khrimian, G. Krawczyk, T. P. Kuhar, D. H. Lee, W. R. Morrison, D. F. Polk, C.

Rodriguez-Saona, P. W. Shearer, B. D. Short, P. M. Shrewsbury, J. F. Walgenbach, D. C. Weber, C. Welty, J. Whalen, N. Wiman, and F. Zaman. **2015**. Attraction of the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae) to traps baited with semiochemical stimuli across the United States. J. Econ. Entomol. 44: 746–756.

Leskey, T. C., and A. L. Nielsen. **2018**. Impact of the invasive brown marmorated stink bug in North America and Europe: history, biology, ecology, and management. Annu. Rev. Entomol. 63: 599–618.

Leskey, T. C., B. D. Short, B. R. Butler, and S. E. Wright. **2012**. Impact of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål), in Mid-Atlantic tree fruit orchards in the United States: case studies of commercial management. Psyche 2012: 1-14.

Leskey, T. C., H. Andrews, A. Bády, L. Benvenuto, I. Bernardinelli, B. Blaauw, P. P. Bortolotti, L. Bosco, E. Di Bella, G. Hamilton, T. Kuhar, D. Ludwick, L. Maistrello, G. Malossini, R. Nannini, L. J. Nixon, E. Pasqualini, M. Preti, B. D. Short, L. Spears, L. Tavella, G. Vétek, and N. Wiman. 2021. Refining pheromone lures for the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae) through collaborative trials in the United States and Europe. J. Econ. Entomol. 114: 1666–1673.

Malek, R., J. M. Kaser, G. Anfora, M. Ciolli, A. Khrimian, D. C. Weber, and K. A. Hoelmer. **2021**. *Trissolcus japonicus* foraging behavior: implications for host preference and classical biological control. Biol. Control 104700.

Martínez, G., R. Soler, and M. Dicke. 2013. Behavioral ecology of oviposition-site selection in herbivorous true bugs, pp. 175-207. In H. J. Brockmann, T. J. Roper, M. Naguib, J. C. Mitani, L. W. Simmons and L. Barrett (eds.), Advances in the Study of Behavior. Elsevier, Amsterdam, The Netherlands.

Milnes, J. M., N. G. Wiman, E. J. Talamas, J. F. Brunner, K. A. Hoelmer, M. L. Buffington, and E. H. Beers. **2016**. Discovery of an exotic egg parasitoid of the brown marmorated stink bug, *Halyomorpha halys* (Stål) in the Pacific Northwest. Proc Entomol Soc Wash. 118: 466–470.

Morrison, W. R., M. Allen, and T. C. Leskey. **2018**. Behavioural response of the invasive

Halyomorpha halys (Hemiptera: Pentatomidae) to host plant stimuli augmented with semiochemicals in the field. Agric. For. Entomol. 20: 62–72.

Morrison, W. R., C. R. Mathews, and T. C. Leskey. **2016**. Frequency, efficiency, and physical characteristics of predation by generalist predators of brown marmorated stink bug (Hemiptera: Pentatomidae) eggs. Biol. Control 97: 120–130.

Morrison, W. R., C. G. Park, B. Y. Seo, Y. L. Park, H. G. Kim, K. B. Rice, D. H. Lee, and T. C. Leskey. **2017**. Attraction of the invasive *Halyomorpha halys* in its native Asian range to traps baited with semiochemical stimuli. J. Pest Sci. 90: 1205–1217.

Nielsen, A. L., S. Chen, and S. J. Fleischer. **2016**. Coupling developmental physiology, photoperiod, and temperature to model phenology and dynamics of an invasive Heteropteran, *Halyomorpha halys*. Front. Physiol. 7: 165.

Ogburn, E. C., R. Bessin, C. Dieckhoff, R. Dobson, M. Grieshop, K. A. Hoelmer, C. Mathews, J. Moore, A. L. Nielsen, K. Poley, J. M. Pote, M. Rogers, C. Welty, and J. F. Walgenbach. **2016**. Natural enemy impact on eggs of the invasive brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), in organic agroecosystems: a regional assessment. Biol. Control 101: 39–51.

Peri, E., G. Salerno, T. Slimani, F. Frati, E. Conti, S. Colazza, and A. Cusumano. **2016**. The response of an egg parasitoid to substrate-borne semiochemicals is affected by previous experience. Sci. Rep. 6: 1–8.

Peterson, H. M., E. Talamas, and G. Krawczyk. **2021**. Survey for adventive populations of the samurai wasp, *Trissolcus japonicus* (Hymenoptera: Scelionidae) in Pennsylvania at commercial fruit orchards and the surrounding forest. Insects 12: 258.

Peverieri, G. S., E. Talamas, M. Claude Bon, L. Marianelli, I. Bernardinelli, G. Malossini, L. Benvenuto, P. F. Roversi, and K. Hoelmer. **2018**. Two Asian egg parasitoids of *Halyomorpha halys* (Stål) (Hemiptera, Pentatomidae) emerge in northern Italy: *Trissolcus mitsukurii* (Ashmead) and *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae). J. Hymenopt. Res. 37–53.

Qiu, L.-F., Z.-Q. Yang, and W. Q. Tao. **2007**. Biology and population dynamics of *Trissolcus* *halyomorphae*. Sci. Silvae Sin. 43: 62–65.

