

# Heat waves affect an invasive herbivore and its parasitoid differentially with impacts beyond the first generation

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**Abstract.** Heat waves are becoming more frequent with climate change, and their impact on lower and higher trophic levels can differ. Higher trophic levels, such as predators and parasitoids, are predicted to be more strongly affected by heat waves than herbivores because they may have smaller thermal windows and because of altered ecological interactions with lower trophic levels. We tested the effects of heat waves of varying intensity (36–42°C), imposed for 4 h during five consecutive days on the egg stage of an invasive herbivore, the brown marmorated stink bug (*Halyomorpha halys*), and on various developmental stages of its parasitoid, *Trissolcus japonicus*. *Halyomorpha halys* eggs had lower hatching success and fewer nymphs emerging with increasing intensity of heat stress, while exposure during the egg and larval stages of *T. japonicus* did not decrease adult emergence rates. Heat waves increased developmental time of parasitoids and decreased adult emergence when pupal stages were exposed. Adult parasitoids experiencing heat stress had lower survival, but their parasitism success overall was not affected. In addition, we detected transgenerational effects, whereas emergence success and the number of adult parasitoids emerging in the second generation declined when the parental generation was exposed to 42°C. Contrary to expectations, the results suggest that *H. halys* may be more sensitive to heat stress than *T. japonicus*, at least during early development. Our findings indicate that biological control services provided by *T. japonicus* can be maintained in the face of increasing heat wave events and point to the importance of following populations beyond the first generation to better understand the long-term consequences of heat stress.

**Key words:** biological control; brown marmorated stink bug; climate change; *Halyomorpha halys*; transgenerational effects; *Trissolcus japonicus*.

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## INTRODUCTION

Global climate change is increasing mean temperatures and the frequency, length, and intensity of extreme weather events, such as heat waves (Ebi and Meehl 2007, Houghton 2009). Insects, such as 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 increase overwintering survival, seasonal activity windows, and geographic ranges (Parmesan et al. 1999, Bale et al. 2002, Hickling et al. 2006, Musolin and Fujisaki 2006, Deutsch et al. 2008, Berg et al. 2010). However, heat waves, defined as periods when maximum temperatures are above seasonal norms by 5°C

for at least five consecutive days (De Boeck et al. 2010), can cause direct mortality and can have negative fitness consequences for surviving individuals (Roux et al. 2010, Bannerman et al. 2011, Sentis et al. 2013, Zhang et al. 2019).

Susceptibility to thermal stress can vary between trophic levels across a food chain (Voigt et al. 2003, Hance et al. 2007, Aguilar-Fenollosa and Jacas 2014). 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 (Hance et al. 2007, Montserrat et al. 2013, Aguilar-Fenollosa and Jacas 2014, Schreven et al. 2017). However, evidence for these expectations is contradictory. For example, the parasitoid wasp *Aphidius avenae* Haliday suffered high mortality after 1 h 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-d period reduced aphid population growth but did not affect the fecundity of the parasitoid *Aphidius matricariae* Haliday (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 were reduced, and developmental time of the ladybeetle *Coleomegilla maculata* lengi Timberlake 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 detrimental, heat waves can still have larger effects on natural enemies because of “bottom-up amplification” effects from the lower trophic levels (Aguilar-Fenollosa and Jacas 2014). Higher trophic levels have to cope with not only 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. This can alter trophic and other ecological interactions (Aguilar-Fenollosa and Jacas 2014, Frank 2021). 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 or asynchrony with host phenology with increasing temperatures (Frank 2021). For example, parasitism was shown to be less effective at higher temperatures in the African cotton leafworm *Spodoptera littoralis* Boisduval by the braconid wasp *Microplitis rufiventris* Kokujev and also in *Drosophila melanogaster* Meigen by the parasitoid *Asobara tabida* Nees von Esenbeck (Fellowes 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 (Meineke et al. 2014). 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 bug, *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), and one of its natural enemies, the parasitoid *Trissolcus japonicus* Ashmead (Hymenoptera: Scelionidae), to simulated 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 (Lee et al. 2013, Haye et al. 2015, Faúndez and Rider 2017, Leskey and Nielsen 2018). It is highly polyphagous, feeding on over 100 plant species including economically important crops, such as apples, peaches, peppers, and beans (Lee et al. 2013, Leskey and Nielsen 2018). Optimal development of *H. halys* occurs between 25°C and 30°C, and it cannot complete development when kept below 15°C or over 35°C (Haye et al. 2014, Nielsen et al. 2017).

