BIOLOGICAL CONTROL OF THE INVASIVE BROWN MARMORATED STINK BUG (*HALYOMORPHA HALYS*) (HEMIPTERA: PENTATOMIDAE) IN MICHIGAN BY INTRODUCED AND NATIVE PARASTIOIDS IN A CHANGING CLIMATE

By

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ABSTRACT

BIOLOGICAL CONTROL OF THE INVASIVE BROWN MARMORATED STINK BUG (*HALYOMORPHA HALYS*) (HEMIPTERA: PENTATOMIDAE) IN MICHIGAN BY INTRODUCED AND NATIVE PARASTIOIDS IN A CHANGING CLIMATE

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This thesis focuses on evaluating the potential of native natural enemies and an exotic parasitoid to provide biological control of an invasive pest, the brown marmorated stink bug (*Halyomorpha halys* Stål) in Michigan and explores the consequences climate change might have both on the pest and its natural enemies.

The first chapter provides background on the invasion of *H. halys* and reviews research on the native parasitoid community and their potential to provide biological control for this pest.

The second chapter focuses on augmentative releases of the biological control agent, *Trissolcus japonicus* (Ashmead), which is an exotic parasitoid, discovered in Michigan in 2018. The importance of release size and of release frequency were tested during two years of augmentative releases and the native parasitoid community was surveyed. Overwintering success and reproduction of *Tr. japonicus* were confirmed but population growth was found slow. Parasitism by native parasitoids was below 5%, which is too low to provide control of *H. halys*.

In the third chapter the effects of heat waves were evaluated on *H. halys* and *Tr. japonicus*. Eggs of *H. halys* were found to be more sensitive to heat waves than larval stages of *Tr. japonicus*. While heat waves did not reduce parasitism rates, *Tr. japonicus* adult mortality increased and their development was delayed with intensifying heat waves. These results suggest that *Tr. japonicus* could maintain its biocontrol potential even in the face of climate change.

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CHAPTER 1: The status of *Halyomorpha halys* invasion and its biological control in the U.S.

Background

Brown marmorated stink bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) is a severe agricultural pest in Eastern and Mid-Atlantic states and is an emerging pest in Michigan, threatening fruit and vegetable production. Native to East Asia, *H. halys* was first detected in the US in the late 1990s and since has established in 44 states and four Canadian provinces (Hoebeke and Carter, 2003; stopbmsb.org). It was discovered in Michigan in 2010 and has since spread to all but four counties (Figure. 1.1) (Wilson et al. 2018; www.misin.msu.edu). *H. halys* is extremely polyphagous, being able to feed on over 200 different plant species including tree fruits, vegetables, field crops, ornamentals, and native vegetation (Leskey et al., 2012a). Economic losses attributed to *H. halys* feeding were first recorded in 2006 in Mid-Atlantic orchards and by 2009, *H. halys* damage on apples was estimated to cost $37 million (Leskey et al. 2012b). Given that Michigan is the third largest producer of apples in the USA, *H. halys* is poised to become a major threat to the Michigan apple industry (MI Agricultural Statistics 2017-2018). In addition to becoming a crop pest, *H. halys* is also a nuisance pest due to its habit of overwintering in human-made structures, and in some instances, people can find tens of thousands of stink bugs in their homes during the fall (Leskey et al., 2012).

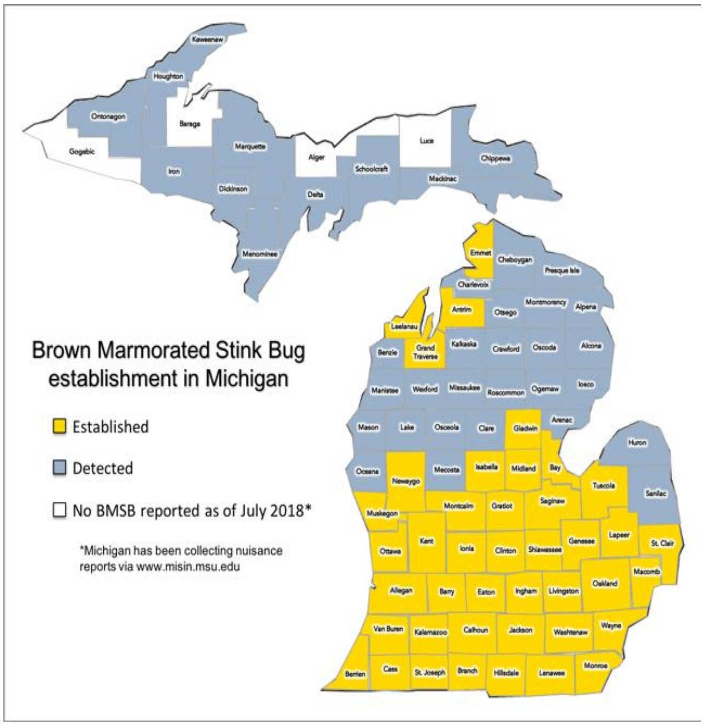

Figure.1.1. The distribution on *H. halys* in Michigan. Misin.msu.edu accessed Oct 2020.

Management of *H. halys* currently relies on the use of broad-spectrum pesticides applied throughout the growing season (Leskey et al. 2012b). The increase in the amount and frequency of pesticide applications for *H. halys* management has interfered with integrated pest management (IPM) programs by indiscriminately killing natural enemies and resulted in outbreaks of secondary pests that were previously successfully kept at bay by biological control (Leskey et al. 2012a; Rice et al. 2014). Despite continued chemical control within cropping areas large populations persist in natural areas and other plant hosts where they can recolonize cropping system. Thus, alternative control methods that include biological control by either native or introduced natural enemies are likely to be essential for managing this pest in the long term.

Surveys of natural enemies covering mostly Eastern and Central states in the U.S. found that at least eight native endoparasitoids from three families (Scelionidae, Eupelmidae, and

Encyrtidae) and four genera (*Telenomus*, *Trissolcus*, *Anastatus* and *Ooencyrtus*) already attack *H. halys* eggs in the field (reviewed in Abram et al. 2017). The ability of different parasitoid species to develop on this new host varies among crops and regions (Abram et al. 2017; Cornelius et al. 2016a; Cornelius et al. 2016b; Dieckhoff et al. 2017; Jones et al. 2017; Ogburn et al. 2016). To date, rates of native parasitoid emergence from viable *H. halys* eggs are generally low, below 5% in most cases, and vary among environments between 0 – 59% (Abram et al. 2017; Dieckhoff et al. 2017). A 9-year survey in Delaware found no indication that parasitism by native parasitoids increased over time (Dieckhoff et al. 2017). These data indicate that native parasitoids alone may not provide effective biological control of *H. halys* in the near future.

Classical biological control, the introduction of host-specific natural enemies from the native range of exotic species is a critical component of sound long-term management of many invasive species. The exotic egg parasitoid, *Trissolcus japonicus* Ashmead (Samurai wasp) (Hymenoptera: Scelionidae) originating from Eastern Asia, the native range of *H. halys*, has been under investigation as a candidate for a classical biological control program since 2007 (Talamas et al. 2015). Adult female wasps lay their eggs into *H. halys* eggs and their larvae consume the entire stink bug embryo. *Tr. japonicus* has shown great potential to be a successful biological control agent because it can parasitize 50-70% of *H. halys* eggs (Yang et al. 2009; Talamas et al. 2013). However, studies in quarantine revealed that *Tr. japonicus* can attack and develop on at least seven native stink bug species in Oregon (Hedstrom et al. 2017) and 15 native species in Michigan (Botch and Delfosse, 2018). Because *Tr. japonicus* attacks native stink bugs it has not been field released.

Even though field release is not permitted adventive (accidentally introduced) populations of *Tr. japonicus* were found first in MD in 2014 and, within a few years, in some

Mid-Atlantic and Western states (Talamas et al. 2015; Hedstrom et al. 2017; Herlihy et al. 2016; Milnes et al. 2016; stopbmsb.org). Molecular analyses confirmed that the adventive populations are genetically distinct from those in quarantine, indicating these were unrelated and independent introductions rather than accidental escapees from quarantine colonies (Milnes et al. 2016). Given its effectiveness on *H. halys* in Asia, the accidental introduction and spread of *Tr. japonicus* in the U.S., we need to further examine its potential as an effective agent of natural *H. halys* population suppression.

Despite the presence of *Tr. japonicus* in the field in several states, interstate redistribution of this parasitoid is not permitted. However, biological control programs can be initiated within states where *Tr. japonicus* has been detected using the captured individuals for rearing. The mass rearing and redistribution of *Tr. japonicus* is already underway in several Eastern and Western states to augment populations (BMSB SCRI Annual Report, 2018). *Tr. japonicus* arrived at neighboring Ohio in 2017 (stopbmsb.org) and was first found in Michigan in 2018 at a single location despite widespread sampling within the state (Jarrett et al. 2019). Given the pattern of detection across the U.S., *Tr. japonicus* appears to be expanding its populations westward from an initial introduction point on the east coast and was found in Michigan very early upon its arrival. Thus, its density was likely very low around 2018, and its state-wide distribution very limited. The second chapter of this thesis focuses on augmentative releases of *Tr. japonicus* using a laboratory rearing that had been initiated from live field captures in Michigan.

CHAPTER 2: Augmentative field releases of the exotic parasitoid *Trissolcus japonicus* and survey of native parasitoids attacking brown marmorated sting bug

Introduction

Classical biological control has a long history, with introductions of biological control agents dating back to the 1880s (Cock et al. 2016), but we still know relatively little about how to release biological control agents against invasive insects to ensure their establishment. Less than 33% of predator and parasitoid introductions against insect pests led to establishment worldwide (Cock et al. 2016), while in North America, an estimated 54% of released parasitoid species have established since 1985 (Driesche et al. 2020). Multiple mechanisms may influence establishment, including abiotic conditions, species-specific biological traits, stochastic processes, Allee effects, or the level of genetic variation within the released population. However, the most consistent predictor of establishment success across a wide range of species is found to be propagule pressure: the combined size and number of introductions (Blackburn et al. 2015; Lockwood et al. 2005; Simberloff 2009). Recent experimental laboratory studies using insects also confirm that higher release sizes increase establishment success (Hufbauer et al. 2013; Szűcs et al. 2014; Szűcs et al. 2017). This could be due to multiple factors including a reduction in Allee effects, demographic, and environmental stochasticity at larger release sizes (Boyce et al. 2006; Lande, 1993). Additionally, more individuals in a founding group usually harbor higher genetic diversity, which could reduce the probability of inbreeding, increasing chances of adaptation to novel environmental conditions and ensuring longer-term persistence of populations (Drake, 2006; Forsman, 2014; Rius and Darling, 2014).

For insect biological control, Hopper and Roush (1993) reviewed releases against species within the order Lepidoptera and found indirect evidence that release size was positively correlated with establishment success of natural enemies (Hopper and Roush, 1993). However, the three field studies, that we know of, that experimentally manipulated release size of parasitoid classical biological control agents did not find the same relationship (Borowiec et al. 2018; Fauvergue and Hopper 2009; Fauvergue et al. 2007). Establishment rates were similar for *Neodryinus typhlocybae* (Ashmead) (Hymenoptera: Dryinidae) against plant hoppers regardless of release sizes (1, 10 or 100 females) (Fauvergue et al. 2007). The release of 1,200 or 11,400 aphid mummies parasitized with *Aphelinus asychis* Walker (Hymenoptera: Aphelinidae) also yielded similar parasitoid establishment success (Fauvergue and Hopper, 2009). Neither release size nor release frequency explained establishment success of *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) against the chestnut gall wasp (Borowiec et al. 2018). In weed biological control, experimental field introductions have shown that larger releases of biological control agents are more likely to establish and persist (Grevstad, 1999a; Grevstad, 2006; Memmott et al. 2005; Memmott et al. 1998). However, there are examples of a single female founding populations (Grevstad, 1999a) and of releases made with 65,000 individuals failing (Grevstad et al. 2011).

Establishment success may be impacted not only by the number of individuals in a single release (propagule size) but also by the frequency of releases (propagule number). Models considering propagule number provide contrasting recommendations for biological control release strategies. Grevstad (1999b) recommends several smaller introductions to a variable environment and a single large release when Allee effects are present in a homogenous environment. In contrast, if the hosts are established only at a few sites, a few large releases were predicted to yield success (Shea and Possingham, 2000). For parasitoid releases, Hopper and Roush (1993) recommended releasing over 1000 individuals at a single site and time. If the number of individuals available for releases is limited, single larger releases were predicted to maximize establishment, but if environmental stochasticity is substantial, then several smaller releases spread over time may be the optimal approach (Hopper and Roush, 1993).

Given the scarcity of experimental field studies and the contrasting recommendations of models and laboratory experiments, there are no specific guiding principles on how to release parasitoid biological control agents. The lack of information on release strategies can be particularly problematic for biocontrol agents that are difficult or expensive to rear in large numbers, and haphazard releases can lead to the loss of significant time and financial investments in case they do not establish. New releases of classical biological control agents that are imported from the native range of a pest would be ideal to assess experimentally how best to release parasitoids. However, there has been a steep decline in exotic parasitoid approvals and releases in the past few years (Driesche et al. 2020), and thus few opportunities for such studies. Nevertheless, exotic parasitoids sometimes follow invasive pests and their redistribution could provide insights into the effectiveness of different release strategies for establishment.

Trissolcus japonicus (Ashmead) (Hymenoptera: Scelionidae) is an exotic egg parasitoid that was introduced accidentally, likely along with its host, the brown marmorated stink bug (*Halyomorpha halys* (Stål)) (Hemiptera: Pentatomidae) from eastern Asia. *Halyomorpha halys* was found in the US in 1996, while adventive populations of *Tr. japonicus* were first discovered in 2014 in Maryland and in subsequent years in multiple mid-Atlantic and Western states (Hedstrom et al. 2017; Herlihy et al. 2016; Hoebeke and Carter, 2003; Milnes et al. 2016; Northeastern IPM Center, 2020; Talamas et al. 2015). Since its introduction, *H. halys* has spread across 44 states and four Canadian provinces and has become a primary agricultural pest of multiple fruit and vegetable crops (Abram et al. 2019, Gariepy and Talamas, 2019; Leskey and Nielsen, 2018; Northeastern IPM Center, 2020). A suite of native parasitoids from three families (Scelionidae, Eupelmidae, and Encyrtidae) and four genera (*Telenomus*, *Trissolcus*, *Anastatus* and *Ooencyrtus*) were found to attack *H. halys* eggs in North America, but their rates of parasitism had been too low (<10%) to provide control (reviewed in Abram et al. 2017). *Trissolcus japonicus* has been the primary candidate for a classical biological program (Talamas et al. 2015), because it can parasitize 50–80% of *H. halys* eggs in its native range (Yang et al. 2009; Zhang et al. 2017). Host-specificity tests have shown that it can attack over 10 native stink bug species but strongly prefers *H. halys* both in the laboratory and in the field (Botch and Delfosse, 2018; Hedstrom et al. 2017; Milnes and Beers, 2019). Thus, once it fortuitously arrived, multiple states started conducting augmentative releases to increase its geographic distribution and population densities to accelerate biological control (Jentsch 2017; Milnes and Beers, 2019).