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

Quinn, N. F., E. J. Talamas, A. L. Acebes-Doria, T. C. Leskey, and J. C. Bergh. **2019b**. Vertical sampling in tree canopies for *Halyomorpha halys* (Hemiptera: Pentatomidae) life stages and its egg parasitoid, *Trissolcus japonicus* (Hymenoptera: Scelionidae). Environ. Entomol. 48: 173–180.

Quinn, N. F., E. J. Talamas, T. C. Leskey, and J. C. Bergh. **2021**. Seasonal captures of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) and the effects of habitat type and tree species on detection frequency. Insects 12: 1–12.

Rostás, M., D. Ruf, V. Zabka, and U. Hildebrandt. **2008**. Plant surface wax affects parasitoid’s response to host footprints. Naturwissenschaften 95: 997–1002.

Salerno, G., E. Conti, E. Peri, S. Colazza, and F. Bin. **2006**. Kairomone involvement in the host specificity of the egg parasitoid *Trissolcus basalis* (Hymenoptera: Scelionidae). Eur. J. Entomol. 103: 311–318.

Stahl, J., F. Tortorici, M. Pontini, M. C. Bon, K. Hoelmer, C. Marazzi, L. Tavella, and T. Haye. **2019**. First discovery of adventive populations of *Trissolcus japonicus* in Europe. J. Pest Sci. 92: 371–379.

Talamas, E. J., M. V. Herlihy, C. Dieckhoff, K. A. Hoelmer, M. L. Buffington, M. C. Bon, and D. C. Weber. **2015a**. *Trissolcus japonicus* (Ashmead) (Hymenoptera, Scelionidae) emerges in North America. J. Hymenopt. Res. 43: 119–128.

Talamas, E. J., N. F. Johnson, and M. Buffington. 2015b. Key to Nearctic species of *Trissolcus* Ashmead (Hymenoptera, Scelionidae), natural enemies of native and invasive stink bugs (Hemiptera, Pentatomidae). J. Hymenopt. Res. 43: 45-110.

Tognon, R., J. R. Aldrich, M. L. Buffington, E. J. Talamas, J. Sant’Ana, and F. G. Zalom. **2017**. *Halyomorpha halys* (Heteroptera: Pentatomidae) egg surface chemicals inhibit North American *Telenomus* and *Trissolcus* (Hymenoptera: Scelionidae) parasitism. Biol. Control

114: 39–44.

Weber, D. C., T. C. Leskey, G. C. Walsh, and A. Khrimian. **2014**. Synergy of aggregation pheromone with methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate in attraction of *Halyomorpha halys* (Hemiptera: Pentatomidae). J. Econ. Entomol. 107: 1061–1068.

Yang, S.-Y., Y.-Z. Zhong, J.-P. Zhang, X.-P. Wang, and F. Zhang. **2016**. A comparative scanning electron microscopy study on antennal sensilla of *Trissolcus japonicus* and *Trissolcus plautiae*, egg parasitoids of stink bugs (Pentatomidae). Ann. Entomol. Soc. Am. 109: 112–120.

Zhang, J., F. Zhang, T. Gariepy, P. Mason, D. Gillespie, E. Talamas, and T. Haye. **2017**. Seasonal parasitism and host specificity of *Trissolcus japonicus* in northern China. J. Pest Sci. 90: 1127–1141.

Zhong, Y. Z., J. P. Zhang, L. L. Ren, R. Tang, H. X. Zhan, G. H. Chen, and F. Zhang. **2017**. Behavioral responses of the egg parasitoid *Trissolcus japonicus* to volatiles from adults of its stink bug host, *Halyomorpha halys*. J. Pest Sci. 90: 1097–1105.