*Trissolcus japonicus* is a solitary oligophagous endoparasitoid that attacks eggs of several stink bug species within the family Pentatomidae (Hedstrom et al. 2017, Botch and Delfosse 2018, Haye et al. 2020). It is native to Asia and was accidentally introduced to North America and Europe (Talamas et al. 2015, Stahl et al. 2019). 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, Zhang et al. 2017). The optimal developmental window of *T. japonicus* is between 27°C and 30°C with an upper

threshold of 36°C beyond which no development was observed (Li and Liu 2004, Qiu et al. 2007). While the developmental thresholds for *H. halys* and *T. japonicus* are similar (35°C and 36°C, respectively), it is unknown how intermittent heat stress beyond these upper limits will affect either species. Since *T. japonicus* is considered the most promising biological control agent of *H. halys* through the introduced range, it is important to explore how survival of both species and parasitism rates may be affected by heat waves.

We simulated heat waves of varying intensity (36–42°C), applied for 4 h during the afternoon of five consecutive days, and exposed *H. halys* eggs and different developmental stages of *T. japonicus*. We assessed nymphal emergence of *H. halys* and adult emergence and parasitism rates of *T. japonicus*. In addition, we measured transgenerational effects by evaluating parasitism success of the offspring of wasps that had been exposed to heat waves. We predicted that the parasitoid will be more negatively affected than the herbivore by heat waves based on expectations regarding differential responses of different trophic levels.

## METHODS

### Insect rearing

*Halyomorpha halys* egg masses used for the experiments were either shipped from the Phillip Alampi Beneficial Insect Laboratory (PABIL) (New Jersey Department of Agriculture, Trenton, New Jersey, USA) or came from a rearing colony at Michigan State University (MSU) that was initiated from eggs from the above source. In the MSU laboratories, *H. halys* were kept in mesh cages (40 × 20 × 20 cm) 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 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 three females and two males that were captured on the MSU campus in August 2018 by rearing from 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 d under these conditions to complete development. *Trissolcus japonicus* eggs hatch on day 1 upon parasitism (~16 h), and development proceeds through three larval instars during days 1–5, a prepupa (days 5–8), and a pupal stage (13–20 d) prior to adult emergence (Li 2002, Giovannini et al. 2021).

### Effect of heat waves on *H. halys* eggs

In the first experiment, to assess the effect of heat stress on *H. halys* embryos, unparasitized eggs (<72 h) were exposed to five different heat wave treatments: 25°C (control), 36°C, 38°C, 40°C, and 42°C. Heat waves were imposed for 4 h between 12:00 and 16:00 for five consecutive days. Temperatures were returned to 25°C following the heat wave treatment. A completely randomized design was used with eight replications for each heat wave treatment. One replication consisted of one egg mass that contained on average 28 individual eggs. Egg masses were placed in 60 × 15 mm stackable petri dishes in temperature-controlled incubators that were kept at 16:8 L:D photoperiod and 50–65% humidity. Hatching success and the number of first instar *H. halys* nymphs emerging were recorded during and for four days following the heat wave treatments. The experiment was repeated in two independent trials.

### Effect of heat waves on *H. halys* eggs parasitized by *T. japonicus*

In the second experiment, to assess the effect of heat waves on *T. japonicus* development and emergence, *H. halys* eggs that had been parasitized by *T. japonicus* five days prior to the start of the experiment and thus were 5- to 10-d-old prepupae and pupae were exposed to heat waves using the same experimental procedures as described above. The experiment was repeated in two independent trials with eight replications for each trial. Emergence success and the number of emerging adults were measured in both the first and second trials. In addition, developmental time of *T. japonicus* was assessed in the first trial.