In Michigan, *Tr. japonicus* was first found in 2018 at a single location despite extensive sampling across the state (Jarrett et al. 2019). The rate of discovery within the U. S. suggests that it has spread from the east coast to the Midwest and that it was discovered in Michigan soon after its arrival from neighboring Ohio, where it had been found just a year before, in 2017. A colony of *Tr. japonicus* was built from this initial capture and augmentative releases were conducted in 2019 and 2020 across the state, testing the importance of release size in year one, and of release frequency in year two for establishment. Fresh or frozen *H. halys* sentinel eggs and yellow sticky cards placed at two different heights were used to monitor the presence of *Tr. japonicus* before and after releases and the diversity of native parasitoids attacking *H. halys* in Michigan. In addition, potential non-target effects of *Tr. japonicus* were evaluated on a native beneficial stink bug species, *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae).

The overall objective of the study was to increase the geographic distribution and population densities of *Tr. japonicus* across Michigan to accelerate biological control of *H. halys*. The hypotheses were that larger releases in 2019 and multiple releases in 2020 would establish at higher rates than smaller or single releases, respectively. In addition, the expectation was that there would not be a difference in parasitism between fresh and frozen *H. halys* sentinel eggs (Abram et al. 2017) and that eggs and traps placed higher in the canopy would capture more parasitoids than those closer to the ground (Quinn et al. 2019).

Materials and Methods

Insect rearing

The colony of *Tr. japonicus* was started from five individuals, two males and three females that emerged from a single *H. halys* sentinel egg mass placed at the Student Organic Farm at Michigan State University (MSU) campus (42.6749, -84.4897) in August 2018 (Jarrett et al. 2019). Wasps were reared by providing either fresh (< 72 h old) or frozen (at -80˚C) *H. halys* egg masses to groups of 5–15 mixed sex individuals for 3–7 days for oviposition in 10-dram vials. A drop of honey was placed on the lids to provision wasps. Vials were kept in an incubator at 20˚C and 70% humidity with a photoperiod of 16:8 L:D. Adult wasps emerged 14–21 days following oviposition.

Halyomorpha halys eggs used for rearing *Tr. japonicus* originated either from colonies maintained on MSU campus or from the New Jersey Department of Agriculture Phillip Alampi

Beneficial Insect Laboratory rearing facility. Adult *H. halys* was reared in mesh cages (30 x 30 x 60 cm) (Educational Science, League City, TX, U.S.) by keeping 40-60 mixed-sex adults in each cage at 25°C, 50–75% humidity and 16:8 L:D photoperiod in a climate controlled room. Nymphs were housed in 236 ml clear plastic containers (Ziploc® large rectangle). A six square cm hole was cut on the lids and covered with gauze to provide ventilation. All stages were provided water via dental wicks (Dynarex N/S #2 Medium) inserted into 60 ml cups (Gordon Food Service) filled with water. Their diet included organic green beans, snap peas, broccoli, carrots, apples, and mixed nuts. The native stink bug, *P. maculiventris*, used to assess non-target effects of *Tr. japonicus*, was reared in groups of 40–50 in 1.2 L clear, round plastic containers (Gordon Food Service) at 25˚C, 50–75% humidity and 16:8 L:D photoperiod in a climate chamber. Water was provided via dental wicks inserted in 60 ml cups filled with water. Their diet included wax worms (Top Hat Cricket Farm Inc., Portage, Michigan) and organic green beans. For both stink bug colonies, eggs were collected two to three times weekly and used either to rear parasitoids, as sentinel eggs in the field, returned to the colony, or were frozen at -80˚C for later use.

Experimental design

To test how different release methods may impact establishment success of *Tr. japonicus*, a 2-year field study was conducted. In year one, in 2019, the importance of release size was evaluated. For this study 12 field sites were chosen in southwestern and central Michigan (Table 2.1, Figure. 2.1). A randomized complete block design was used with three release treatments (0, 100, or 900 individuals) assigned randomly within each block, replicated over four blocks. The blocks represented sites that were in geographic proximity (Figure. 2.1). Releases took place in 2019 and were monitored in 2019 and in 2020. Establishment success of the different release treatments was evaluated using a mix of fresh and frozen *H. halys* sentinel eggs in 2019 (Table 2.2) and yellow sticky traps (Trécé® AM no-Bait traps) in 2020 to capture *Tr. japonicus*. Half of the sentinel eggs and the yellow sticky traps were placed at 1.5 m and the other half at 3.5 m height to evaluate parasitoid activity at different heights. The density of *H. halys* was measured using four pyramid traps at each of the 12 study sites both in 2019 and 2020. Non-target effects of *Tr. japoni*cus on native stink bugs were evaluated by placing *P. maculiventris* sentinel eggs in 2019 at sites where 900 *Tr. japonicus* had been released, where the potential for attack was highest. Parasitism by native species was evaluated by identifying parasitoids emerging from *H. halys* sentinel eggs in 2019.

To test how the release frequency may impact establishment success, 12 new study sites were identified in southeastern Michigan in 2020 (Table 2.1). A randomized complete block design was used with three release treatments: 0 (control), 500 wasps released at one date, or 250 wasps at one date and 50 more wasps four weeks later. Release treatments were replicated over four sites in four blocks that were assigned based on geographic proximity (Figure. 2.1). Establishment success was evaluated by monitoring *Tr. japonicus* numbers with frozen *H. halys* sentinel eggs and with yellow sticky traps in 2020. Half of the sentinel eggs and the yellow sticky traps were placed at 1.5 m and the other half at 3.5 m height to evaluate parasitoid activity at different heights. The density of *H. halys* was measured using four pyramid traps at each of the 12 study sites.

Experimental procedures

The main criterion for choosing sites was to have relatively high densities of *H. halys* in the region as assessed by a multi-year monitoring effort prior to this study. Another criterion was to have at least one side of the field bordered with a woodlot that can provide habitat for *H. halys* and *Tr. japonicus* and where pesticide drift would be minimized. Most study sites were apple orchards (n = 16) managed either conventionally (n = 14) or using low input or organic methods (n = 2). Eight sites had a mix of crops of both different fruits and vegetables and were either managed conventionally (n = 1) or with low input or organic means (n = 7) (Table 2.1).

Adult wasps were released in both years within the wood lots, at least 10 m from the field edges using 500 ml plastic deli cups (Gordon Food Service) with the lids removed, raised into the canopy on a 3 m long pole. Releases were aimed to coincide with peak *H. halys* oviposition that is estimated to occur between 460 and 734 growing degree days (base 14.17˚C starting with January 1), based on estimates modeled for Geneva, New York by Nielson et al. (2016). These degree days corresponded to June 9–27 in 2019 and June 9–25 in 2020 in East Lansing, Michigan. Single releases took place on June 22 or at 521 degree days in 2019 (enviroweather.msu.edu). For the 2020 field season the first releases (n = 250 or 500) took place on July 1 or at 873 degree days and the second releases (50 individuals added to the 250 prior release) took place on Aug 4 or at 1674 degree days.

Table. 2.1. Study sites, experimental design and site characteristics. All sites designated as 'Mixed' had both a diversity of fruits and vegetables grown. Sites characterized as 'Low input' use a mix of conventional and organic management methods.

City	Block	Release size	Release date	Latitude	Longitude	Crop	Management
Bath	1	0	2019	42.8566	-84.4083	Mixed	Low input
Charlotte	1	100	2019	42.6337	-84.7877	Mixed	Organic
St. Johns	1	900	2019	43.1207	-84.6252	Apple	Conventional
Conklin	2	0	2019	43.1526	-85.8195	Apple	Conventional
Grand Rapids	2	100	2019	43.0629	-85.7396	Apple	Conventional
Sparta	2	900	2019	43.1113	-85.7580	Apple	Conventional
Grand Junction	3	0	2019	42.3450	-86.0502	Apple	Conventional
Covert	3	100	2019	42.3066	-86.2537	Apple	Conventional
Bangor	3	900	2019	42.2949	-86.1816	Apple	Conventional
Benton Harbor	4	0	2019	42.0829	-86.3521	Mixed	Conventional
Coloma	4	100	2019	42.1615	-86.3123	Mixed	Low input
Hartford	4	900	2019	42.1852	-86.1749	Apple	Conventional
Burton	5	0	2020	43.0256	-83.5747	Apple	Low input
Davison	5	250+50	2020	42.9692	-83.4894	Apple	Low input
Flint	5	500	2020	43.0321	-83.6785	Apple	Organic
Washington	6	0	2020	42.8003	-83.0698	Apple	Conventional
Washington	6	250+50	2020	42.7866	-83.0215	Apple	Conventional
Armada	6	500	2020	42.8608	-82.9493	Apple	Conventional
Britton	7	0	2020	42.1425	-83.6286	Mixed	Conventional
Milan	7	250+50	2020	41.9598	-83.9226	Mixed	Conventional
Chelsea	7	500	2020	42.2579	-84.1103	Mixed	Organic
Linden	8	0	2020	42.6898	-83.8726	Apple	Abandoned
Fenton	8	250+50	2020	42.6919	-83.7498	Apple	Conventional
Howell	8	500	2020	42.8059	-83.7945	Apple	Conventional

Halyomorpha halys sentinel eggs

Halyomorpha halys sentinel eggs were used to monitor the presence of *Tr. japonicus* and of native parasitoids during the season when parasitoids were released; in 2019 at the southwestern release sites and in 2020 at the southeastern release sites. Sentinel eggs were not used at the 2019 release sites to monitor in 2020, only yellow sticky traps. In 2019, a mix of fresh (< 72 h old) and frozen eggs were used, while in 2020 only frozen eggs were used (Table 2.2). Ten *H. halys* sentinel egg masses were deployed bi-weekly between May 24 and Sept 9 in 2019 for a total of 840. Half of the egg masses were glued (Elmer's® extra strength non-toxic) to leaves of the surrounding vegetation at 1.5 m height and half were glued to leaves that were raised to 3.5 m height on bamboo poles to test how height may influence parasitism rates (Quinn et al. 2019). Eggs were left in the field for 48 hours then brought back to the lab and incubated at 20˚C, 50–75% humidity and 16:8 L:D photoperiod in a climate chamber. Eggs were monitored for parasitoid emergence for 2 months. At the 12 field sites where wasp releases took place in 2020, monitoring proceeded in a similar manner as described for the 2019 releases by placing 10 *H. halys* sentinel egg masses at each of the 12 field sites bi-weekly between July 2 and Sept 15 for a total of 430.

Podisus maculiventris sentinel eggs

Potential non-target effects of *Tr. japonicus* were monitored in 2019 by placing at least 5 fresh *P. maculiventris* egg masses, depending on availability, at 3.5 m height biweekly at sites that received 900 wasps. *Podisus maculiventris* egg masses were placed on the same leaves as the *H. halys* sentinel eggs to provide a direct choice for oviposition to parasitoids. In 2019, 285 *P. maculiventris* egg masses were deployed between July 2 and Sept 3. No *P. maculiventris* eggs were deployed in 2020.

Yellow sticky traps

Yellow sticky traps were used for monitoring *Tr. japonicus* and native parasitoids in 2020 both at the 2019 and 2020 release sites. At the 2019 release sites, eight traps were placed every three weeks between June 11 and Sept 4 with four at 1.5 m and four at 3.5 m height for a total of 384. At the 2020 release sites, 8 traps were placed at each site biweekly, at the same time when *H. halys* sentinel eggs were retrieved, between June 13 and Sept 15 for a total of 400 across the 12 sites. At a few monitoring dates certain sites could not be accessed because of pesticide applications in the orchards and thus data are missing for sentinel eggs and yellow sticky traps.

Pyramid traps

The density of *H. halys* was monitored using pyramid traps in 2019 at the 12 release sites, and in 2020 at 24 sites to include all sites where *Tr. japonicus* releases had taken place in the two years combined. At each study site, four pyramid traps were placed 7 m apart in the woodlot where releases had taken place. Each trap was baited with a dual stink bug lure (Trécé Pherocon® Dual Lure). The cone at the top of the traps was lined on the inside with a piece of mesh infused with deltamethrin (D-Terrence® net, Vestergaard S.A., Lausanne, Switzerland) to kill any stink bugs captured. At the 2019 release sites traps were checked biweekly between May 24 and Sept 9 in 2019, and every three weeks between June 25 and Oct 7 in 2020. At the 2020 release sites, traps were checked biweekly between July 1 and Oct 9 in 2020.

