Assessing Life History Parameters of *Trissolcus japonicus* and *Anastatus reduvii*, Parasitoids of the Brown Marmorated Stink Bug

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INTRODUCTION

The Brown marmorated stink bug, *Halyomorpha halys* (Stål), is a major agricultural pest. It was first detected in the United States in mid-1990's and has since spread across Europe and North America (Rice et al., 2014). As part of an integrated effort to manage *H. halys*, methods of biological control were investigated. Biological control takes advantage of natural enemies such as predators, parasitoids, or pathogens to reduce pest populations.

Fig. 1a) *Trissolcus japonicus*. Photo by Oregon Department of Agriculture

Trissolcus japonicus (Ashmead) is a parasitoid native to South Korea, China, Taiwan and Japan that is now found in the United States (Talamas et. al., 2015). It is known for its short developmental time (Avila & Charles, 2018) and high rates of parasitism of *H. halys* eggs (Fig. 1a). *Trissolcus japonicus* is a specialist parasitoid of stink bugs (Pentatomidae).

Anastatus reduvii (Howard) is a native generalist across orders of insects (Fig. 1b) and is found in the Northeastern United States. It is a major parasitoid of *H. halys* in Maryland nurseries (Jones et al., 2017). *Trissolcus japonicus* and *A. reduvii* are believed to be strong candidates for biological control of *H. halys*.

Fig. 1b) *Anastatus reduvii*. Photo by Emily Ogburn, NCSU

Objectives

The goal of this study was to obtain a better understanding of *T. japonicus* and *A. reduvii* effectiveness as biological control agents of *H. halys* by analyzing their life history parameters. Several features measured regarding their longevity and reproduction potential were investigated. Specific factors included: 1) fecundity and progeny emergence, 2) mean lifespan of progeny, and 3) male/female sex ratio of progeny.

METHODS

Parasitoid Rearing

Colonies of 25-50 *T. japonicus* or *A. reduvii* were maintained in polystyrene containers and kept in growth chambers at 28° C and 66% relative humidity. Parasitoid colonies were fed honey.

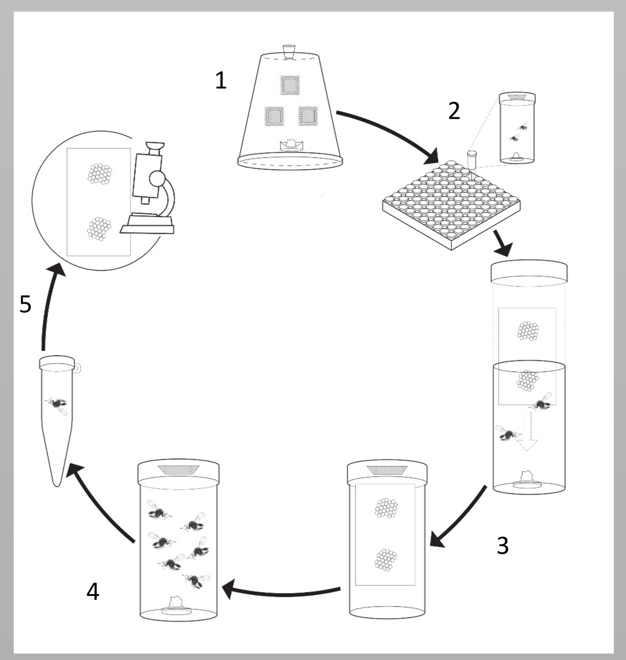

Fig. 2. Layout of laboratory tasks used to measure parasitoid longevity and reproduction potential.

Experimental Procedure (Fig. 2)

1. Laboratory colonies reared growth chambers.
2. Ten pairs of male and female *T. japonicus* were placed into independent 10-dram ("F0 vials"). Ten female *A. reduvii* were separated and placed in F0 vials; female-only due to the inconsistency of *A. reduvii* producing males within colonies.
3. An egg card containing two frozen *H. halys* egg masses were placed in each F0 vial. After 48 hrs, egg cards were removed and placed into a separate vial ("F1 vials") containing progeny. A new egg card was placed in each F0 vial every 48 hours until female death.
4. Progeny emergence vials were checked daily for emerged parasitoids (< 24 hrs in age) and placed into separate vials ("F1 longevity vials").
5. F1 longevity vials checked daily for mortality.

Egg Masses (Fig. 3)

All egg masses were dissected to measure rates of oviposition and parasitism. Discolored eggs with exit holes were classified as parasitoid emergence (Fig. 3a). Unhatched eggs containing parasitoids inside were classified as non-egressed parasitoids. Eggs with no signs of parasitism were classified as non-parasitized (Fig. 3b).