For the first trial, the experiment continued beyond monitoring the first generation of *T. japonicus* emerging from *H. halys* eggs to assess carry-over or transgenerational effects of the 5-d

exposure to heat waves. One adult female parasitoid was randomly chosen from each replication that had adult *T. japonicus* emergence. Each female wasp was given one *H. halys* egg mass for five days to parasitize 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 *T. japonicus* were monitored from these egg masses for four weeks. The number of replications depended on the number of replications from which *T. japonicus* adults successfully emerged from the first generation at each temperature treatment. At 25°C, 36°C, 38°C, and 40°C, there were eight, at 40°C five, and at 42°C two replications.

#### **Effect of heat waves on *T. japonicus* eggs, larvae, adults, and parasitism success**

In the third experiment, fresh (<72 h old) *H. halys* eggs together with adult female *T. japonicus* were exposed to evaluate the effects of heat waves on the egg and larval (1–5 d old) stages of *T. japonicus*, parasitism success, and adult parasitoid survival. The experiments took place in 120-mL deli cups with a fine mesh top to allow airflow. A 4 cm long dental wick remoistened every day, and a green bean leaf with a drop of honey was placed in each cup to provide water, shelter, and provision for the parasitoids. The heat wave treatments were as described previously. There were eight replications for each heat wave treatment with one replication consisting of one *H. halys* egg mass and one adult female *T. japonicus*. Egg masses were followed for 4 weeks, and the developmental time, emergence success, and the number of adult *T. japonicus* emerging were recorded. In addition, the survival of female *T. japonicus* individuals used for parasitism was monitored during the 5-d exposure to heat waves.

Transgenerational effects were assessed similarly as described for Experiment 2. One emerging *T. japonicus* female that had been exposed to heat waves as an egg and then as a 1- to 5-d-old larva was randomly chosen from each replication of each temperature treatment. These females were then provided one *H. halys* egg mass each to parasitize at ambient temperatures, and emergence success and the number of emerging *T. japonicus* were monitored in the second

generation. At 25°C, there were eight, at 36°C four, at 38°C six, at 40°C five, and at 42°C two replications.

#### **Statistical analyses**

Binary logistic regression was used in analyses where the response variable was binary (i.e., emergence success or no success, or survival versus no survival). Binary logistic regression was used to compare the probability of successful emergence of nymphs from *H. halys* eggs in Experiment 1 and emergence of *T. japonicus* adults from experiments 2 and 3 both in the first and in the second generation. 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. Similarly, differences in 5-d survival of *T. japonicus* adults exposed to different heat waves in Experiment 3 were compared with logistic regression. Temperature was included as a continuous variable in all logistic models. To evaluate the accuracy of the logistic regression models, the position of receiver operating characteristic (ROC) curves was 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 the predictive power the model has (SAS Institute Inc. 2016).

The number of *H. halys* nymphs in Experiment 1 and the number of *T. japonicus* adults emerging from the different heat wave treatments in experiments 2 and 3 in the first and second generations were compared using the method of standard least squares. 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  $\text{base}10(x + 0.5)$ . Tukey's HSD post hoc test was used to assess significant differences between temperature treatments. The two independent trials were analyzed separately. For trial 1 of experiments 2 and 3, *T. japonicus* developmental time was compared among heat wave treatments using standard least squares where temperature was coded as a categorical variable. All analyses were conducted using JMP Pro (JMP®, Version 13.0.0).

## RESULTS

### Effect of heat waves on *H. halys* emergence

*Halyomorpha halys* egg hatching success was significantly reduced by heat waves in both the first ( $\chi^2 = 15.44$ ,  $P < 0.0001$ ) (Fig. 1a) and second ( $\chi^2 = 29.24$ ,  $P < 0.0001$ ; Fig. 1b) trials. Hatching 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 and 12.5% at 40°C, and no hatch was observed at 38°C and 42°C (Fig. 1a). In the second trial, hatching success was overall higher with at least a few nymphs emerging from all replicates at 36°C, 50% of egg masses producing at least one nymph at 38°C, 37.5% at 40°C, and no hatch at 42°C (Fig. 1b). The number of *H. halys* nymphs emerging were negatively affected by heat waves in both the first ( $F_{4,3.05} = 7.448$ ,  $P = 0.0002$ ) and second trials ( $F_{4,12.91} = 33.599$ ,  $P < 0.0001$ ) showing significant declines at any heat wave treatments compared with the control temperature (Fig. 2a).