4.7 Figures and Tables

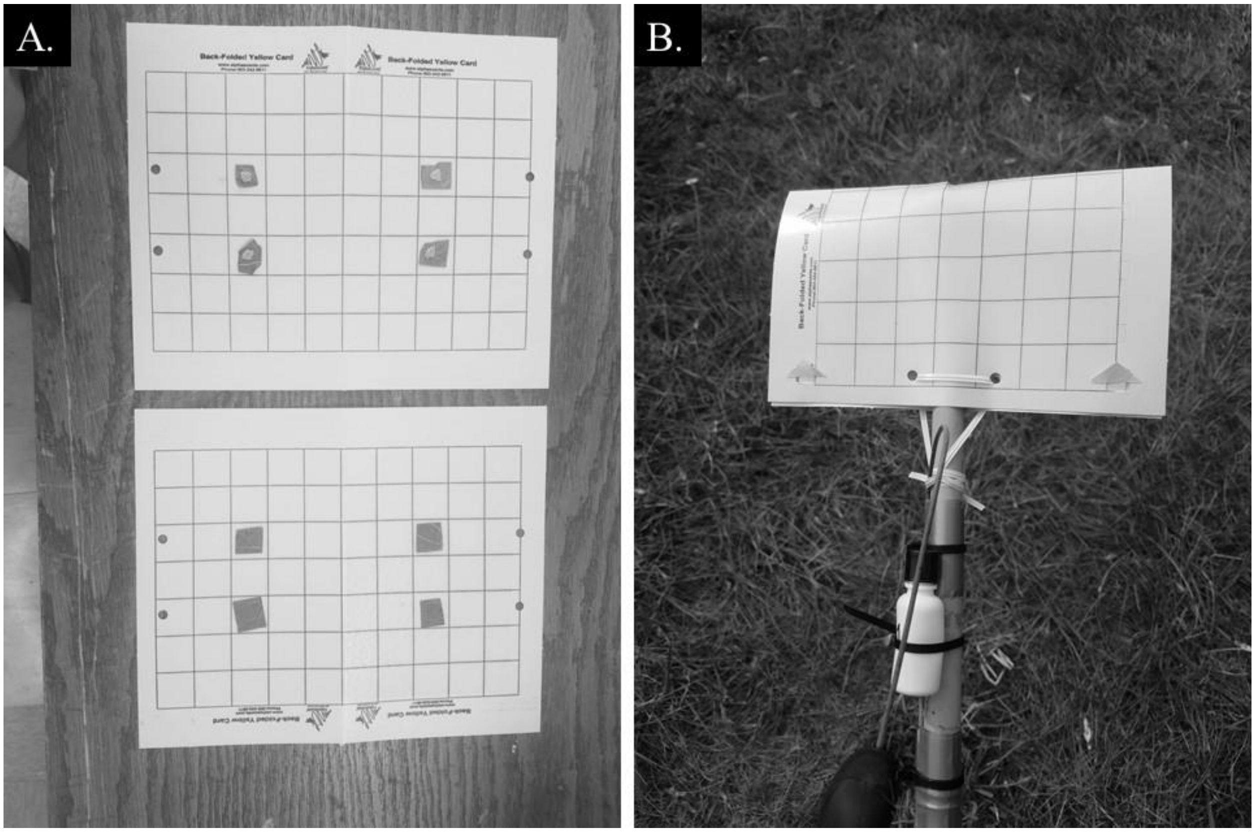

Fig. 4.1. Double-sided yellow sticky cards augmented with, A) *H. halys* egg masses (upper: treatment) and bean leaf pieces contaminated by *H. halys* (lower: control) and, B) synthetic tridecane in a polyethylene vial.