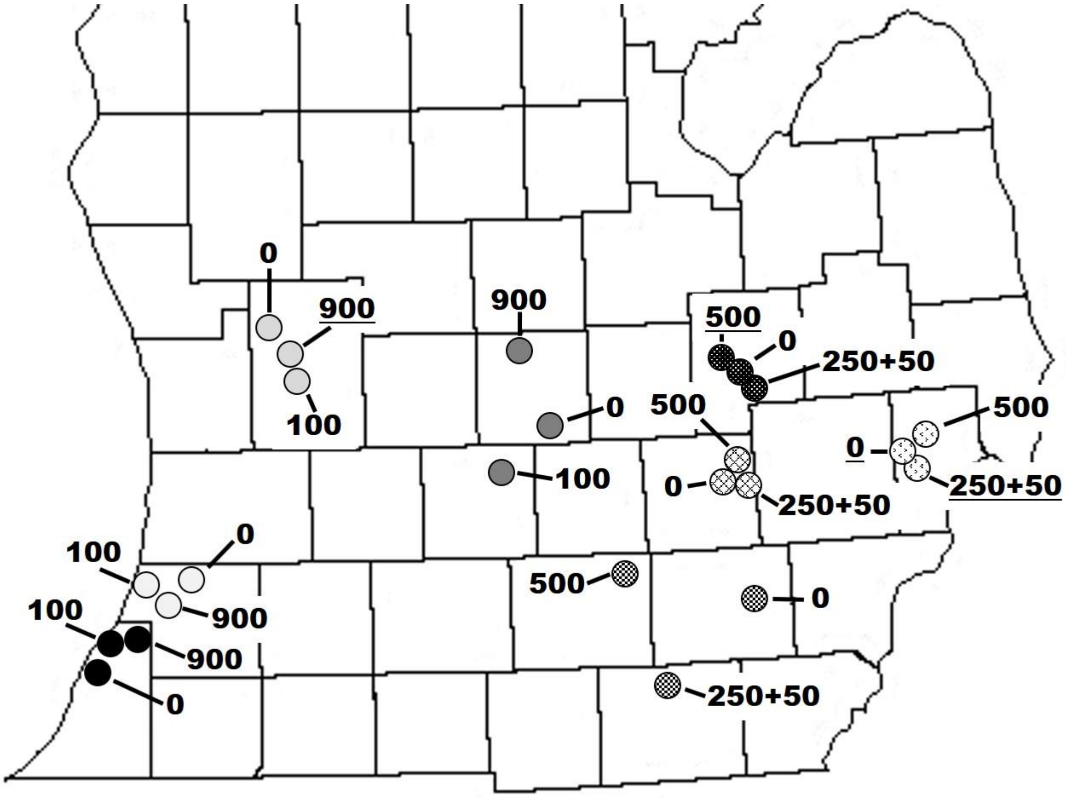

Figure. 2.1**.** Study sites for *Tr. japonicus* releases in 2019 (solid fill) and in 2020 (pattern fill) in lower Michigan. Different shaded circles indicate the 4 regional blocks each year within which sites were assigned to different *Tr. japonicus* release treatments. In 2019, sites received either 0 (control), 100 or 900 adult parasitoids as part of a single release. In 2020, sites received either 0 (control) or 500 in a single release or 250 individuals first and then an additional 50 at a second release date. The four underlined release numbers indicate the sites where *Tr. japonicus* was recaptured in 2020. For exact coordinates see Table. 2.1.

Statistical analyses

Halyomorpha halys *sentinel eggs*

The rate of parasitism was assessed at the egg mass level by calculating the percentage of egg masses that yielded any parasitoids across the 12 sites of the 2019 and the 12 sites of the 2020 releases. Parasitism rate on the individual egg level was calculated by using 28 eggs for the average size of an egg mass for *H. halys*. A generalized linear mixed model (GLMM) with binomial distribution (emergence vs. no emergence) and logit link function was used to assess differences in parasitism rates between frozen and fresh sentinel eggs and those placed either lower or higher in the canopy in 2019. Height and the state of eggs were fixed variables and block was a random variable.

Linear mixed models were used to assess how the state of the *H. halys* sentinel eggs (fresh or frozen), their placement (low or higher), and sampling date (month) may influence the number and species identity of emerging parasitoids (both native and *Tr. japonicus*) in 2019. Fixed effects in the model included date, placement, state of the eggs and parasitoid species identity. All interactions were non-significant (at $\alpha = 0.05$) and did not improve AIC values, therefore were not included in the final model. These analyses were done only for 2019 because in 2020 parasitoid captures were very low. Emerging parasitoid numbers were log transformed to meet the model assumption of normal distribution and block was included as a random effect. The correlation between the number of egg masses attacked and the number of *H. halys* captured at each study site in 2019 was assessed using a linear regression.

Podisus maculiventris *sentinel eggs*

The rate of parasitism was assessed at the egg mass level by calculating the percentage of egg masses that yielded any parasitoids across the four sites where 900 *Tr. japonicus* had been released and where *P. maculiventris* sentinel eggs had been deployed. Parasitism rate on the individual egg level was calculated by using 14 eggs for the average size of an egg mass (Legaspi 2004).

Yellow sticky traps

Generalized linear mixed models with binomial distribution and logit link function were used to compare parasitoid capture success (yes or no) of traps placed at different heights at the 2019 and 2020 release site separately. Height and the state of eggs were fixed variables and block was a random variable. The number of captured parasitoids by traps placed lower or higher was compared using linear mixed models. Fixed effect in the model was the placement (low vs. high) and block was included as a random variable.

Pyramid traps

A general linear model was used with sampling date (2019 or 2020 categorical variable), block and their interactions as fixed effects to compare the number of *H. halys* captured in 2019 and 2020 at the 2019 release sites. Only data for the months of June, July and August were included in the analysis when monitoring efforts were similar between the years since monitoring stopped in early September in 2019 but continued through September in 2020. Block (groups of three sites in proximity; see Table 2.1) was included as a fixed effect to assess quantitatively regional differences in *H. halys* numbers. A similar analysis was conducted for stink bug captures at the 2020 release sites with block as a fixed factor. Stink bug numbers were log transformed (Log + 0.5) to meet model assumption of normal distribution. All analyses were performed using JMP Pro Version 13.0.0 (SAS Institute 2016).

Results

Halyomorpha halys *sentinel eggs*

In 2019, 840 *H. halys* sentinel egg masses were deployed and 727 were successfully retrieved. Parasitoids emerged from 30 egg masses from 9 of the 12 study sites (Table 2.2). This constitutes 4.12% parasitism rate on the egg mass level over all sites, egg types and placements. A total of 241 parasitoids emerged from the parasitized egg masses resulting in 1.18% parasitism rate on the individual egg level. More than one parasitoid species emerged from three of the 30 egg masses parasitized in 2019. For those three observations the number of emerged parasitoids were divided equally between the two species and were included as such in the dataset.

In 2019, there was no difference in parasitism success of frozen versus fresh sentinel eggs (F = 3.5286 df = 1, 724 p = 0.0607) or eggs placed lower or higher in the canopy (F = 0.4713, df = 1, 720.7, p = 0.4926). The placement of eggs (F = 0.3636, df = 1, 24.35, p = 0.5521), the date (F = 0.6433, df = 2, 24.55, p = 0.5342) or the species identity of parasitoids (F = 0.8981, df = 3, 24.14, p = 0.4565) did not influence the number of emerging parasitoids (Figure. 2.2). However, more parasitoids emerged from frozen (non-transformed mean = 9.1 ± 1.91 SE) than from fresh (4.16 ± 2.12) eggs (F = 5.4686, df = 1, 23.79, p = 0.0281) (Figure. 2.3, Table 2.2). The number of *H. halys* captured over the season and the number of sentinel egg masses attacked at each site were positively correlated at the 2019 release sites ($R^2 = 0.61$, p = 0.0027) (Figure. 2.4).

Table. 2.2. Outcome of monitoring using *H. halys* and *P. maculiventris* sentinel egg masses deployed at 12 field sites each in 2019 and 2020.

	2019 Release		2020 Release
	H. halys	*P. maculiventris*	*H. halys*
Sentinel eggs deployed	840	285	430
Sentinel eggs retrieved	727	133	321
Sentinel eggs placed lower (1.5 m)	371 (51%)	0	168 (52%)
Sentinel eggs placed higher (3.5 m)	356 (49%)	133 (100%)	153 (48%)
Frozen sentinel eggs	532 (73%)	39 (30%)	321 (100%)
Fresh sentinel eggs	195 (27%)	133 (70%)	0
Egg masses parasitized	30 (4.12%)	3 (2.26%)	6 (1.87%)
Sentinel eggs placed lower parasitized	17 (57%)	0	2 (33%)
Sentinel eggs placed higher parasitized	13 (43%)	3 (100%)	4 (67%)
Frozen egg masses parasitized	17 (57%)	2 (67%)	6 (100%)
Fresh egg masses parasitized	13 (43%)	1 (33%)	0
Number of emerging parasitoids	241	12	39
Parasitoids emerging from frozen eggs	171 (71%)	10 (83%)	39
Parasitoids emerging from fresh eggs	70 (29%)	2 (17%)	0
Parasitism rate on egg level	1.18%	0.60%	0.43%

In 2019, all emerging parasitoids were native species representing three families and four genera: *Telenomus* (Scelionidae), *Trissolcus* (Scelionidae), *Anastatus* (Eupelmidae) and *Ooencyrtus* (Encyrtidae). The number of emerging parasitoids were as follows: *Anastatus* spp. n = 97, *Tr. euschisti* (Ashmead) n = 55, *Te. podisi* Ashmead n = 40, and *Ooencyrtus* spp. n = 29 (Figure. 2.5).

In 2020, of the 430 *H. halys* sentinel egg masses deployed, 321 were retrieved. Six egg masses yielded a total of 39 parasitoids for 1.87% parasitism rate at the egg mass and 0.43% parasitism on the individual egg level across all sites (Table 2.2). *Trissolcus japonicus* emerged from one egg mass (n = 7) that was deployed on July 30 in a mixed orchard in Flint, Michigan following augmentative releases of 500 adults on July 1st. *Trissolcus euschisti* emerged from three egg masses (n = 24), *Te. podisi* from one egg mass (n = 2) and Encyrtidae spp. from one egg mass (n = 6) (Figure. 2.2).

Podisus maculiventris *sentinel eggs*

Of the 285 *P. maculiventris* sentinel egg masses deployed in 2019, 133 were retrieved successfully. Three yielded 12 total parasitoids resulting in 2.25% parasitism rate of egg masses across all sites. Parasitism rate on the individual egg level was 0.6%. All parasitoids emerging from *P. maculiventris* eggs were native species from the genera *Telenomus* (n = 11) and *Ooencyrtus* (n = 1).

Yellow sticky traps

Of the 384 yellow sticky traps placed at the 12 field sites in 2020 where parasitoids had been released in 2019, 51 captured parasitoids that are likely to attack stink bugs. Encyrtidae spp. were captured most often (n = 46), followed by *Tr. euschisti* (n = 14), *Te. podisi* (n = 11), *Tr. thyantae* (Ashmead) (n = 2), *Tr. brochymenae* (Ashmead) (n = 2), *Anastatus* spp. (n = 2) and an unidentified *Trissolcus* spp. (n = 1). *Trissolcus japonicus* was captured at a single location close to Sparta, Michigan where 900 individuals had been released in 2019 (Figure. 2.5). The number of parasitoids captured by traps placed lower or higher in the canopy did not differ (F = 0.8447, df = 1, 379.1, p = 0.3587).

Of the 400 yellow sticky traps placed at the 12 study sites in 2020 where parasitoids had been released in 2020, 69 captured parasitoids that likely attack stink bugs. The parasitoid species captured were similar to that captured in 2020 at the 2019 release sites with Encyrtidae being most numerous (n = 62), followed by *Tr. euschisti* (n = 19), *Te. podisi* (n = 8), *Anastatus* spp. (n = 3), *Te. persimilis* Ashmead (n = 1) and *Tr. brochymenae* (n = 1) (Figure. 2.5). *Trissolcus japonicus* was recaptured at two sites, one where 250 and later 50 individuals had been released close to Washington, Michigan and at a control site that is located just 4.3 km from the Washington release site. The number of parasitoids captured by traps placed lower or higher in the canopy did not differ (F = 0.0176, df = 1, 395, p = 0.8945).

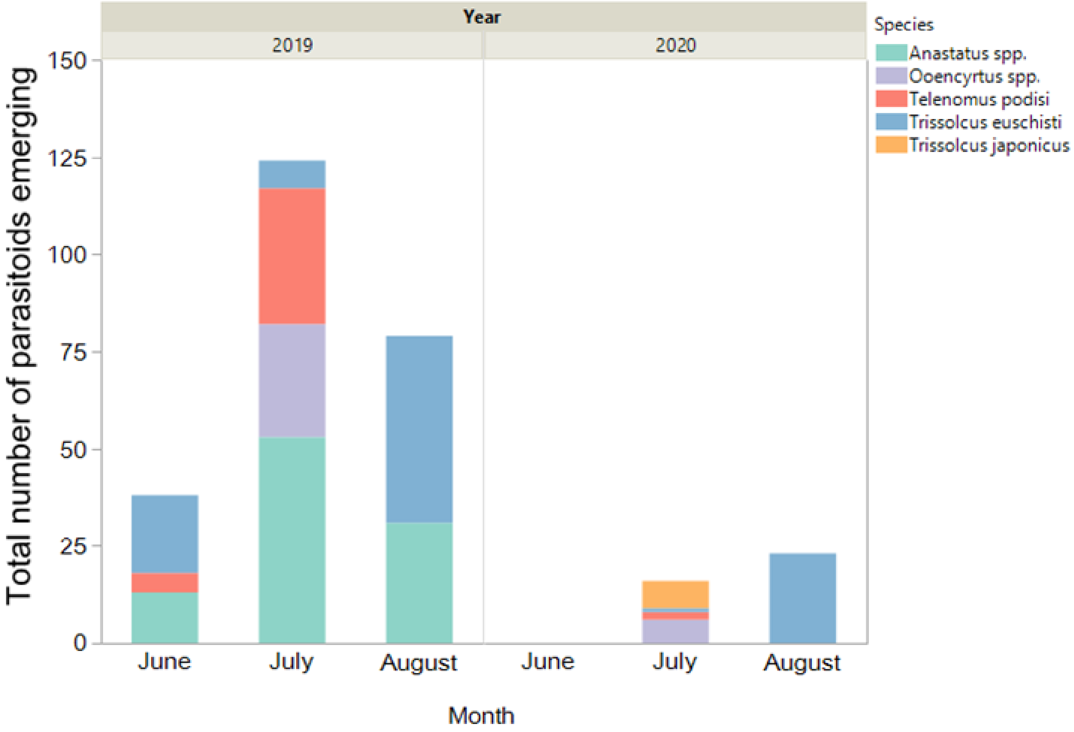

Figure. 2.2. Total number of parasitoids of five species (or genera) emerging from *H. halys* sentinel eggs. The year of sampling is shown at the top of the graph. Parasitoids were released and monitored using sentinel eggs in southwestern Michigan in 2019 (left panel) and in southeastern Michigan in 2020 (right panel). There was no difference in the mean number of parasitoids emerging between months (p = 0.5342) or for the different parasitoid species (p = 0.4565) in 2019. Note that releases and sampling took place later in 2020 than in 2019 and only frozen eggs were used in 2020 for monitoring, which may explain the relatively lower parasitoid captures.