### Effect of heat waves on *H. halys* eggs parasitized by *T. japonicus*

*Trissolcus japonicus* adult emergence success was reduced by heat waves in both the first ( $\chi^2 = 17.20$ ,  $P < 0.0001$ ) (Fig. 1c) and second ( $\chi^2 = 7.79$ ,  $P = 0.0052$ ) (Fig. 1d) trials when exposed as 5- to 10-d-old pupae/prepupae during development in *H. halys* eggs. The results of the two trials were similar showing adult emergence from all replicates exposed to 36°C and 38°C. Emergence success at 40°C was 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 (Fig. 1c, d). 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 ( $F_{4,8.98} = 13.287$ ,  $P < 0.0001$ ) trials (Fig. 2b). Adult emergence was similar at 25°C, 36°C, and 38°C in both trials and only started to decrease at temperatures of 42°C in trial 2 and at 40°C and 42°C in trial 1 (Fig. 2b). Developmental time increased with increasing temperatures ( $F_{4,134.23} = 20.713$ ,  $P < 0.0001$ ) taking almost twice as long at 42°C (16.3 d  $\pm$  0.73, mean  $\pm$  SE) than at 25°C (9  $\pm$  0.45) (Appendix S1: Fig. S1).

Transgenerational effects were not apparent for females that had been exposed as 5- to 10-d-old prepupae/pupae to heat waves (Figs. 1g, 2d). Emergence success of the second generation (F2)

of parasitoids produced by exposed *T. japonicus* females was similar, regardless of the intensity of heat waves the mothers experienced ( $\chi^2 = 2.07$ ,  $P = 0.1501$ ) (Fig. 1g). The number of parasitoids emerging in the second generation was not affected either by the different temperatures the mothers experienced during their early development ( $F_{4,0.61} = 0.5372$ ,  $P = 0.7096$ ; Fig. 2d).

### Effect of heat waves on *T. japonicus* eggs, larvae, adults, and parasitism success

The survival of females exposed to heat waves for five days during parasitism of *H. halys* eggs decreased significantly with increasing temperatures ( $\chi^2 = 14.54$ ,  $P < 0.0001$ ; Fig. 1e). All females survived at 25°C, females started dying at 36°C on day 2 with half of the replicates dying by day 5, and all females died after a single 4-h exposure to 42°C after day 1 (Appendix S1: Fig. S2). Despite the increasing mortality of *T. japonicus* with increasing heat waves, females were able to parasitize *H. halys* eggs to similar extents resulting in similar adult emergence success across the different heat wave treatments ( $\chi^2 = 0.874$ ,  $P = 0.3498$ ) (Fig. 1f). While the number of adults that emerged differed among the heat wave treatments ( $F_{4,3.5} = 2.853$ ,  $P = 0.038$ ), this pattern was driven by lower emergence rates at 36°C than at 38°C and there were no differences in emergence rates between 25°C and 40°C or 42°C (Fig. 2c). Development took significantly longer when parasitism took place during heat waves of 42°C (18 d  $\pm$  0.46) than at lower temperatures (25°C: 13  $\pm$  0.46) ( $F_{4,141.02} = 21.067$ ,  $P < 0.0001$ ) (Appendix S1: Fig. S3).

There was a transgenerational effect both for emergence success ( $\chi^2 = 3.957$ ,  $P = 0.0467$ ) (Fig. 1h) and in terms of the number of second-generation adults emerging ( $F_{4,4.91} = 5.14$ ,  $P = 0.0052$ ; Fig. 2d), when the heat wave exposure occurred during parasitism and was experienced by eggs and 1- to 5-d-old *T. japonicus* larvae. Both emergence success and the number of adults emerging declined in the second generation when the mothers experienced the highest heat stress (42°C; Figs. 1h, 2d).