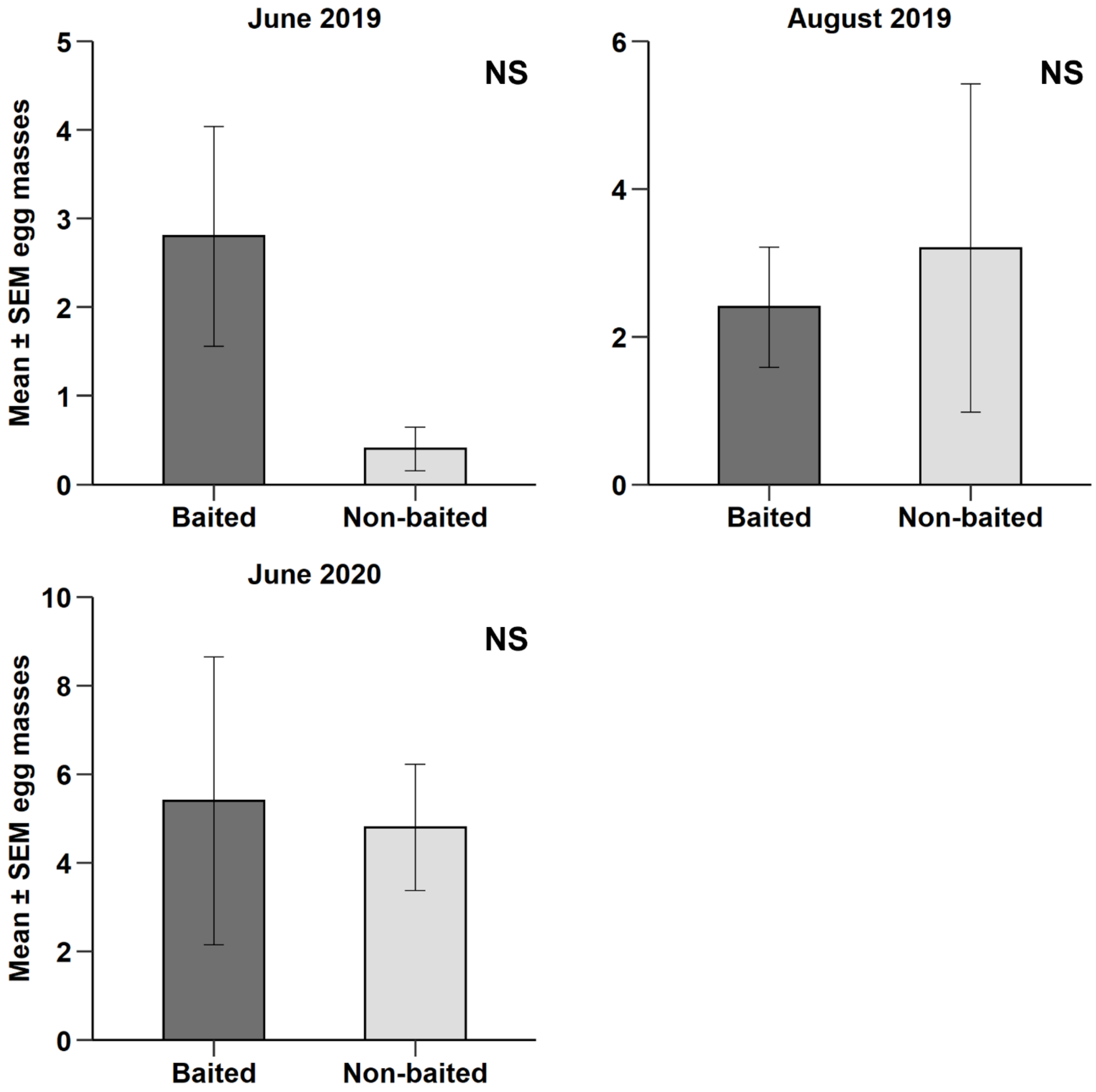

Fig. 4.2. *Halyomorpha halys* egg masses collected from pairs of felled pheromone-baited and non-baited *Ailanthus altissima* in June and August, 2019 (n = 5 tree pairs per sampling period) and June, 2020 (n = 5 tree pairs) in Virginia.

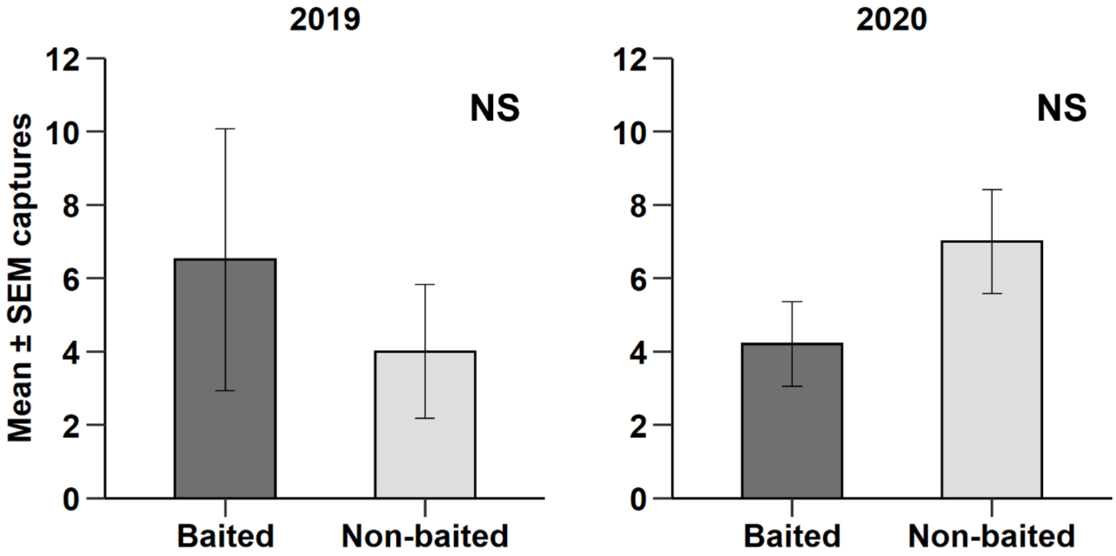

Fig. 4.3. *Trissolcus japonicus* captures in yellow sticky cards deployed in the mid-canopy of pairs of pheromone-baited and non-baited *Ailanthus altissima* from 21 June to 12 July and 24 July to 14 August, 2019 (n = 6 tree pairs) and from 1 July to 22 July and 29 July to 19 August, 2020 (n = 5 tree pairs).

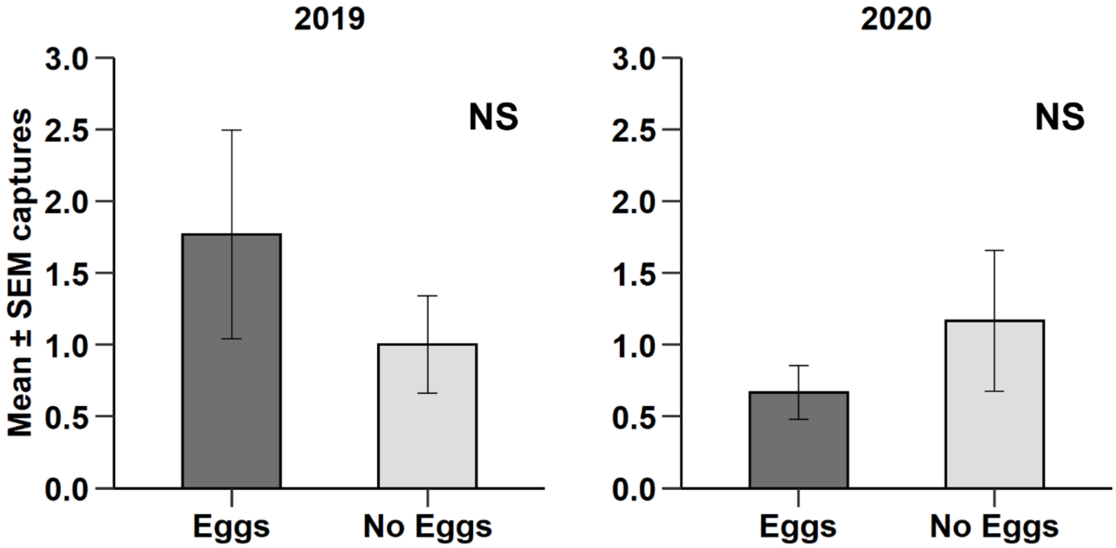

Fig. 4.4 *Trissolcus japonicus* captures in pairs of yellow sticky cards baited with fresh *H. halys* egg masses and non-baited yellow sticky cards deployed for 72-h in the mid-canopy of individual *Ailanthus altissima* from 9 July to 14 August, 2019 (n = 12 pairs) and from 12 June to 21 August, 2020 (n = 13 pairs).