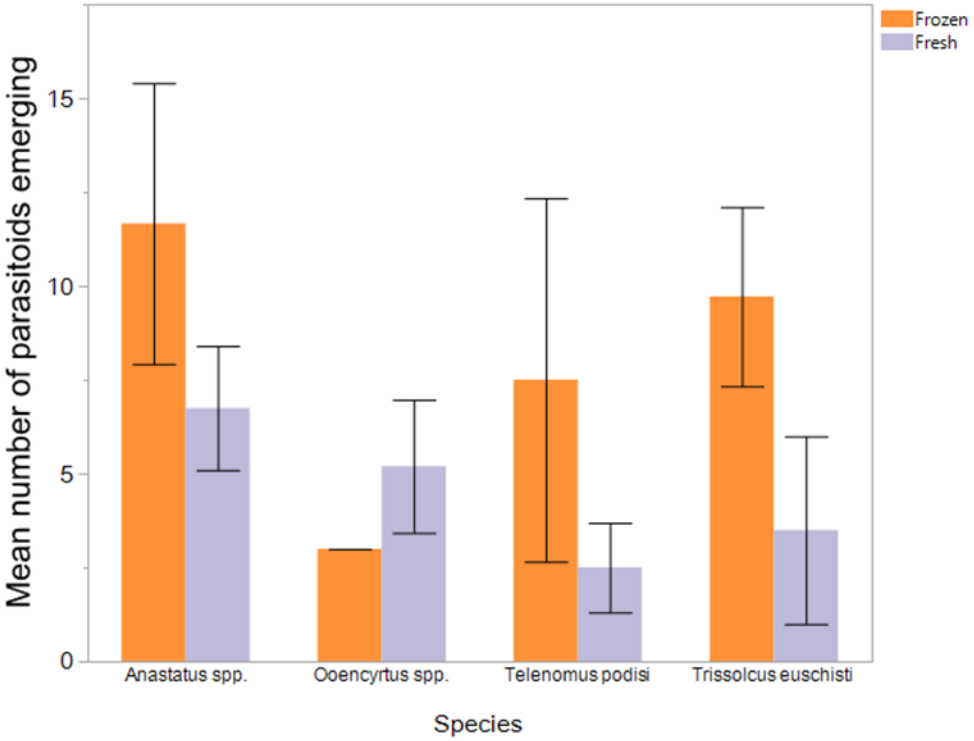

Figure. 2.3. Mean (± SE) number of parasitoids emerging from frozen or fresh *H. halys* sentinel eggs deployed in 2019. Across species more parasitoids emerged from frozen than from fresh sentinel eggs (p = 0.0281) but within parasitoid species there was no difference between numbers emerging from frozen versus fresh eggs.

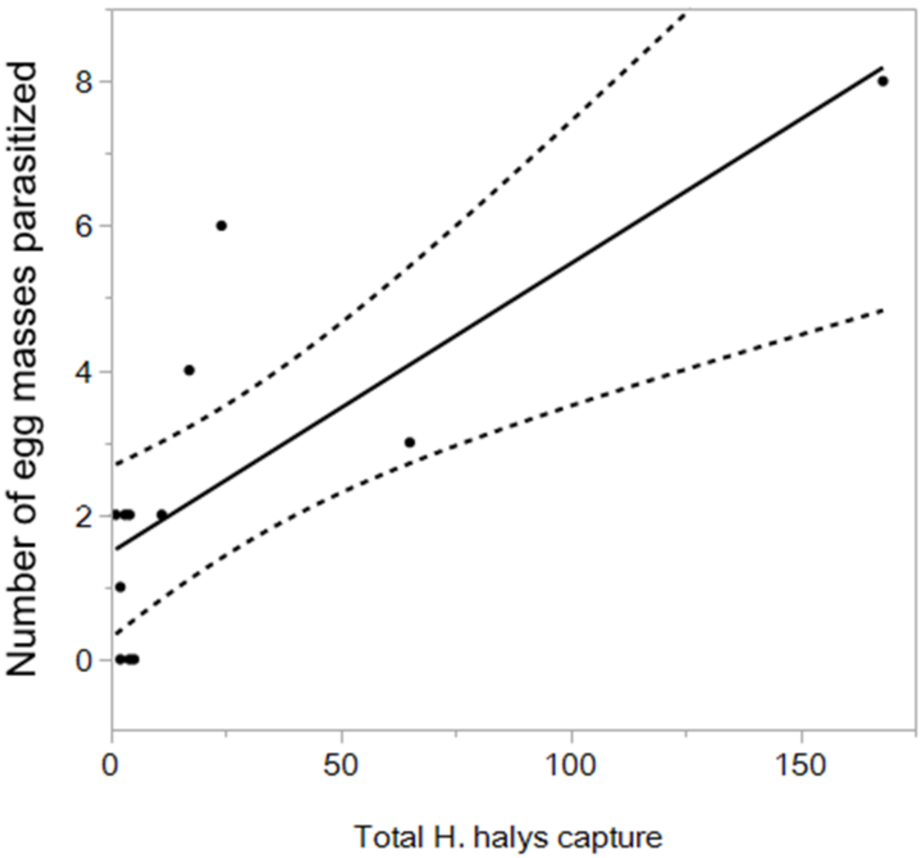

Figure. 2.4. The relationship between the number of *H. halys* sentinel egg masses parasitized and the number of *H. halys* captured by pyramid traps at the 12 study sites in 2019 (R^2 = 0.61). Dotted lines represent 95% confidence interval curves.

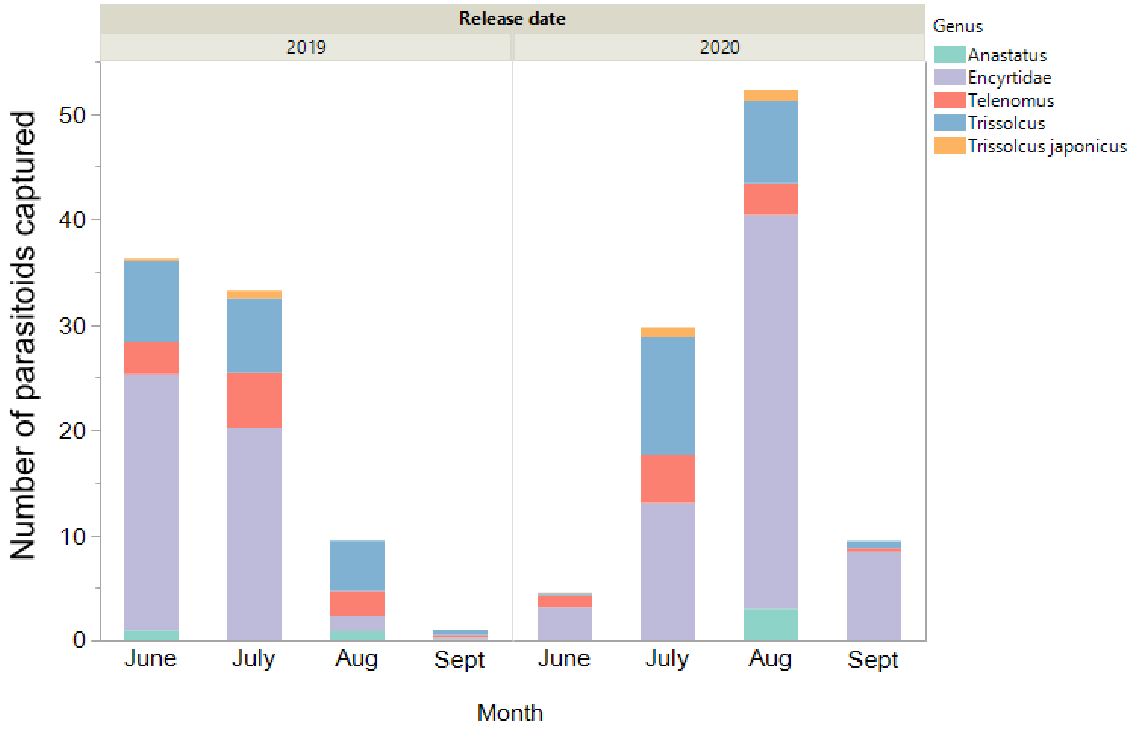

Figure. 2.5. Parasitoid captures by yellow sticky traps in 2020 at 12 study sites where *Tr. japonicus* was released in 2019 (left panel) and 12 sites where released in 2020 (right panel). Traps were changed every 3 weeks at the 2019 release sites and every 2 weeks at the 2020 release sites. To display monthly total captures the number of parasitoids captured by each trap was divided in proportion of the number of days spent in the field for and given month.

Halyomorpha halys *numbers*

Halyomorpha halys densities across the 12 sites where *Tr. japonicus* had been released in 2019 were higher in 2020 (non-transformed mean: 6.4 ± 1.18 SE) than in 2019 (1.12 ± 0.97) (year: F = 33.5459, df = 1, p < 0.0001). There were also regional differences in *H. halys* densities among sites (block: F = 21.5173, df = 3, p < 0.001). The mean *H. halys* captures were significantly lower in block 2 (the three sites in the Grand Rapids region; see Table 2.1 and Figure. 2.1) (non-transformed mean: 0.052 ± 0.05 SE) than the other three blocks at the 2019 release sites (block 1: 6.15 ± 1.58, block 3: 5.27 ± 1.52, block 4: 3.58 ± 1.52) (Figure. 2.6). *H.*

halys captures were higher at three of the four blocks in 2020 than in 2019, but in block two they remained low (block x year interaction: F = 2.8, df = 3, p = 0.0048).

There were also regional differences in *H. halys* densities at the 2020 release sites (block: F = 10.8042, df = 3, p < 0.0001). Block five, that included sites around Flint, Michigan (see Table 2.1 and Figure. 2.1) had significantly higher *H. halys* densities (50.8 ± 7.2) than the other blocks (block 6: 9.22 ± 7.2, block 7: 10.83 ± 7.2, block 8: 7.6 ± 7.2) (Table 2.1, Fig 2.1).

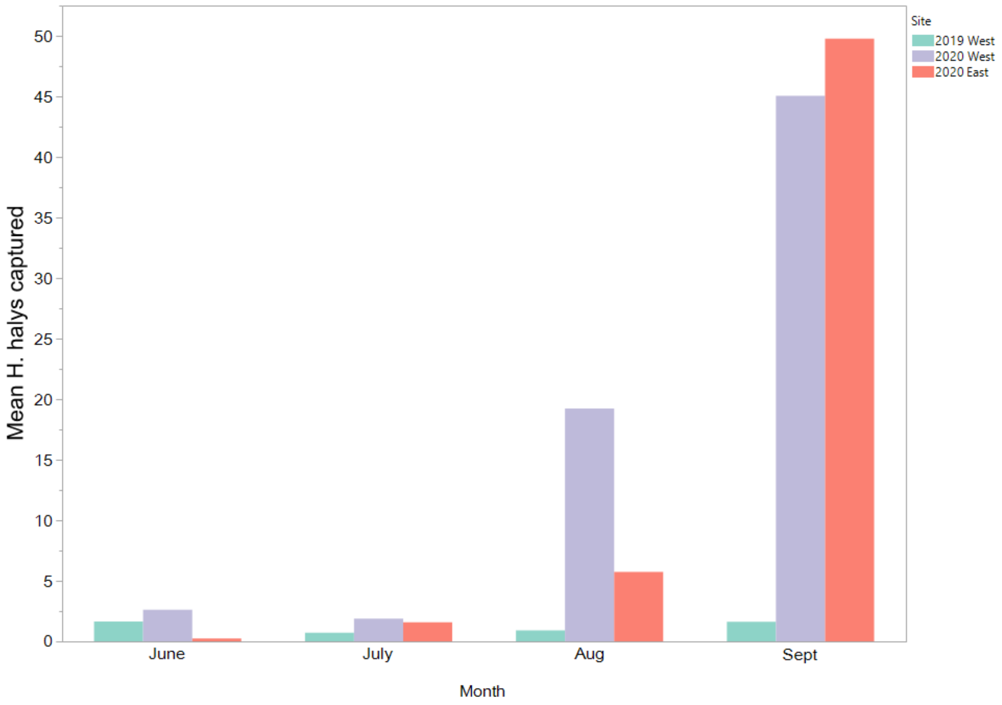

Figure. 2.6. Mean number of *H. halys* captured by pyramid traps at sites where *Tr. japonicus* was released either in 2019 or 2020. The 2019 release sites were sampled both in 2019 and 2020 (2019 West and 2020 West). The 2020 release sites were sampled in 2020 (2020 East). For the 2019 release sites more *H. halys* was captured in 2020 than in 2019 between June and August. Here mean captures are shown for the entire sampling season, which ended in early September in 2019 but continued until the end of September in 2020.

Discussion

The importance of release size or release frequency mediating establishment of the egg parasitoid *Tr. japonicus* could not be determined during the course of this two-year study because of low recapture rates. Of the 7,200 adult parasitoids released over two years only 10 individuals were recaptured at four of the 24 study sites (Figure. 2.1). In 2020, *Tr. japonicus* was found on one yellow sticky trap at a site where 900 individuals had been released a year prior. This could indicate successful overwintering. For the 2020 releases, *Tr. japonicus* was recaptured at three sites each representing a different release treatment (0, 250+50, or 500 individuals). The emergence of seven individuals from an *H. halys* sentinel egg mass shows that *Tr. japonicus* is reproducing and the yellow sticky trap captures indicate summer activity and dispersal.

The release sizes used in 2019 and 2020 at individual sites that ranged from 100–900 (Table. 2.1, Figure. 2.1) are comparable to other studies testing the importance of release size of parasitoids for establishment. For example, 1, 10, or 100 female *N. typhlocybae* were introduced against a planthopper in southern France (Fauvergue et al. 2007) and 100–1000 *To. sinensis* were released at each site against the chestnut gall wasp (Borowiec et al. 2018). Both the above studies with *N. typhlocybae* and *To. sinensis* found parasitoid establishment and increasing population sizes in the same year and one or two years after the original releases (Borowiec et al. 2018; Fauvergue et al. 2007). However, our results that show very limited distribution and density of *Tr. japonicus* a few years after initial detection are not unusual. For example, a 2017 study in New Jersey that placed 236 sentinel eggs in commercial peach and apple orchards, similarly to this study, found only three egg masses parasitized by *Tr. japonicus* (Kaser et al. 2018). In Virginia only three of the 135 sentinel egg masses were parasitized by *Tr. japonicus* in 2016

(Quinn et al. 2019). These studies, conducted within two to three years of the initial detection of *Tr. japonicus* in nearby Maryland in 2014 (Talamas et al. 2015), indicate very low densities within a few years after discovery.

Population growth of introduced populations can be impeded by a multitude of environmental, demographic and genetic factors (Fauvergue et al. 2012). Small introduced populations are subject to environmental and demographic stochasticity that can cause large fluctuations in population sizes and can lead to extinction (Fauvergue et al. 2012; Lande 1988; Lande 1993). Allee effects, whereby at low population densities individuals have lower survival or mating success, can slow population growth and can also lead to extinction (Courchamp et al. 2008; Courchamp et al. 1999; Lande 1998). Introduced populations often go through bottlenecks that can reduce genetic diversity, adaptive potential and population growth as well (Fauvergue et al. 2012). Thus, it is not surprising that *Tr. japonicus* densities in Michigan are still low given its initial discovery in 2018 (Jarrett et al. 2019), followed by two years of augmentative releases.