## DISCUSSION

Heat waves are predicted to have stronger negative effects on natural enemies than on herbivores. We found that the eggs of the herbivore,

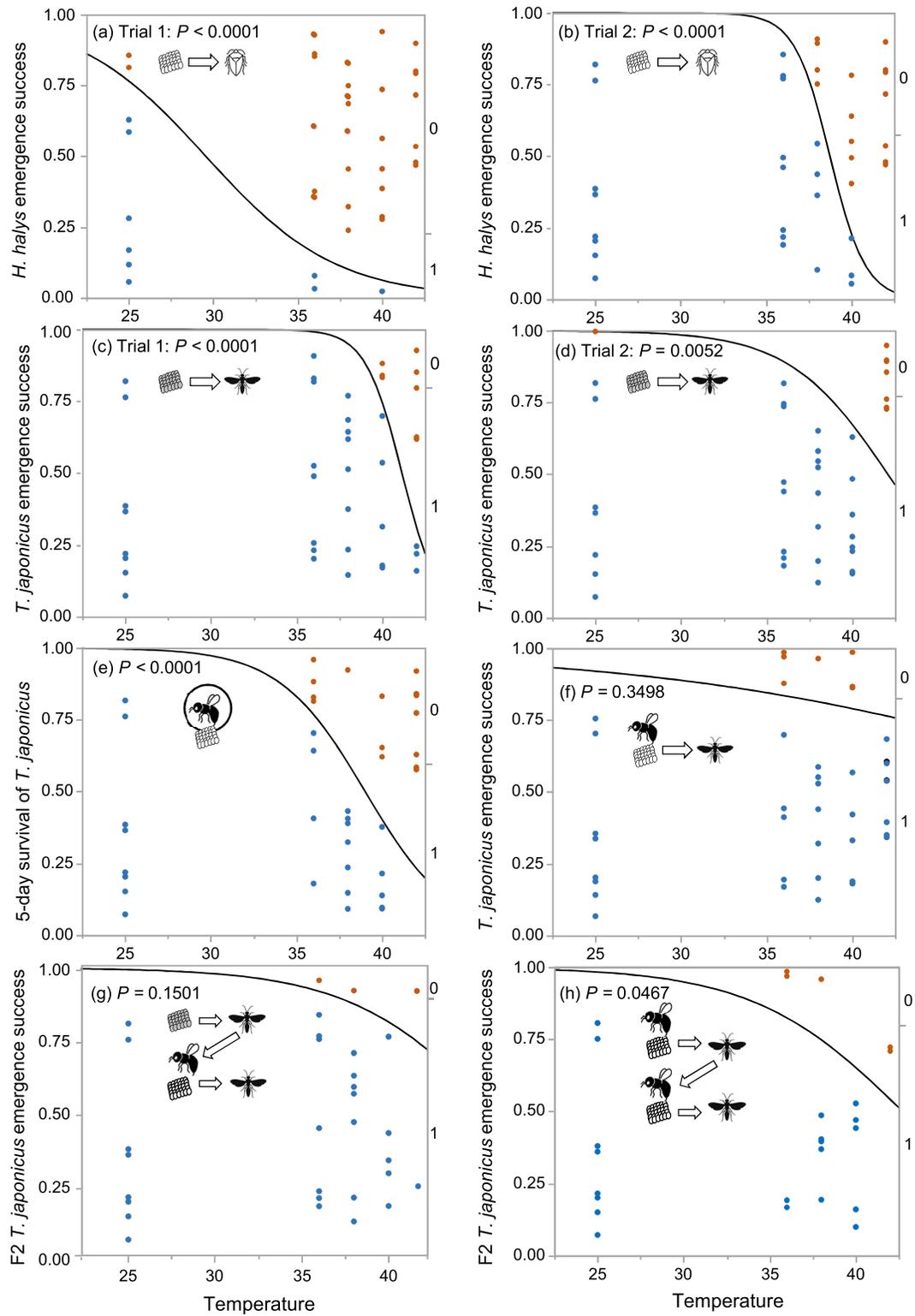


Fig. 1. (a, b) Emergence success of *Halyomorpha halys* nymphs from egg masses, each with 28 eggs, exposed to an ambient temperature (25°C) or four heat wave treatments (36°C, 38°C, 40°C, and 42°C) in two independent