Table 4.1. Percentages of *H. halys* egg masses collected from pairs of felled *Ailanthus altissima* with previously hatched nymphs, previously hatched parasitoids, predation, or unhatched that yielded nymphs, parasitoids, or remained unhatched. One tree per pair was baited in mid-canopy with a commercial *H. halys* aggregation pheromone lure.

Measurement	**June 2019** **n = 16**	**August 2019** **n = 28**	**June 2020** **n = 51**
Hatched[1] (%)	25.0	67.9	41.2
Predated (%)	6.3	10.7	0
Unhatched-nymphs (%)	56.3	3.6	33.3
Unhatched-parasitoids (%)	12.5	14.2	23.5
Unhatched-no emergence (%)	0.0	3.6	2.0

[1] All previously hatched egg masses indicated the emergence of *H. halys* nymphs (i.e., presence of egg bursters). No egg masses were collected that indicated the previous emergence of parasitoids.

Table 4.2. Number of adult parasitoids that emerged from *H. halys* egg masses collected from the foliage of pairs of felled *Ailanthus altissima* in Frederick County, VA. One tree per pair was baited in mid-canopy with a commercial *H. halys* aggregation pheromone lure.

Date	**Treatment**	***Trissolcus***			***Anastatus* (Eupelmidae)**
		japonicus	***thyantae***	***euschisti***	***spp.***
June 2019	Baited	0	13	0	2
	Non-baited	0	0	0	0
	% of total	0.0	86.7	0.0	13.3
Aug 2019	Baited	49	0	0	2
	Non-baited	13	0	0	0
	% of total	96.9	0.0	0.0	3.1
June 2020	Baited	50	0	0	0
	Non-baited	52	0	18	61
	% of total	56.4	0.0	9.9	33.7

Table 4.3. Number of Scelionid parasitoids captured in yellow sticky cards deployed in pairs of *H. halys* pheromone-baited and non-baited *Ailanthus altissima* in Frederick County, VA for two, three-week intervals between June and August, 2019 and 2020. Percentages based on species for which more than one specimen was captured across both years.

Year and treatment	*Trissolcus*				*Telenomus*	
	japonicus	*euschisti*	*thyantae*	*brochymenae*	*podisi*	spp.
2019						
Baited	39	9	3	4	8	2
Non-baited	24	10	6	3	9	2
% of total	***52.9***	***16.0***	***7.6***	***5.9***	***14.3***	***3.3***
2020						
Baited	21	24	1	6	30	3
Non-baited	35	4	0	2	19	1
% of total	***38.3***	***19.2***	***0.7***	***5.5***	***33.6***	***2.7***

Table 4.4 Scelionid *H. halys* parasitoids caught in egg-baited and non-baited YSC deployed in the mid-canopy of *A. altissima* for 72-hr intervals in stands *of Ailanthus altissima* in Frederick County, VA from 9 July to 14 August, 2019 and 12 June to 21 August, 2020.

Treatment	*Trissolcus*				*Telenomus*	
	japonicus	*euschisti*	*thyantae*	*brochymenae*	*podisi*	spp.
2019						
Eggs	23	2	3	1	1	0
No Eggs	13	2	0	0	1	0
2020						
Eggs	8	0	0	1	33	5
No Eggs	14	2	1	0	32	3

Chapter 5

Summary, Conclusions, and Suggestions for Future Research

5.1 Results summary and conclusion

Halyomorpha halys remains an important invasive pest of concern throughout much of the northern hemisphere. *Trissolcus japonicus,* an important natural enemy of *H. halys* in Asia, offers the possibility of contributing to the long-term, sustainable management of *H. halys* in invaded regions. The discovery of adventive *T. japonicus* populations in the United States and elsewhere has afforded opportunities to investigate its ecology and behavior in the field. Fortuitously, and as the studies within this thesis reveal, *T. japonicus* continues to be well established in Frederick County, Virginia. This created and strengthened opportunities to address questions about optimal surveillance methods for it, founded in aspects of its foraging ecology.

Earlier work concluded that the distribution of *H. halys* adults, nymphs, and egg masses were vertically stratified within the canopy of wild hosts (Quinn et al. 2019a). Additionally, *T. japonicus* emerged most frequently from egg masses collected from the mid-canopy, where *H. halys* egg masses were most prevalent, and never from egg masses found in the lower canopy. From this, Quinn et al. (2019b) developed a sampling technique using yellow sticky cards (YSC) attached to a bamboo pole and deployed in the mid-canopy of wild hosts, assuming this would maximize the likelihood of *T. japonicus* captures. Subsequent monitoring studies in Frederick County, VA have used this method, yet, there have been ongoing questions about the need for the extra time and effort required to deploy YSC in the upper portions of the tree canopy, especially given that sampling using YSC in the lower canopy also has yielded substantial detections of *T. japonicus* in other studies in the US (Holthouse et al. 2021, Peterson et al. 2021).