The climate in Michigan is predicted to be within the ‘highly’ and ‘moderately’ suitable range for *Tr. japonicus* establishment (Avila and Charles, 2018), therefore it is unlikely that abiotic conditions would prevent its widespread establishment. In fact, *Tr. japonicus* population growth appears quicker on the west coast of the USA, that is deemed only as ‘marginally’ suitable for establishment by climate models (Avila and Charles, 2018). In Washington state, where *Tr. japonicus* was first detected in 2015 (Milnes et al. 2016), a study conducted in 2017 found that 67% of sentinel egg masses and 77% of individual *H. halys* eggs had been parasitized (Milnes and Beers, 2019). This may suggest that a ‘marginally’ suitable climate is not hindering population buildup of *Tr. japonicus* orthat this climate model have underestimated the optimal range of *Tr. japonicus* (Avila and Charles, 2018)

Trissolcus japonicus establishment and population growth in Michigan may have been impacted by the low density of *H. halys* in 2019 (Figure. 2.6). Spring 2019 was unusually cold and wet that could have caused mortality or delayed development of *H. halys*, which might partly explain the lack of *Tr. japonicus* captures that year. There is some evidence that even native parasitoid captures by *H. halys* sentinel eggs are positively correlated with *H. halys* density (Figure. 2.4), which suggest that parasitoids tend to accumulate where stink bug populations are higher. However, the most likely explanation for the lack of recapture in 2019 and the low recapture rates in 2020 is that *Tr. japonicus* densities are very low across Michigan and within one to two years the released 7,200 adults cannot build up large enough populations that are reliably detectable with sentinel eggs or yellow sticky traps.

The native parasitoid community, representing four genera (*Trissolcus*, *Anastatus*, *Telenomus*, and *Ooencyrtus*) that attacked *H. halys* sentinel eggs in Michigan is similar to those found across North America (Abram et al. 2017). Average rates of parasitism by native parasitoids that were between 1.87–4.12% at the egg mass level and 0.43–1.18% at the individual egg level in Michigan also align well with results of a review that found that in 87% of surveys the parasitism levels were under 5% (Abram et al. 2017). In our surveys, more parasitoids emerged from frozen than from fresh *H. halys* eggs, but the likelihood of parasitism did not differ between frozen and fresh eggs (Figure. 2.3). In Maryland, surveys also found higher emergence from frozen eggs, which may be explained by the lack of adaptation by native parasitoids to the immune response presented by viable *H. halys* eggs (Herlihy et al. 2016). However, a review of 98 datasets for parasitism by native species did not find any difference in attack rates between fresh and frozen sentinel eggs (Abram et al. 2017).

Parasitoid activity did not exhibit a distinct trend across the season as both the sentinel egg and yellow sticky trap captures showed varying peaks of activity among the study sites and sampling years (Figures. 2.3, 2.6). In Delaware, the rate of parasitism was positively correlated with average temperatures and the warmest month, July, was found to yield the highest parasitism rates (Dieckhoff et al. 2017). On the other hand, parasitism rates in Maryland steadily increased over the season, peaking in early September (Jones et al. 2017). A recent study indicated that *Tr. japonicus* captures are most likely in the middle and upper canopy of trees, while native parasitoids may be captured at any location (low, mid, or upper canopy) (Quinn et al. 2019). Our results corroborate that native parasitoids are equally likely to be captured both in the low and mid-canopy. However, three of our four *Tr. japonicus* recaptures occurred at 1.5 m height, which does not support the recommendation to sample only in the mid and high canopy for *Tr. japonicus* (Quinn et al. 2019). These discrepancies indicate that it may be difficult to generalize patterns with regards to when peak parasitism is expected, whether fresh or frozen eggs are more likely to be attacked, and at which heights parasitism by native or exotic parasitoids may be more prevalent. Differences in climate, site characteristics, sampling methods and a multitude of other factors between studies may account for the different findings. However, there is general agreement across studies that the current rates of egg parasitism by native parasitoids is unlikely to provide sufficient biological control to suppress *H. halys* populations (Abram et al. 2017; Cornelius et al. 2016; Dieckhoff et al. 2017; Jones et al. 2014; Ogburn et al. 2016; Rice et al. 2014).

While *Tr. japonicus* is considered the most promising biocontrol agent because of its high parasitism rates of 50–80% of *H. halys* eggs in the native range (Yang et al. 2009; Zhang et al. 2017), there are concerns regarding potential non-target effects. Host-specificity tests in the laboratory have revealed that *Tr. japonicus* can attack over ten native stink bug species (Botch and Delfosse, 2018; Hedstrom et al. 2017). *Trissolcus japonicus* was also recovered from native stink bug eggs from the field including from *P. maculiventris*, which is an important predatory stink bug in North America (Gariepy and Talamas, 2019; Milnes and Beers, 2019). We deployed over 100 *P. maculiventris* eggs at four sites where 900 *Tr. japonicus* had been released in 2019 but only native parasitoids attacked those eggs. In a survey in 2018, when 51 *P. maculiventris* eggs were placed alongside *H. halys* sentinel eggs we also did not find parasitism by *Tr. japonicus* (Jarrett et al. 2019). However, given the low density of *Tr. japonicus* in Michigan these results may not be representative of its potential impact on native species and it should be reevaluated once population densities increase. Since parasitism of native stink bugs by *Tr. japonicus* appears much lower (0.4–8%) in the field than that of *H. halys* (77%) its redistribution continues within multiple states (Jentsch, 2017; Milnes and Beers, 2019). *Trissolcus japonicus* is expected to be an important component of integrated pest management programs targeting *H. halys* both in North America and in Europe (Abram et al. 2020) and the augmentative releases conducted as part of this study will likely contribute to its widespread establishment and population growth across Michigan.

CHAPTER 3: The effects of heat waves on *Halyomorpha halys* survival and parasitism success by *Trissolcus japonicus*

Introduction

Global climate change is leading to a rise in mean temperatures and increases in the frequency, length and intensity of extreme weather events, such as heat waves (Ebi and Meehl, 207; Houghton, 2009; IPCC, 2012). Insects, like other ectotherms are particularly sensitive to temperature changes because they have limited ability for thermoregulation. Changes in temperature can directly affect the physiology and behavior of insects including development, movement, reproduction, and foraging abilities (Angilletta et al. 2002; Chown and Terblanche, 2006). Gradual increases in temperature are predicted to benefit insects in temperate regions as it can lead to higher overwintering survival, extend seasonal activity windows and can result in the expansion of geographic ranges (Bale et al. 2002; Berg et al. 2010; Deutsch et al. 2008; Hickling et al. 2006; Musolin and Fujisaki, 2006; Parmesan, 1999). However, heat waves, defined as periods when maximum temperatures are above seasonal norms by 5˚C for at least 5 consecutive days (De Boeck et al. 2010), can cause direct mortality and can have negative fitness consequences for surviving individuals (Bannerman et al. 2011; Roux et al. 2010; Sentis et al. 2013; Zhang et al. 2019).

The susceptibility to thermal stress can vary between different trophic levels across a food chain (Aguilar-Fenollosa and Jacas 2014; Hance et al. 2007; Voigt et al. 2003). It is predicted that natural enemies (e.g. parasitoids and predators) will be more adversely affected than herbivorous insects, partly because they appear to have smaller thermal windows within which they can grow and reproduce (Aguilar-Fenollosa and Jacas, 2014; Hance et al. 2007;

Montserrat et al. 2013; Schreven et al. 2017), however, the evidence for these expectations is contradictory. For example, the parasitoid wasp *Aphidius avenae* Haliday (Hymenoptera: Braconidae) suffered high mortality after one hour of exposure to a heat stress event of 36˚C and the surviving individuals had lower fecundity (Roux et al. 2010). On the other hand, heat waves of up to 40˚C imposed over a 7-day period reduced aphid population growth but did not affect the fecundity of the parasitoid *Aphidius matricariae* Haliday (Hymenoptera: Aphidiidae) (Bannerman et al. 2011). In a tri-trophic system, the biomass of sweet pepper plants did not change, the abundance and fecundity of the aphid *Myzus persicae* Sulzer (Homoptera: Aphididae) were reduced, and development time of the ladybeetle *Coleomegilla maculata* lengi Timberlake (Coleoptera: Coccinellidae) increased and larval weight decreased in response to heat waves (Sentis et al. 2013). Thus, the responses can vary widely depending on species, the community context and differences in experimental procedures.

Even if the direct effects of heat stress are not necessarily detrimental to natural enemies, they can still be affected by heat waves to a larger extent than herbivores because of 'bottom-up amplification' effects from the lower trophic levels (Anguilar-Fenollosa and Jacas, 2014). Higher trophic levels have to cope not only with the direct effects of heat waves but also the indirect effects stemming from the differential responses of lower trophic levels to the same heat stress, which can alter trophic and other ecological interactions (Anguilar-Fenollosa and Jacas, 2014). Within higher trophic levels the vulnerability of different groups also varies. Generalist predators that can easily switch between prey are predicted to be less sensitive to changes in trophic interactions than specialist parasitoids with narrow host ranges whose development is tightly linked to the phenology of their hosts (Foden et al. 2008). In addition, the effectiveness of parasitoids can further be reduced because of changes in host resistance with increasing temperatures. For example, parasitism was shown to be less effective at higher temperatures in *Spodoptera littoralis* Boisduval (Lepidoptera: Noctuidae) by the braconid wasp *Microplitis rufiventris*, Kokujev (Hymenoptera: Braconidae) and also in *Drosophila melanogaster* Meigen (Diptera: Drosohilidae) by *Asobara tabida* Nees von Esenbeck (Hymenoptera: Braconidae) (Fellows et al. 1999; Hegazi and Khafagi, 2005). Because of the asymmetrical effects of climate change on natural enemies and herbivores, biological control services provided by higher trophic levels can be disrupted. This is especially important in the case of invasive insects that have relatively few natural enemies in the introduced range.

In this study, we evaluated the sensitivity of an invasive insect, the brown marmorated stink, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae) and one of its natural enemies, the parasitoid *Trissolcus japonicus* Ashmead (Hymenoptera: Scelionidae) to stimulated heat waves. *Halyomorpha halys* occupies a large geographic range, as it is native to Asia and has invaded North America, Europe, and more recently, South America (Faundez and Rider 2017; Haye et al. 2015; Lee et al. 2013; Leskey and Nielsen 2018). It is highly polyphagous feeding on over 100 plant species including economically important crops (Lee et al. 2013; Leskey and Nielsen 2018). Optimal development of *H. halys* occurs between 25-30˚C and it cannot complete development when kept below 15˚C or over 35˚C (Haye et al. 2014; Nielsen et al. 2008).

Trissolcus japonicus, is a solitary oligophagous endoparasitoid that attacks eggs of several stink bug species within the family Pentatomidae (Botch and Delfosse 2018; Haye et al. 2020; Hedstrom et al. 2017; Yang et al. 2009). It is native to Asia and was accidentally introduced to North America and Europe (Stahl et al. 2019; Talamas et al. 2015). It is one of the most important egg parasitoids of *H. halys* in its native range with parasitism rates of 50-80% (Yang et al. 2009). The optimal development window of *Tr. japonicus* is between 27-30˚C with an upper threshold of 36˚C beyond which no development had been observed (Li and Liu 2004; Qiu et al. 2007). While the developmental thresholds for *H. halys* and *Tr. japonicus* are similar (35 and 36˚C) it is unknown how intermittent heat stress beyond these upper limits will impact either species. Since *Tr. japonicus* is considered the most promising biological control agent of *H. halys* in the introduced range it is important to explore how survival of both species and parasitism rates may be impacted by heat waves.

We simulated heat waves of varying intensity (36 – 42˚C), applied for 4 hours during the afternoon of five consecutive days and assessed emergence of *H. halys* and *Tr. japonicus* from unparasitized and parasitized *H. halys* eggs, respectively. We also evaluated how heat waves may affect parasitism rates by exposing adult female *Tr. japonicus* with *H. halys* eggs to the same heat waves. In addition, we measured post-generational effects by assessing parasitism success of the offspring of wasps exposed to heat waves. We predicted that the parasitoid will be more sensitive than the herbivore to heat waves based on expectations regarding differential responses of different trophic levels.

Materials and Methods

Background on study species

Halyomorpha halys overwinters as adults and terminates diapause at 12.7 hours of photoperiod (Nielsen et al. 2017). Reproductive development is mediated by temperature and differs between locations with oviposition commencing anywhere between 150 – 450 growing degree days (May/June in temperate climates) assuming 14.17˚C as the lower developmental threshold (Nielsen et al. 2017; Nielsen et al. 2008). Females lay on average 244 eggs with a median of 28 eggs per cluster over a period of four months (Nielsen et al. 2008). Eggs hatch in a week, and development proceeds through five instars with adult emergence within 41 – 48 days at 25˚C (Nielsen et al. 2008). In the invaded range *H. halys* typically completes one generation a year but as it spreads south it can become multivoltine (Nielsen et al. 2008).

Trissolcus japonicus overwinters as adults and can start development at 12.2˚C but most growth takes place at temperatures between 18 and 33˚C (Li and Liu 2004; Qiu et al. 2007). Females prefer to oviposit in stink bug eggs that are 1 – 3 days old and lay 42 eggs on average over their lifetime (Yang et al. 2009). Egg to adult development can be completed within 12 days at 25˚C resulting in up to 10 generations in a year in the native range in subtropical areas and multiple generations in temperate regions including in North America (Avila and Charles, 2008; Yang et al. 2018).

Insect rearing

Halyomorpha halys egg masses used for the experiments were either shipped from the Phillip Alampi Beneficial Insect Laboratory (New Jersey Department of Agriculture, Trenton, NJ) or came from a rearing at Michigan State University (MSU) that was initiated from eggs from the above source. In the MSU labs *H. halys* were kept in mesh cages (40 x 20 x 20 cm) (Educational Science, League City, TX, U.S.) at 25˚C and 60-75% humidity with a photoperiod of 16:8 L:D in a climate-controlled room. They were given water through dental wicks (Dynarex N/S #2 Medium) and raised on a diet of organic green beans, snap peas, broccoli, carrots, apples, and mixed nuts.