(Fig. 1. *Continued*)

trials. (c, d) Emergence success of *Trissolcus japonicus* from *H. halys* egg masses that were parasitized 5 d prior to heat waves and exposed as 5- to 10-d-old prepupa/pupa in two independent trials. (e) Survival of adult *T. japonicus* exposed to heat wave treatments for five days during parasitism of *H. halys* eggs. (f) Emergence success of *T. japonicus* from *H. halys* egg masses that were parasitized during heat waves and exposed as eggs and 1- to 5-d-old larvae. (g, h) Second-generation (F2) emergence rates of *T. japonicus* where the maternal generation (F1) was exposed either as 5- to 10-d-old prepupa and pupa (g) or 1- to 5-d-old larvae (h) to heat waves. The curve is the logistic regression curve that indicates the probability of successful emergence at the different temperature treatments. Dots are randomly scattered in a vertical position. Blue dots under the curve indicate replicated egg masses from which at least one *H. halys* nymphs or *T. japonicus* adults emerged. Red dots above the curve are replications without any emergence. The reference categories on the right y-axis show the probability of successful (1) or unsuccessful emergence (0) across all temperature treatments.

*H. halys*, were more sensitive to heat stress than eggs of their egg parasitoid, *T. japonicus*. Heat waves did not reduce parasitism rates, but increased adult parasitoid mortality and delayed parasitoid development. We also detected trans-generational effects, whereas the emergence success and number of adults produced by *T. japonicus* in the second generation were reduced when the maternal generation was exposed to 42°C as eggs and larvae.

When *H. halys* eggs were exposed to heat waves, hatching success declined to 0–50% at 38°C with little survival and few nymphs hatching beyond these temperatures (40–42°C) (Figs. 1a, b, 2a). It has been shown that increasing temperatures can reduce developmental 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, Kingsolver et al. 2011, Chiu et al. 2015, Pincebourde and Casas 2015, Klockmann et al. 2017). Thus, it is possible that nymph and adult stages of *H. halys* will be less sensitive to heat waves (Haye et al. 2014). Nevertheless, negative effects of the same heat waves on the egg or larval stages of *T. japonicus* were not evident (Figs. 1f, 2c).

There were some differences between the two independent trials with trial 1 showing overall lower survival and numbers of *H. halys* nymphs

hatching compared with trial 2. These differences likely stem from the quality of *H. halys* eggs used in the two experiments. Infection by the microsporidian *Nosema maddoxi* can reduce *H. halys* egg viability (Preston et al. 2020), and signs of infection have been evident in our laboratory colonies from which eggs were used in trial 1. In trial 2, most *H. halys* eggs originated from the Philip Alampi Beneficial Insect Laboratory where *N. maddoxi* has not been present (A. Lovero *pers. comm.*). Since *N. maddoxi* is found in up to 28% of *H. halys* in the wild (Hajek et al. 2018), it would be interesting to test explicitly how infection by this microsporidian may mediate responses of *H. halys* to heat waves. Another possible mechanism that may have affected responses of *H. halys* to heat waves could stem from mortality of their gut-associated symbionts that are sensitive to heat, and without which stink bug fitness is lowered (Prado et al. 2010).

Heat waves may directly increase egg mortality of *H. halys*. In addition, the death of *H. halys* eggs might also aid *T. japonicus* population growth indirectly. Yang et al. (2018) found that developmental time of *T. 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 can result in high egg mortality, developmental success of *T. 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