To resolve questions about the effect of trap location in the tree canopy on *T. japonicus* detections (Chapter 2), pairs of YSC were deployed in the mid- and lower canopy of individual mature, female *A. altissima*. In 2020 and 2021, no significant differences in weekly captures of *T. japonicus* were found between traps deployed in the mid- and lower canopy. While more time and labor efficient than sentinel eggs (Quinn et al. 2019b), deploying YSC in the mid-canopy also creates costs of time and effort, which are mitigated considerably by using YSC in the lower canopy. My finding that YSC in either canopy location were equal in terms of their effectiveness and sensitivity for sampling *T. japonicus*, improves the flexibility, efficiency, and convenience of their use for surveillance of adventive *T. japonicus* populations.

Given that adventive *T. japonicus* is expected to impact *H. halys* populations in North America to at least some degree, and that it is a prime candidate for a potential classical biocontrol program, there is a need to better understand the spatiotemporal relationship between adventive *T. japonicus* and *H. halys*. In Chapter 3, adventive *T. japonicus* and *H. halys* were sampled simultaneously at individual sites in 2020 and 2021 to examine correlations between the seasonal phenology and relative abundance of each. In both years, a significant positive correlation between captures of *T. japonicus* and total *H. halys* (adults and nymphs) was found. In 2021, with an increased number of sampling sites, significant correlations also were observed between captures of *T. japonicus* and *H. halys* adults, and between *T. japonicus* and *H. halys* second instar nymphs. Importantly, these studies were the first to document a significant relationship between the relative abundance of adventive *T. japonicus* and *H. halys* in North America. Seasonal trends in captures of *T. japonicus* (e.g. onset, peaks, and cessation) aligned with those recorded previously in this area by Quinn et al. (2021), lending further strength to these findings. Peak captures were recorded in July and August, in addition to a smaller initial peak in late May early June that aligned with oviposition by overwintered *H. halys* (Nielsen and Hamilton 2009). Sampling ended earlier in 2020 than 2021, due to the resumption of JED's academic year and to a lack of assistance owing to the COVID pandemic, but it is reasonable to assume similar late season declines in *T. japonicus* abundance as were observed in 2021 and previously (Quinn et al. 2021). The spatial and temporal overlap between *H. halys* and adventive *T. japonicus* bodes well for the persistence of adventive populations (Godfray et al. 1994), and such data may be used to provide context when interpreting results from future augmentative releases of *T. japonicus*, relative to local *H. halys* populations.

Developing effective surveillance tools for detecting and tracking *T. japonicus* will be critical as the range of adventive populations in North America and Europe continues to expand. Quinn et al. (2019b) described the limitations of using sentinel egg masses to track the spread and distribution of *T. japonicus*, and demonstrated that yellow sticky cards can serve as an effective alternative sampling tool for this purpose. Optimizing the likelihood of detecting *T. japonicus* populations is an important consideration, and may be accomplished through a deeper understanding of the biotic factors that influence its foraging for *H. halys* egg masses. Quinn et al. (2021) addressed the effects of habitat type and *H. halys* host tree species on its detection frequency, but other aspects of its foraging ecology remain poorly understood.

In Chapter 4, following the results of Quinn et al. (2019a), which showed higher densities of *H. halys* and its egg masses in the upper portions of the tree canopy, the effect of baiting the mid-canopy of *A. altissima* with the *H. halys* aggregation pheromone was examined, under the hypothesis that aggregated adults in the canopy would deposit more egg masses and/or leave more tarsal "footprints", to which *T. japonicus* responds (Boyle et al. 2020). Felling pheromone-baited and non-baited trees and inspection of their foliage for *H. halys* egg masses yielded no significant differences in egg mass abundance between baited and non-baited trees, aligning with results from previous ground-level visual surveys in southwestern Virginia (Formella et al. 2020). Moreover, a companion study that compared *T. japonicus* captures in pheromone-baited and non-baited *A. altissima* resulted in no significant differences. Given that *H. halys* tarsal footprints elicited behavior indicative of concentrated, localized searching by female *T. japonicus* (Boyle et al. 2020, Malek et al. 2021), we originally speculated that increased concentrations of *H. halys* footprints in pheromone-baited trees may promote *T. japonicus* foraging and thus result in more encounters with YSC. The lack of effect of baiting trees with pheromone on *T. japonicus* captures may be due to tarsal footprints serving as short-range foraging cues, and not as longer-range attractants. The combined results from these experiments indicate that baiting trees with the *H. halys* aggregation pheromone will not enhance the likelihood of detecting *T. japonicus.*

While YSCs are certainly effective for monitoring *T. japonicus*, it is possible that their sensitivity might be further enhanced by incorporating olfactory cues from its host with the yellow, visual stimulus. Under laboratory conditions, *T. japonicus* has been shown to respond to *n*-tridecane, a compound in adult *H. halys* secretions (Zhong et al. 2017, Malek et al. 2021). Similarly, in host-range tests *T. japonicus* has demonstrated a strong preference for *H. halys* egg masses (Dieckhoff et al. 2017, Milnes and Beers 2019a), and can exploit host cues that some native parasitoids fail to recognize (Tognon et al. 2017). Thus, captures of *T. japonicus* in YSC augmented with these host-associated stimuli were compared with YSC that were not augmented. Neither study yielded significant differences in *T. japonicus* captures between baited and non-baited YSC. While laboratory bioassays these stimuli have elicited positive behavioral responses by female *T. japonicus* (Zhong et al. 2017, Boyle et al. 2020, Malek et al. 2021), environmental or chemical constraints on their bioactivity and volatility may limit the range over which foraging female *T. japonicus* are attracted by them in the field. Based on my results and experimental approach, these stimuli do not appear to show utility for enhancing the sensitivity of YSC for *T. japonicus* surveillance.