Trissolcus japonicus used for the experiments originated from a laboratory rearing at MSU. The colony was founded by two males and three females that were captured on MSU campus in August 2018 by a *H. halys* sentinel egg mass (Jarrett et al. 2019). Adults were kept in

10-dram plastic vials at 20˚C and 70% humidity with a photoperiod of 16:8 L:D in groups of 10-20 with a drop of honey for provision. One or two *H. halys* egg masses were offered for oviposition to 5-10 wasps for three days then placed in a new vial. Parasitoids took 14-20 days under these conditions to complete development.

Experimental design

Three experiments were conducted to evaluate the effects of heat waves on early life stages of *H. halys* and *Tr. japonicus* and on parasitism success of *H. halys* by *Tr. japonicus*. In Experiment 1, the impacts of heat waves on *H. halys* emergence were evaluated by exposing unparasitized *H. halys* egg masses to heat waves. In Experiment 2, the impacts of heat waves were evaluated on *H. halys* eggs parasitized by *Tr. japonicus*. In Experiment 3, the impact of heat waves on parasitism of *H. halys* eggs by *Tr. japonicus* were evaluated by exposing fresh *H. halys* eggs together with one *Tr. japonicus* female. These three experiments were run at the same time within the same climate chambers for given temperature treatments. Experiments 1 and 2 were repeated in a second independent trial and will be referred to as Experiment 1 trial 2 and Experiment 2 trial 2.

Two additional experiments were conducted to assess transgenerational effects of heat waves by evaluating the performance of the offspring of *Tr. japonicus* that had been exposed to heat waves. Experiment 4 used *Tr. japonicus* adults that emerged from parasitized *H. halys* eggs that were exposed to heat waves as part of Experiment 2, and Experiment 5 used *Tr. japonicus* adults that emerged from *H. halys* eggs that were parasitized during heat waves as part of Experiment 3.

For Experiments 1-3, five different heat wave treatments were applied: 25˚C (control), 36˚C, 38˚C, 40˚C, and 42˚C, for four hours between 12 – 4 pm for five consecutive days. Temperatures were returned to 25˚C following the heat wave treatments. Heat wave treatments were chosen to represent a range around the highest temperature (40.5˚C) recorded in Grand Rapids, Michigan based on data from www.weatherbase.com for the years 2014 - 2019. A completely randomized design was used with eight replications for each heat wave treatments for Experiments 1-3 and in the second trials of Experiments 1-2. The number of replications varied for Experiments 4 and 5 and will be described in the below subsections.

Experiment 1: Impact of heat waves on H. halys *emergence*

To assess the impact of heat waves on *H. halys* emergence, fresh *H. halys* eggs (< 72 h) were exposed to the above described heat wave treatments. Eggs were placed in 60 x 15 mm stackable Petri dishes (VWR®) in temperature-controlled incubators that were kept at 16:8 L:D photoperiod and 50-65% humidity. Emergence success and the number of emerging first instar *H. halys* nymphs were recorded during and for four days following the heat wave treatments. This experiment was repeated in two independent trials.

Experiment 2: Impact of heat waves on H. halys *eggs parasitized by* Tr. japonicus

To assess the impact of heat waves on *Tr. japonicus* development and emergence, *H. halys* eggs that had been parasitized by *Tr. japonicus* five days prior to the start of the experiment were exposed to the above described heat wave treatments. The experimental procedures were the same as described for Experiment 1. Emergence success and the number of emerging adults were measured in both the first and second trials. In addition, development time of *Tr. japonicus* was assessed in the first trial.

Experiment 3: Impact of heat waves on H. halys *egg parasitism by* Tr. japonicus

To assess the impact of heat waves on parasitism success by *Tr. japonicus*, one fresh (< 72 h old) *H. halys* egg mass together with one adult female *Tr. japonicus* were exposed to heat wave treatments. The experiments took place in 120 ml deli cups with a fine mesh top to allow air flow. A 4 cm long dental wick remoistened every day, and a green bean leaf with a drop of honey were placed in each cup to provide water, shelter and provision for the parasitoids. The heat wave treatments were as described previously. Egg masses were followed for 4 weeks and the development time, development success (or emergence success) and the number of adult *Tr. japonicus* emerging were recorded. In addition, the survival of female *Tr. japonicus* individuals used for parasitism was monitored during the 5-day exposure to heat waves. Adults surviving past the heat waves were kept at 25˚C for three days and then a fresh *H. halys* egg mass was given to each for five days to assess the effects of heat waves on their ability for further parasitism. Emergence success and the number of emerging *Tr. japonicus* was monitored from these egg masses.

Experiments 4 and 5: Transgenerational effects

To measure carry-over or transgenerational effects of heat waves one adult female parasitoid was randomly chosen from each replication that had adult *Tr. japonicus* emergence from egg masses parasitized prior to (Experiment 2 trial 1) or during (Experiment 3) heat waves. Experiment 4 refers to transgenerational effects resulting from Experiment 2 and Experiment 5 refers to transgenerational effects resulting from Experiment 3. Each female wasp was given one *H. halys* egg mass to parasitize for five days at ambient temperatures and parasitized eggs were kept at 25˚C, 16:8 L:D photoperiod and 50-65% humidity in a climate chamber. Emergence success and the number of emerging *Tr. japonicus* were monitored from these egg masses for four weeks. The number of replications varied between temperature treatments for both Experiments 4 and 5 and depended on the number of replications from which *Tr. japonicus* adults successfully emerged in Experiments 2 and 3, respectively. The number of replications is shown in Figure. 3.11.

Statistical analyses

Experiment 1: Impact of heat waves on H. halys *emergence*

Binary logistic regression was used to compare the probability of successful emergence of *H. halys* nymphs from eggs exposed to the different heat wave treatments. The response variable was emergence success, coded as either 0 in case of no emergence or 1 if at least one individual emerged from an egg mass. Temperature was included as a continuous variable. To evaluate the accuracy of the logistic regression model the position of Receiver Operating Characteristic (ROC) curves were inspected and the area under the curve (AUC) index was evaluated. ROC curves resembling a diagonal line indicate inaccurate models. The further the ROC curves are from the diagonal line and the higher the AUC is (the closer to 1) the better predictive power the model has (SAS Institute 2016). Similar logistic regression models were used for all subsequent analyses for Experiments 2-5 where the response variable was binary (i.e. emergence success or no success, or survival versus no survival).

The number of nymphs emerging from the different heat wave treatments were compared using one-way ANOVA. For this analysis, replications both with and without any emergence were included and temperature was included as a categorical variable. Data for emergence counts were log transformed using base10(x+0.5) to meet the model assumption of normal distribution, but non-transformed means are used to visualize the results. Tukey's HSD post hoc test was used to assess significant differences between temperature treatments. The two independent trials were analyzed separately. Similar one-way ANOVAs and post hoc tests were used for subsequent analyses for Experiments 2-5 to compare the number of emerging *Tr. japonicus*. All analyses were run in JMP Pro, version 13.0.0 (SAS Institute, 2016).

Experiment 2: Impact of heat waves on H. halys *eggs parasitized by* Tr. japonicus

Binary logistic regression, as described for Experiment 1, was used to compare the probability of successful emergence of *Tr. japonicus* from parasitized eggs exposed to the different heat wave treatments. One-way ANOVA, as described for Experiment 1, was used to compare the number of emerging *Tr. japonicus* adults between the different heat wave treatments. The two independent trials were analyzed separately. One-way ANOVA was used to compare development time of *Tr. japonicus* at different temperatures in trial 1 with temperature coded as a categorical variable.

Experiment 3: Impact of heat waves on H. halys *egg parasitism by* Tr. japonicus

The probability of successful emergence and the number of emerging adults of *Tr. japonicus* at the different heat wave treatments were evaluated with binary logistic regression and one-way ANOVA as described for Experiment 1. One-way ANOVA was used to compare development time of *Tr. japonicus* when exposed as a 1-5 day of larvae to different heat waves with temperature coded as a categorical variable. The 5-day survival of *Tr. japonicus* females used for parasitism during heat waves was assessed with a binary logistic regression where those that survived to day five were coded as 1 and those that did not were coded as 0. The number of emerging offspring of females exposed to different heat waves were compared with one-way ANOVA as described for Experiment 1.

Experiments 4 and 5: Transgenerational effects

The probability of successful second-generation offspring emergence of mothers exposed to heat waves either as 5-10 days old larvae (Experiment 4) or 1-5 days old larvae (Experiment 5) were evaluated using logistic regression as described for experiment one. The number of second-generation *Tr. japonicus* adults emerging for Experiment 4 and 5 in relation to maternal exposure to different heat waves were compared with one-way ANOVAs separately for the two experiments.

Results

Experiment 1: Impact of heat waves on H. halys *emergence*

Halyomorpha halys egg emergence success was significantly reduced by heat waves in both the first (χ^2=15.44, df = 1, p < 0.0001) (Figure. 3.1.A) and second trial (χ^2=29.24, df = 1, p < 0.0001) (Figure. 3.1.B). Emergence success in the first trial, defined as at least one egg hatching from a cluster of 28 in a replication decreased to 25% at 36˚C, 12.5% at 40˚C and no emergence was observed at 42˚C (Figure. 3.1.A). In the second trial overall emergence rates were higher with at least a few nymphs emerging from all replicates at 36˚C with 50% survival at 38˚C, 37.5% at 40˚C and no survival at 42˚C (Figure. 3.1.B). The number of emerging *H. halys* nymphs were negatively affected by heat waves in both the first ($F_{4,3.05} = 7.448$, $p = 0.0002$) and second trials ($F_{4,12.91} = 33.599$, $p < 0.0001$) (Figure. 3.2).

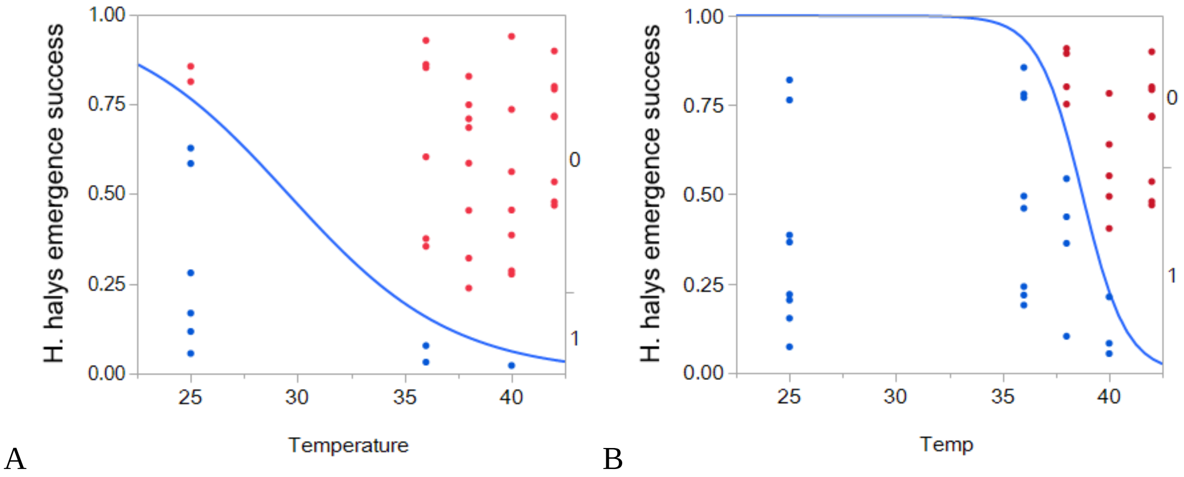

Figure. 3.1. Results of Experiment 1. Emergence success of *H. halys* nymphs from eggs exposed to an ambient temperature (25˚C) or four heat wave treatments (36, 38, 40, and 42˚C) in two independent trials (A: trial 1, B: trial 2). The curve is the logistic regression curve that indicates the probability of successful emergence at the different temperature treatments. Dots are randomly jittered in a vertical position. Blue dots under the curve indicate replicated egg masses, each with 28 eggs, from which at least one nymph emerged. Red dots above the curve are replications without any adult emergence. The reference categories on the right y axis show the probability of successful (1) or unsuccessful emergence (0) across all temperature treatments.

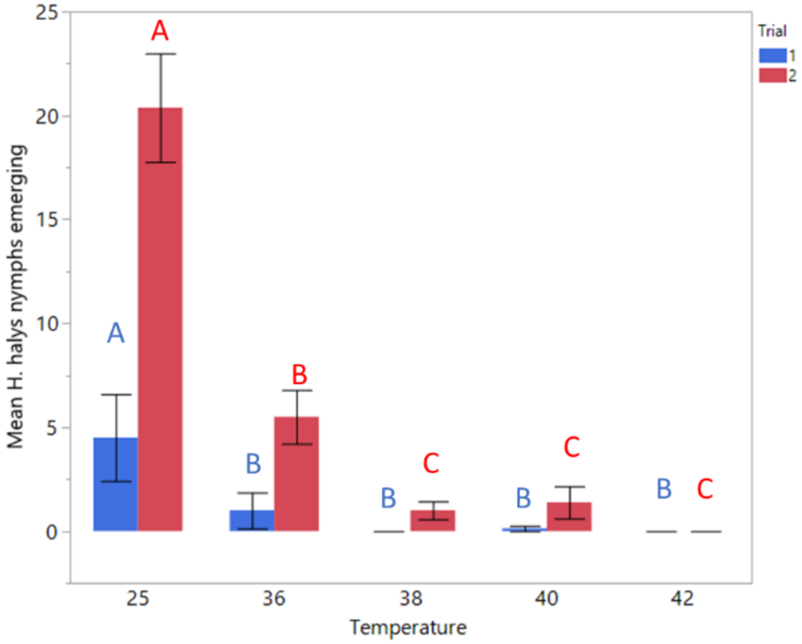

Figure. 3.2. Results of Experiment 1. Mean (± SE) *H. halys* nymphs emerging from egg masses, each with 28 eggs, that were exposed to an ambient temperature (25˚C) or four heat wave treatments (36, 38, 40, and 42˚C) in two independent trials (Blue: trial 1, Red: trial 2). Letters indicate significant difference between temperatures within each trial.

Experiment 2: Impact of heat waves on H. halys *eggs parasitized by* Tr. japonicus

Trissolcus japonicus emergence success was negatively impacted by heat waves in both the first (χ^2=17.20, $p < 0.0001$) (Figure. 3.3.A) and second trials (χ^2=7.79, $p = 0.0052$) (Figure. 3.3.B). The results of the two trials were similar showing adult emergence from all replicates exposed to 36 and 38˚C (Figure. 3.3.A). Emergence rates at 40˚C were 100% in trial 2, dropped only to 75% in trial 1, and even at 42˚C 37.5% of replications had adult emergence in trial 1 (Figure. 3.3.B). The number of adults emerging was reduced by heat waves in both the first ($F_{4,11.91} = 16.099$, $p < 0.0001$) and second trials ($F_{4,8.98} = 13.287$, $p < 0.0001$). Adult emergence was similar at 25, 36, and 38˚ C in both experiments and only started to decrease at temperatures of 40 and 42˚C (Figure. 3.4). Development time increased with increasing temperatures ($F_{4,134.23} = 20.713$, $p < 0.0001$) taking almost twice as long at 42˚C than at 25˚C (Figure. 3.5).