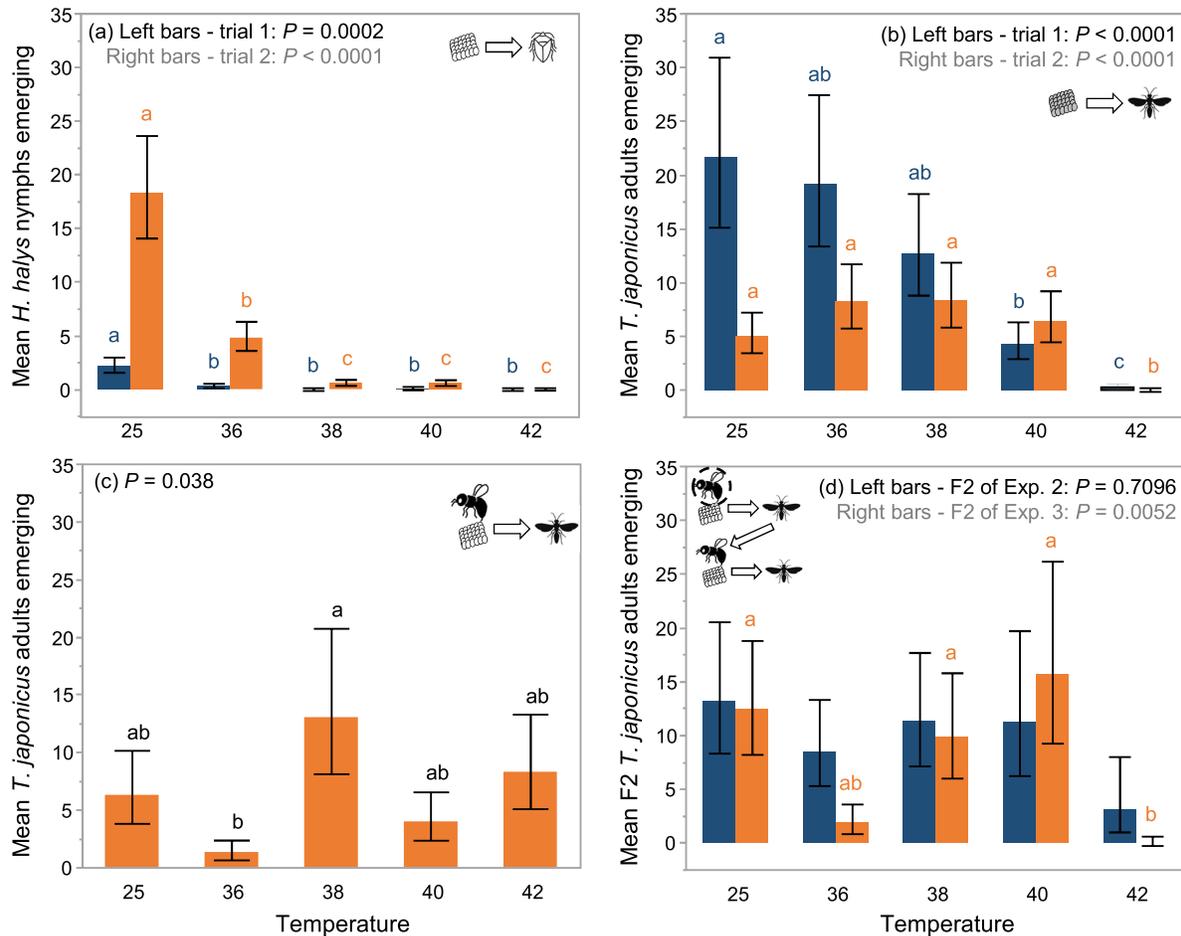


Fig. 2. Number of *Halyomorpha halys* nymphs (a) and *Trissolcus japonicus* adults emerging (b, c, d) following exposure to an ambient temperature (25°C) or four heat wave treatments (36°C, 38°C, 40°C, and 42°C) during different life stages. (a) Mean number of *H. halys* nymphs emerging from egg masses, each with 28 eggs, that were exposed in two independent trials. (b) Mean number of *T. japonicus* adults emerging from *H. halys* egg masses that were parasitized five days prior to heat waves and exposed as 5- to 10-d-old prepupa/pupa in two independent trials. (c) Mean number of *T. japonicus* adults emerging from *H. halys* egg masses that were parasitized for five days during heat waves and exposed as eggs and 1- to 5-d-old larvae. (d) Mean number of second-generation (F2) *T. japonicus* adults emerging from mothers that were exposed either as 5- to 10-d-old prepupa/pupa (blue left bars) or 1- to 5-d-old larvae (orange right bars) to heat waves. Back-transformed means  $\pm$  SE are shown, and letters indicate significant difference between temperatures within each experiment.

waves may aid control of *H. halys*. However, it is unlikely that intermittent heat waves would result in significant reduction in *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 *T. japonicus* development is very close to that for *H. halys* (36°C vs. 35°C, respectively; Li and Liu 2004, Qiu et al.