5.2 Suggestions for future research

In the studies reported here, *T. japonicus* was the most common Scelionidae captured on YSC, but as noted by Abram et al. (2020), its impacts on the suppression of *H. halys* populations have yet to be quantified. However, even though *H. halys* still is considered an agricultural pest in its native range (Lee et al. 2013), it has not reached the same population densities in Asia as it has in parts of North America and Europe. This may be a function of the combined effects of a guild of co-evolved natural enemies in Asia that have been absent in the invaded ranges, at least until adventive *T. japonicus* was discovered. Whether *T. japonicus* alone can provide adequate regulation to suppress *H. halys* populations below some economic threshold remains an open question, but such questions may be addressed via a thorough understanding of the host-parasitoid population dynamics of *H. halys* and *T. japonicus*, both in their native and introduced ranges and over longer periods of time. A robust study comparing parasitism of *H. halys* egg masses sampled from trees felled *A. altissima* in areas where *T. japonicus* has and has not established may address some knowledge gaps concerning the impact of adventive *T. japonicus* on *H. halys* populations. Because *A. altissima* is an invasive species, and considered noxious, we have found that landowners are eager to have them removed. Given the abundance of *T. japonicus* documented in this thesis, Frederick County, Virginia may serve as an ideal location for comparison with nearby counties where *T. japonicus* has not yet shown evidence of a similar level of establishment (Bergh unpublished data). Recently, Chen et al. (2021) developed a molecular-based approach for identifying *T. japonicus*-parasitized *H. halys* egg masses using a species-specific primer and PCR amplification. This important new finding could be very helpful in the interpretation of egg mass data collected from felled trees. Moreover, predation of *H. halys* eggs is quite common, and collection of egg masses from felled trees, or use of sentinel eggs, can provide important comparisons between levels of predation and parasitism.

It is possible that *T. japonicus* alone cannot adequately regulate *H. halys* populations (Abram et al. 2020), and more emphasis should be placed on investigating the combined impacts of the broader natural enemy community on the regulation of invasive *H. halys*. Stage-structured matrix modeling indicates that egg parasitism combined with mortality of reproductive adults, either through adult parasitism or alternative IPM-based tactic, would be more effective at suppressing a stink bug such as *H. halys* than mortality from egg parasitism alone (Abram et al. 2020). Historically, biological control programs have yielded more success with the release of two or more agents (Stiling and Cornelissen 2005), and the role of native parasitoids should not be overlooked, given that egg mortality also results from incomplete development of native parasitoids (Abram et al. 2016). Thus, the relationship between *T. japonicus* and native parasitoids with respect to abundance and interspecific interactions is also a space for further investigation. In North America, the few parasitoids of *H. halys* adults have not received much attention. Moreover, the recent identification of a tachinid parasitoid, *Pentatomophaga latifascia* (Villeneuve), in China (Chen et al. 2020) indicates opportunities for further exploration of the role of parasitoids of other *H. halys* life stages.

While these studies did not show enhanced captures when host-associated stimuli were used with YSC, they did resolve the effect of trap location in the canopy on *T. japonicus* detections. One drawback of YSC that has been noted is excessive captures of non-target insects that sometimes occur. This is not unexpected, given that yellow is broadly attractive to many taxa (Ferreira Santos de Aquino et al. 2012). Holthouse et al. (2021) compared yellow and blue sticky cards and found that while *T. japonicus* captures were greater in yellow cards, captures in each were significantly correlated. Moreover, blue cards contained fewer non-target insects, thus reducing the trap processing time. However, as observed while conducting the present studies, the number of non-target arthropod captures per trap varied greatly among sites and time of the season. Additionally, the greater sensitivity observed with the use of yellow cards may provide benefits that outweigh the drawbacks of non-target captures for certain surveillance objectives. Regardless, further exploration of visual stimuli for sampling *T. japonicus* may further refine the tools for its surveillance.

The role of herbivore-induced plants volatiles in the foraging behavior of *T. japonicus* is another area on which further work is needed. Plant volatiles often mediate long-distance orientation of female parasitoids to suitable host habitats (Conti and Colazza 2012). In laboratory assays, *T. japonicus* was attracted to volatiles from tomato plants attacked by *H. halys* (Bertoldi et al. 2019). The response of adventive *T. japonicus* to such herbivore-induced volatiles in the field has yet to be explored, but integrating these with YSC could potentially further enhance the sensitivity of YSC by utilizing a longer distance attractant.

5.3 References

Abram, P. K., J. Brodeur, V. Burte, and G. Boivin. **2016**. Parasitoid-induced host egg abortion: an underappreciated component of biological control services provided by egg parasitoids. Biol. Control 98: 52–60.

Abram, P. K., N. J. Mills, and E. H. Beers. **2020**. Review: classical biological control of invasive stink bugs with egg parasitoids – what does success look like? Pest Manag. Sci. 76: 1980–1992.

Bertoldi, V., G. Rondoni, J. Brodeur, and E. Conti. **2019**. An egg parasitoid efficiently exploits cues from a coevolved host but not those from a novel host. Front. Physiol. 10: 746.