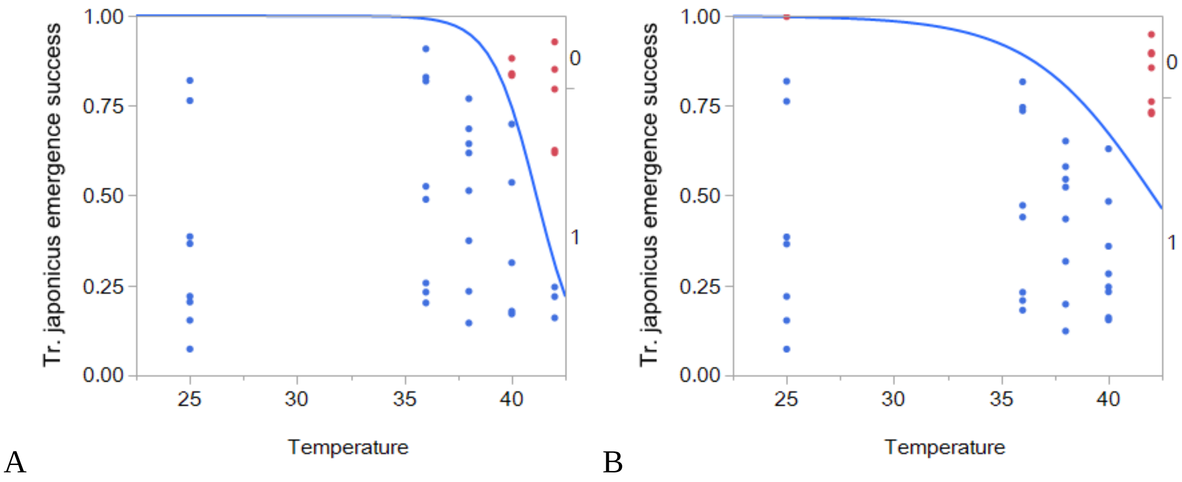

Figure. 3.3. Results of Experiment 2. Emergence success of *Tr. japonicus* from *H. halys* eggs that were parasitized prior to exposure to an ambient temperature (25˚C) or four heat wave treatments (36, 38, 40, and 42˚C) in two independent trials (A: trial 1, B: trial 2). The curve is the logistic regression curve that indicates the probability of successful emergence at the different temperature treatments. Dots are randomly jittered in a vertical position. Blue dots under the curve indicate replicated *H. halys* egg masses, each with 28 eggs, from which at least one adult *Tr. japonicus* emerged. Red dots above the curve are replications without any adult emergence. The reference categories on the right y axis show the probability of successful (1) or unsuccessful emergence (0) across all temperature treatments.

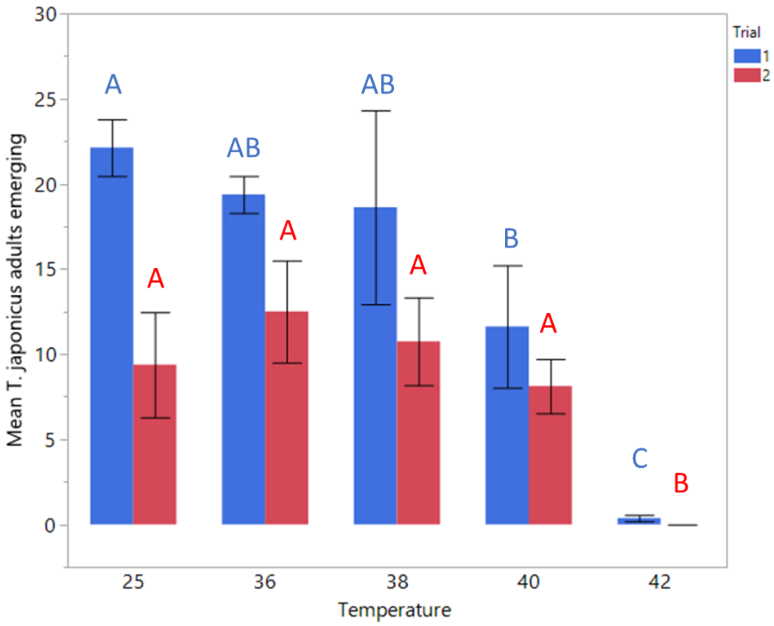

Figure. 3.4. Results of Experiment 2. Mean (± SE) number of *Tr. japonicus* adults emerging from *H. halys* egg masses, each with 28 eggs, that were parasitized prior to exposure to an ambient temperature (25˚C) or four heat wave treatments (36, 38, 40, and 42˚C) in two independent trials (A: trial 1, B: trial 2). Letters indicate significant difference between temperatures within each trial.

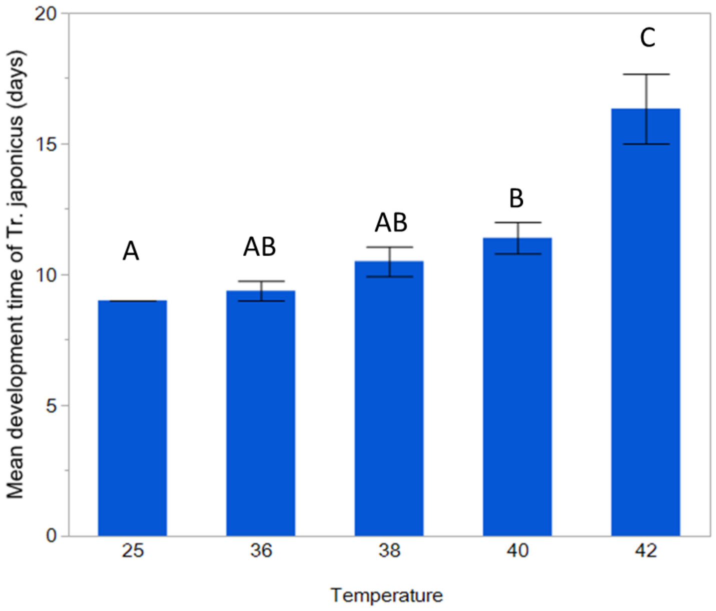

Figure. 3.5. Results of Experiment 2 trial 1. Mean (± SE) development time (days) of *Tr. japonicus* exposed to an ambient temperature (25˚C) or four heat wave treatments (36, 38, 40, and 42˚C). Note that development time here is shown as the time between the first day of heat wave treatments and the first adult emergence. Eggs were parasitized five days prior to the start of the experiment. Letters indicate significant difference between temperatures.

Experiment 3: Impact of heat waves on H. halys *egg parasitism by* Tr. japonicus

Trissolcus japonicus emergence success was similar across the different heat wave treatments (χ^2=0.874, df = 1, p = 0.3498) with adults emerging from most replications exposed to different temperatures as 1-5 days old larvae (Figure. 3.6). The number of adults emerging differed among the different heat wave treatments ($F_{4,3.5} = 2.853$, p = 0.038), a pattern driven by significant difference in emergence rates only between 36˚C and 38˚C (Figure. 3.7). Development took significantly longer when parasitism took place during heat waves of 42˚C than at lower temperatures ($F_{4, 141.02} = 21.067$, p < 0.0001) (Figure. 3.8). The survival of adults exposed to heat waves decreased significantly with increasing temperatures (χ^2=14.54, df = 1, p < 0.0001) with half of the females dying at 36˚C and all at 42˚C by day five of the experiment (Figure. 3.9). When adults that survived the 5-day exposure to heat waves were given a new *H. halys* egg mass to parasitize at ambient temperatures (25˚C) almost all the egg masses yielded similar numbers of adult *Tr. japonicus* ($F_{3, 0.44} = 0.5287$, p = 0.6689).

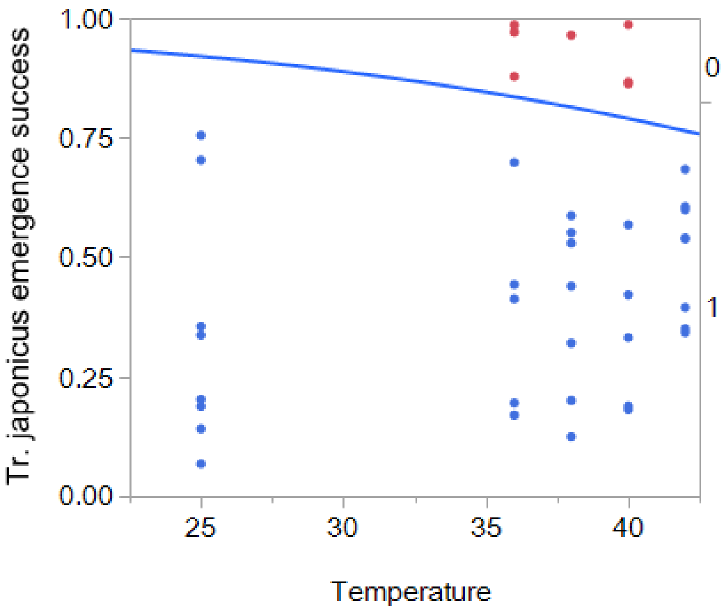

Figure. 3.6. Results of Experiment 3. Emergence success of *Tr. japonicus* from *H. halys* eggs that were parasitized during exposure to an ambient temperature (25˚C) or four heat wave treatments (36, 38, 40, and 42˚C). The curve is the logistic regression curve that indicates the probability of successful emergence at the different temperature treatments. Dots are randomly jittered in a vertical position. Blue dots under the curve indicate replicated *H. halys* egg masses, each with 28 eggs, from which at least one adult *Tr. japonicus* emerged. Red dots above the curve are replications without any adult emergence. The reference categories on the right y axis show the probability of successful (1) or unsuccessful emergence (0) across all treatments.

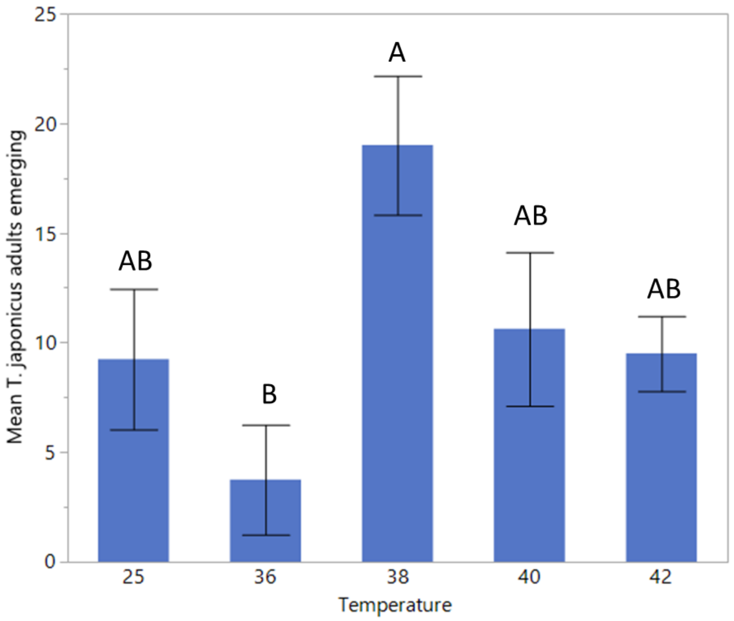

Figure. 3.7. Results of Experiment 3. Mean (± SE) number of *Tr. japonicus* adults emerging from *H. halys* egg masses, each with 28 eggs, that were parasitized during exposure to an ambient temperature (25˚C) or four heat wave treatments (36, 38, 40, and 42˚C). Letters indicate significant difference between temperatures.

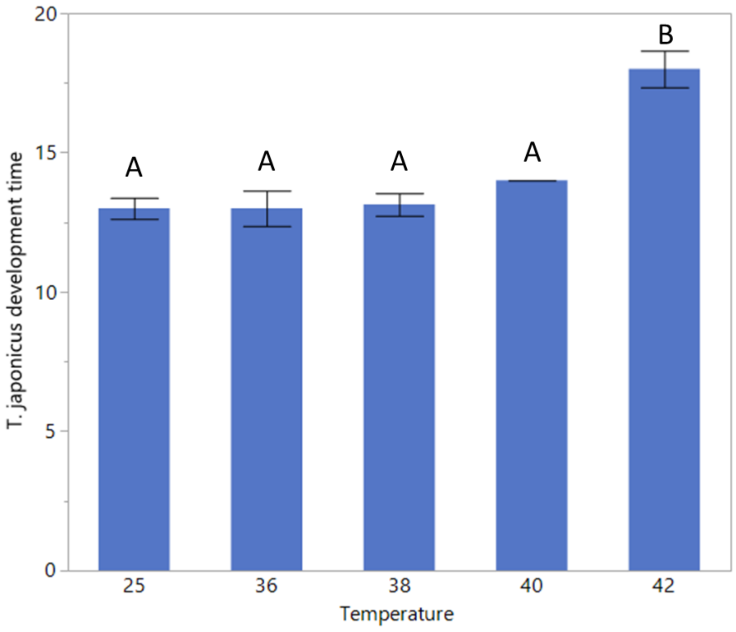

Figure. 3.8. Results of Experiment 3. Mean (± SE) development time (days) of *Tr. japonicus* exposed to an ambient temperature (25˚C) or four heat wave treatments (36, 38, 40, and 42˚C) in the first experiment. Eggs were parasitized during the heat wave treatments. Letters indicate significant difference between temperatures.