2007, Nielsen et al. 2008, Haye et al. 2014), but overall *T. japonicus* appears less vulnerable to intermittent exposure to temperatures beyond this upper limit than *H. halys*. We found high survival rates of *T. japonicus* larvae and pupae at heat waves of up to 40°C with some emergence even after exposure to 42°C (Figs. 1c, d, f, 2b, c). The prepupal and pupal stages in days 5–10 during development (Figs. 1c, d, 2b) were somewhat

more susceptible than 1- to 5-d-old larvae (Figs. 1f, 2c). Adult *T. japonicus* mortality increased with temperature, but more than half of the females survived a 5-d exposure to 40°C and parasitism success remained high during heat waves (Fig. 1f). Even though all females died by day 2 when exposed to 42°C heat waves (Fig. 1e, Appendix S1: Fig. S2), eggs laid during the first day were able to develop and had similar emergence success and number of adults emerging than those not exposed to heat waves (Figs. 1f, 2c). In contrast to our results, females of the parasitoid *A. avenae* that were exposed to 36°C just for 1 h had significantly reduced aphid parasitism rates and their offspring had lower survival (Roux et al. 2010). Similarly, *Trichogrammatoidea bactrae* Nagaraja emergence success was reduced by more than 80% when exposed to 39°C for 8 h as pupa, and the emerging adults lived for less than two days (Wang et al. 2014). On the other hand, parasitism rates of the aphid parasitoid *A. matricariae* were not affected by heat waves of up to 40°C imposed for seven days (Bannerman et al. 2011), which are similar to our results.

Developmental time of *T. japonicus* increased with temperature, almost doubling when experienced 42°C heat waves compared with 25°C (Appendix S1: Fig. S1). At constant temperatures, developmental time in insects usually declines with increasing temperatures; however, this trend is reversed close to the upper thermal limit (Ratte 1985, Liu et al. 1995, Régnière et al. 2012). Our results align with this general trend since all our heat wave treatments were close to or beyond the upper thermal threshold for *T. japonicus*, and accordingly, we saw negative effects on developmental 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), making it hard to generalize. In an endoparasitoid of the cabbage moth, *Diadegma semiclausum* Hellén, a 5°C heat pulse decreased developmental 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 and *A. matricariae*, mummy formation of attacked aphids was delayed but developmental time increased only in *A. matricariae* under heat waves of 40°C (Gillespie et al. 2012). Longer developmental 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 *T. japonicus* is exposed, heat waves may have neutral or negative effects.

Besides the direct effect of heat waves on the generation that is exposed, the experience of the parental generation can 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, developmental 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 affected by heat waves (F1) but the next generation produced by that offspring as well (F2). Looking at the impact of heat waves in sequence, first we found that the survival of exposed F0 adults decreased (Fig. 1e). In their offspring (F1), developmental success appeared unchanged (Fig. 1f); however, their developmental time increased with the intensity of heat waves they experienced as 1- to 5-d-old larvae (Appendix S1: Fig. S3). Finally, in the next generation (F2) that the F1 offspring produced emergence rates and the number of emerging adults declined when their parental generations (F0 and F1) experienced 42°C (Figs. 1h, 2d). 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, Sales et al. 2018, Ma et al. 2020). The aphid parasitoid *A. ervi* showed similar responses as *T. japonicus*, in that the adults had reduced survival and their offspring had increased developmental time and lower developmental 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 the flour beetle, *Tribolium castaneum* Herbst (Sales et al. 2018). In *T. castaneum*, the fecundity and life span of offspring produced by sperm that was exposed to heat waves were also reduced (Sales et al. 2018).

We simulated heat waves based upon 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 heat waves affected eggs of the herbivore host, *H. halys*, more severely than eggs of its parasitoid, *T. japonicus*. Heat waves directly affected both trophic levels, but for *T. japonicus*, the negative effects became apparent only at higher temperatures than for *H. halys*, and only in the pupal and adult stages. 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 effect 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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## DATA AVAILABILITY

Data are available from Dryad: <https://doi.org/10.5061/dryad.9w0vt4bfd>

## SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3796/full>