Boyle, S. M., D. C. Weber, J. Hough-Goldstein, and K. A. Hoelmer. **2020**. Host kairomones influence searching behavior of *Trissolcus japonicus* (Hymenoptera: Scelionidae), a parasitoid of *Halyomorpha halys* (Heteroptera: Pentatomidae). Environ. Entomol. 49: 15–20.

Chen, J., W. Li, Q. Mi, F. Zhang, S. Shi, and J. Zhang. **2020**. A newly reported parasitoid, *Pentatomophaga latifascia* (Diptera: Tachinidae), of adult *Halyomorpha halys* in Beijing, China. Insects 11: 1–8.

Chen, M. N., R. D. Santander, E. J. Talamas, P. J. Jentsch, M.-C. Bon, and S. G. Aćimović. **2021**. Molecular identification of *Trissolcus japonicus*, parasitoid of the brown marmorated stink bug, by species-specific PCR. Insects 12: 467.

Conti, E., and S. Colazza. **2012**. Chemical ecology of egg parasitoids associated with true bugs. Psyche 2012: 1-12.

Dieckhoff, C., K. M. Tatman, and K. A. Hoelmer. **2017**. Natural biological control of *Halyomorpha halys* by native egg parasitoids: a multi-year survey in northern Delaware. J. Pest Sci. 90: 1143–1158.

Ferreira Santos de Aquino, M., A. M. Dias, M. Borges, M. C. B. Moraes, and R. A. Laumann. **2012**. Influence of visual cues on host-searching and learning behaviour of the egg parasitoids *Telenomus podisi* and *Trissolcus basalis*. Entomol. Exp. Appl. 145: 162–174.

Formella, A., S. J. Dorman, S. V Taylor, and T. P. Kuhar. **2020**. Effects of aggregation lure and tree species on *Halyomorpha halys* (Hemiptera: Pentatomidae) seasonal oviposition. J. Econ. Entomol. 113: 203–210.

Godfray, H. C. J., M. P. Hassell, and R. D. Holt. **1994**. The population dynamic consequences of phenological asynchrony between parasitoids and their hosts. J. Anim. Ecol. 63: 1.

Holthouse, M. C., L. R. Spears, and D. G. Alston. **2021**. Comparison of yellow and blue sticky cards for detection and monitoring parasitoid wasps of the invasive *Halyomorpha halys* (Hemiptera: Pentatomidae). J. Insect Sci. 21: 1–1.

Lee, D.-H., B. D. Short, S. V. Joseph, J. C. Bergh, and T. C. Leskey. **2013**. Review of the biology, ecology, and management of *Halyomorpha halys* (Hemiptera: Pentatomidae) in China, Japan, and the Republic of Korea. Environ. Entomol. 42: 627–641.

Malek, R., J. M. Kaser, G. Anfora, M. Ciolli, A. Khrimian, D. C. Weber, and K. A. Hoelmer. **2021**. *Trissolcus japonicus* foraging behavior: implications for host preference and classical biological control. Biol. Control 161: 104700.

Milnes, J. M., and E. H. Beers. **2019**. *Trissolcus japonicus* (Hymenoptera: Scelionidae) causes low levels of parasitism in three North American pentatomids under field conditions. J. Insect Sci. 19: 15.

Nielsen, A. L., and G. C. Hamilton. **2009**. Life history of the invasive species *Halyomorpha halys* (Hemiptera: Pentatomidae) in Northeastern United States. Ann. Entomol. Soc. Am. 102: 608–616.

Peterson, H. M., E. Talamas, and G. Krawczyk. **2021**. Survey for adventive populations of the samurai wasp, *Trissolcus japonicus* (Hymenoptera: Scelionidae) in Pennsylvania at commercial fruit orchards and the surrounding forest. Insects 12: 258.

Quinn, N. F., E. J. Talamas, A. L. Acebes-Doria, T. C. Leskey, and J. C. Bergh. **2019a**. Vertical sampling in tree canopies for *Halyomorpha halys* (Hemiptera: Pentatomidae) life stages and its egg parasitoid, *Trissolcus japonicus* (Hymenoptera: Scelionidae). Environ. Entomol. 48: 173–180.

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

Quinn, N. F., E. J. Talamas, T. C. Leskey, and J. C. Bergh. **2021**. Seasonal captures of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) and the effects of habitat type and tree species on detection frequency. Insects 12: 1–12.

Stiling, P., and T. Cornelissen. **2005**. What makes a successful biocontrol agent? A meta-analysis of biological control agent performance. Biol. Control 34: 236–246.

Tognon, R., J. R. Aldrich, M. L. Buffington, E. J. Talamas, J. Sant'Ana, and F. G. Zalom. **2017**. *Halyomorpha halys* (Heteroptera: Pentatomidae) egg surface chemicals inhibit North American *Telenomus* and *Trissolcus* (Hymenoptera: Scelionidae) parasitism. Biol. Control 114: 39–44.

Zhong, Y. Z., J. P. Zhang, L. L. Ren, R. Tang, H. X. Zhan, G. H. Chen, and F. Zhang. **2017**. Behavioral responses of the egg parasitoid *Trissolcus japonicus* to volatiles from adults of its stink bug host, *Halyomorpha halys*. J. Pest Sci. 90: 1097–1105.