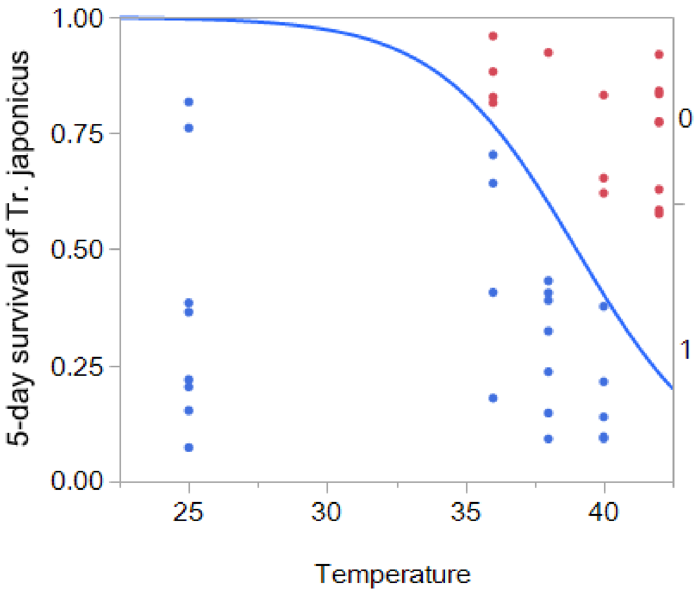

Figure. 3.9. Survival of adult *Tr. japonicus* when exposed to an ambient temperature (25˚C) or four heat wave treatments (36, 38, 40, and 42˚C) for five days. The curve is the logistic regression curve that indicates the probability of successful emergence at the different temperature treatments. Dots are randomly jittered in a vertical position. Blue dots under the curve indicate females that survived and red dots above the curve indicate females that died by day five. The reference categories on the right y axis show the probability of successful (1) or unsuccessful emergence (0) across all temperature treatments.

Experiments 4 and 5: Transgenerational effects

In Experiment 4, exposure of *Tr. japonicus* as 5-10 days old larvae to heat wave temperatures did not affect emergence success of the subsequent generation that developed at ambient temperatures (χ^2=0.874, df = 1, p = 0.3498) (Figure. 3.10.A). The number of parasitoids emerging in the second generation was similar regardless of the different temperatures the mothers experienced during their early development ($F_{4,\ 0.61}$ = 0.5372, p = 0.7096) (Figure. 3.10.B). There was, however, a transgenerational effect both for emergence success (χ^2=3.957, p = 0.0467) and in terms of the number of second-generation adults emerging ($F_{4,4.91}$ = 5.14, p = 0.0052) in Experiment 5, when the heat wave exposure occurred during parasitism and was experienced by 1-5day old *Tr. japonicus* larvae. Emergence success and the number of adults emerging declined in the second-generation with the severity of heat stress experienced by the mothers (Figures. 3.10.B, 3.11). Thus, the latter was driven by the lack of emergence at 42˚C while adult numbers were similar at all other temperatures.

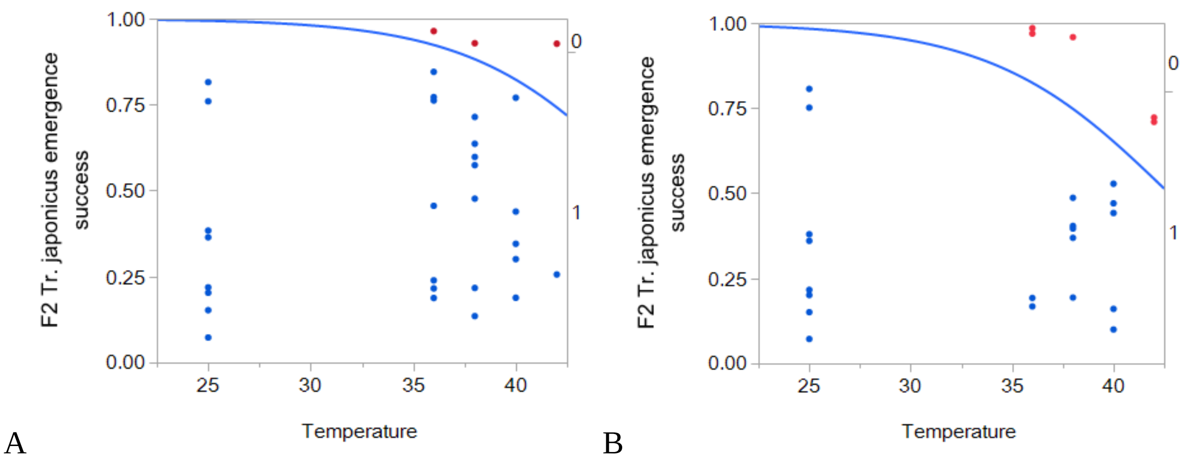

Figure. 3.10. Results of Experiments 4 and 5 testing transgenerational effects Second generation (F2) emergence rates of *Tr. japonicus* where the maternal generation (F1) was exposed either as 5-10 days (A – Experiment 4) or 1-4 days old (B – Experiment 5) larvae to an ambient temperature (25˚C) or four heat wave treatments (36, 38, 40, and 42˚C). Blue dots indicate replicated *H. halys* egg masses, each with 28 eggs, from which at least one adult emerged. Red dots are replications without any adult emergence.

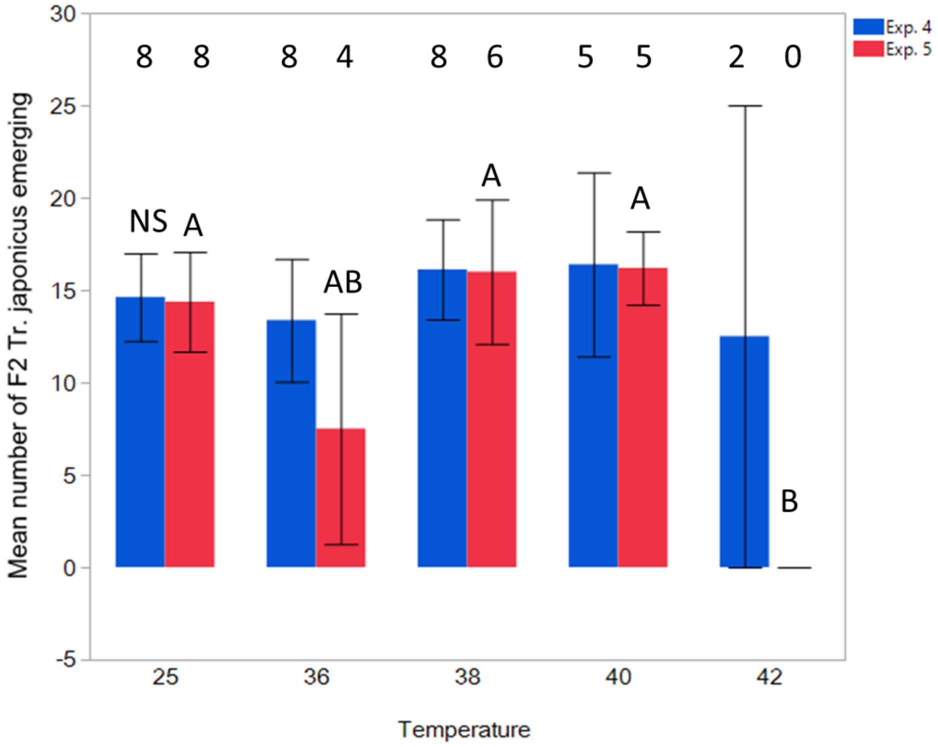

Figure. 3.11. Results of Experiments 4 and 5 testing transgenerational effects. Mean (± SE) number of second-generation (F2) *Tr. japonicus* adults emerging from mothers that were exposed either as 5-10 days (Experiment 4 - Blue) or 1-5 days (Experiment 5 - Red) old larvae to an ambient temperature (25˚C) or four heat wave treatments (36, 38, 40, and 42˚C). Letters indicate significant difference between temperatures within each experiment. Numbers above bars show the number of replications.

Discussion

Heat waves are predicted to have stronger negative effects on natural enemies than on herbivores. We found that the eggs of the herbivore, *H. halys* were more sensitive to heat waves than larvae of their egg parasitoid, *Tr. japonicus*. Heat waves did not reduce parasitism rates, but increased adult parasitoid mortality, and delayed development. We also detected trans-generational effects, whereas the emergence success and number of adults produced by *Tr. japonicus* in the second generation was negatively correlated with the severity of heat waves the maternal generation had experienced as larvae.

We exposed *H. halys* eggs to varying levels of heat waves and found that emergence success declined sharply between 36 and 38˚C with very little survival beyond these temperatures (Figures.3.1, 3.2). It has been shown that increasing temperatures can reduce development time of *H. halys* eggs without much mortality up until 30˚C, but beyond this temperature egg mortality increases with no survival at 35˚C (Nielsen et al. 2008). It appears that even intermittent temperature increases beyond this upper limit can cause significant mortality indicating that the egg stage of *H. halys* is rather sensitive to heat stress. It is well-established that different insect life stages can have differing tolerance to heat stress and that the egg stage can be particularly vulnerable (Bowler and Terblanche, 2008; Chiu et al. 2015; Kingsolver et al. 2011; Klockmann et al. 2017; Pincebourde and Casas, 2015). Thus, it is possible that nymph and adult stages of *H. halys* will be less sensitive to heat waves.

The direct effects of heat waves may well be increased egg mortality of *H. halys* and associated indirect effects might also aid *Tr. japonicus* population growth. Yang et al. (2018) found that development time of *Tr. japonicus* was reduced, emergence rates were higher, and the sex ratio became more female-biased when developing in unfertilized *H. halys* eggs as opposed to fertilized eggs. These positive effects on development are likely due to the unfertilized eggs being inviable and thus lacking resistance to parasitism (Yang et al. 2018). If heat waves of as low as 36˚C result in high egg mortality, development success of *Tr. japonicus* attacking those eggs might increase leading to higher parasitoid population sizes with higher proportion of females in them. Thus, both the direct and indirect effects of heat waves may aid control of *H. halys*. However, it is unlikely that intermittent heat waves would result in significant reduction of *H. halys* populations because they lay eggs over a period of months, therefore only a small portion of eggs may be exposed to high temperatures at any given time.

The upper threshold for *Tr. japonicus* development is very close to that of *H. halys* (36˚C vs. 35˚C, respectively) (Haye et al. 2014; Li and Liu, 2004; Qiu et al. 2007; Nielsen et al. 2008), but overall *Tr. japonicus* appears less vulnerable to intermittent exposure to temperatures beyond this upper limit than *H. halys*. We found high survival rates of *Tr. japonicus* larvae at heat waves up to 40˚C with some emergence even after exposure to 42˚C (Figures. 3.3, 3.6). Older larvae (5-10 days old) (Figures. 3.3, 3.4) was somewhat more susceptible than younger (1-5 days old) larvae, and parasitism success remained high during heat waves (Figures. 3.6, 3.7). Adult *Tr. japonicus* mortality increased with temperature with all females dying after 5 days of exposure to 42˚C heat waves (Figure. 3.9). Nevertheless, the surviving adults were able to parasitize additional eggs without any apparent reduction in efficiency, even those exposed to 40˚C, and there was no difference in offspring survival. In contrast to our results, *A. avenae* females that were exposed to 36˚C just for one hour had significantly reduced aphid parasitism rates and their offspring had lower survival (Roux et al. 2010). However, parasitism rates of *A. matricariae,* another aphid parasitoid, were not affected by heat waves of up to 40˚C imposed for seven days (Bannermann et al. 2011), which are similar to our results.

Development time of *Tr. japonicus* increased with temperature, almost doubling at 42˚C heat waves compared to 25˚C (Figures. 3.5, 3.8). At constant temperatures, development time in insects usually declines with increasing temperatures, however, this trend gets reversed close to the upper thermal limit (Liu et al. 1995; Ratte et al. 1984; Régnière et a. 2012). Our results align with this general trend since all our heat wave treatments were close to or beyond the upper thermal threshold for *Tr. japonicus* and accordingly we saw negative effects on development time. Few other studies looked at the effects of intermittent heat stress on development times in parasitoids (Gillespie et al. 2012; Schreven et al. 2017) so it is hard to generalize. In an endoparasitoid of the cabbage moth, *Diadegma semiclausum* Hellén (Hymenoptera: Ichneumonidae), a 5˚C heat pulse decreased development time but a 10˚C heat pulse, which was closer to the developmental threshold, delayed development (Schreven et al. 2017). In two aphid parasitoids, *A. abdominalis* Dalman (Hymenoptera: Aphelinidae) and *A. matricariae* mummy formation of attacked aphids was delayed but development time only increased in *A. matricariae* under heat waves of 40˚C (Gillespie et al. 2012). Longer development time of parasitoids can increase their exposure to predation and to adverse abiotic conditions that may increase mortality, and it can reduce the number of generations they are able to complete lowering their biocontrol potential. Thus, depending on which life stage of *Tr. japonicus* is exposed, heat waves may have neutral or negative effects.

Besides the direct impact of heat waves on the generation that is exposed, the experience of the parental generation can also carry over to the next generation. Such maternal effects or transgenerational effects are common in insects and have been shown to influence multiple life history traits including diapause, development time or offspring quality (Mousseau and Dingle, 1991). We found that when adults were exposed to heat waves during parasitism (F0), not only was the offspring they produced impacted by heat waves (F1) but the next generation produced by that offspring as well (F2). First, the survival of F0 adults was decreased by heat waves (Figure. 3.10). In their offspring (F1), developmental success appeared unchanged (Figure. 3.11), however, their development time increased with the intensity of heat waves they experienced as 1-5 day old larvae (Figure. 3.8). Finally, in the next generation (F2) that the F1 offspring produced emergence rates and the number of emerging adults declined in relation to the severity of heat stress their parental generations (F0 and F1) experienced (Figures. 3.10, 3.11). There are few examples for transgenerational effects of heat stress in insects and little is known of the mechanisms mediating such effects (Ismaeil et al. 2013; Ma et al. 2020; Sales et al. 2019). The aphid parasitoid *A. ervi* showed similar responses as *Tr. japonicus*, in that, the adults had reduced survival and their offspring had increased development time and lower development success after parental exposure to heat stress (Ismaeil et al. 2013). One possible mechanism may be heat stress damaging the quality of sperm both in males and in inseminated females, which can reduce fertility of the generation experiencing the heat waves as found in *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) (Sales et al. 2019). In *T. castaneum*, the fecundity and lifespan of offspring produced by sperm that was exposed to heat waves was also reduced (Sales et al. 2019).

We simulated heat waves around the highest temperature (40.5˚C) measured within the past five years in Michigan. Temperatures used in the lower range of our heat wave treatments can already be experienced in the region, while the higher ranges may be reached within a few decades if current trends continue. Contrary to expectations, we found that our treatments affected the herbivore host, *H. halys* more severely than its egg parasitoid, *Tr. japonicus*. Heat waves can directly impact both trophic levels but for *Tr. japonicus* the negative effects became only apparent at higher temperatures than for *H. halys*. Clearly, our experiments have limitations as they took place in an artificial environment where neither the host nor the parasitoids could exhibit behaviors that may mediate the impact of heat stress. Nevertheless, our findings highlight the diversity of responses different life stages of a species may have to intermittent heat stress and underscore the importance of following populations beyond the first generation when evaluating the effects of heat waves.

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