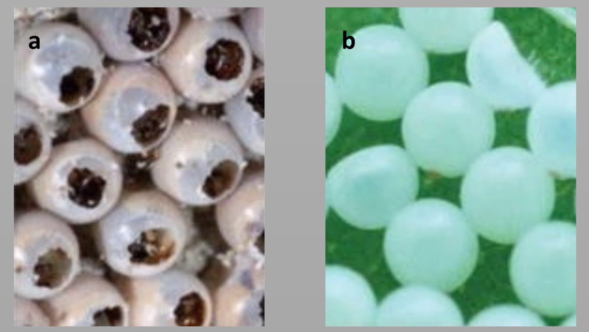

Fig. 3a, b. a) Parasitoid emergence and b) non-parasitized eggs. Photo from Jones et. al, 2017

RESULTS

Progeny Longevity

The life history data of *A. reduvii* and *T. japonicus* were analyzed using R Statistical Program (RStudio, Inc., 2016) and Excel (Microsoft, 2020). Female progeny of *T. japonicus* (40.66 ± 0.95 (SE); n =568) lived significantly longer than males (28.13 ± 0.78 (SE); n =504; p = 2.2e-16). Females of *A. reduvii* lived significantly more days (34.64 ± 0.41 (SE); n =1033) than males (18.96 ± 0.85 (SE); n =134; p = 2.2e-16).

In order to quantify the energy cost of oviposition, female F1 individuals, which were not given egg masses were compared to F0 females, which were given egg masses. Female *T. japonicus* (40.66 ± 0.95 (SE); n=568) lived significantly longer than females who had access to *H. halys* eggs (23.5 ± 2.03 (SE) days; n =10; p = 3.111e-06). However, female *A. reduvii* (34.64 ± 0.41 (SE); n =1033) did not live significantly longer than females who had access to *H. halys* eggs (30± 4.68 (SE) days; n =10; p = 0.2299).

Fecundity and Reproductive Rate

Fecundity was measured by recording the average number of F1 parasitoids that emerged daily during female F0 lifespan. For *T. japonicus* F0, peak fecundity was 2-4 days after F0 emergence (maximum=203, minimum=1) (Fig. 4a). The peak fecundity of *A. reduvii* was 6-10 days after F0 emergence (maximum=124, minimum=1, Fig. 4b).

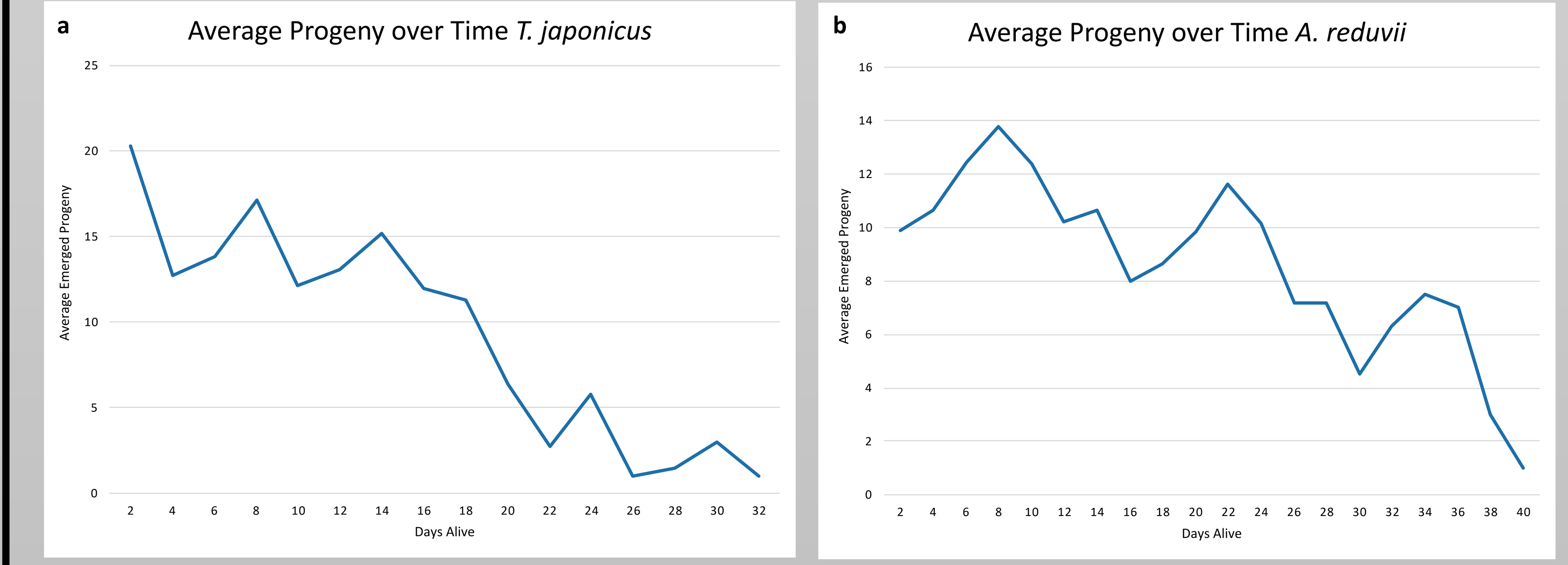

Figure 4a, b. The average fecundity of a) *T. japonicus* and b) *A. reduvii* F0 females (n=10) during their lifetime. Fecundity was measured by calculating the average number of emerged progeny from egg cards provided to replicate females every two days throughout their lifespan.

Male/female Sex Ratio

The mean total female to male ratio of *T. japonicus* F1 parasitoids was 1.3:1. The female to male ratio of *A. reduvii* F1 parasitoids was 7.7:1. The sex ratio of both *T. japonicus* (Fig. 5a) and *A. reduvii* (Fig. 5b) varied over time.

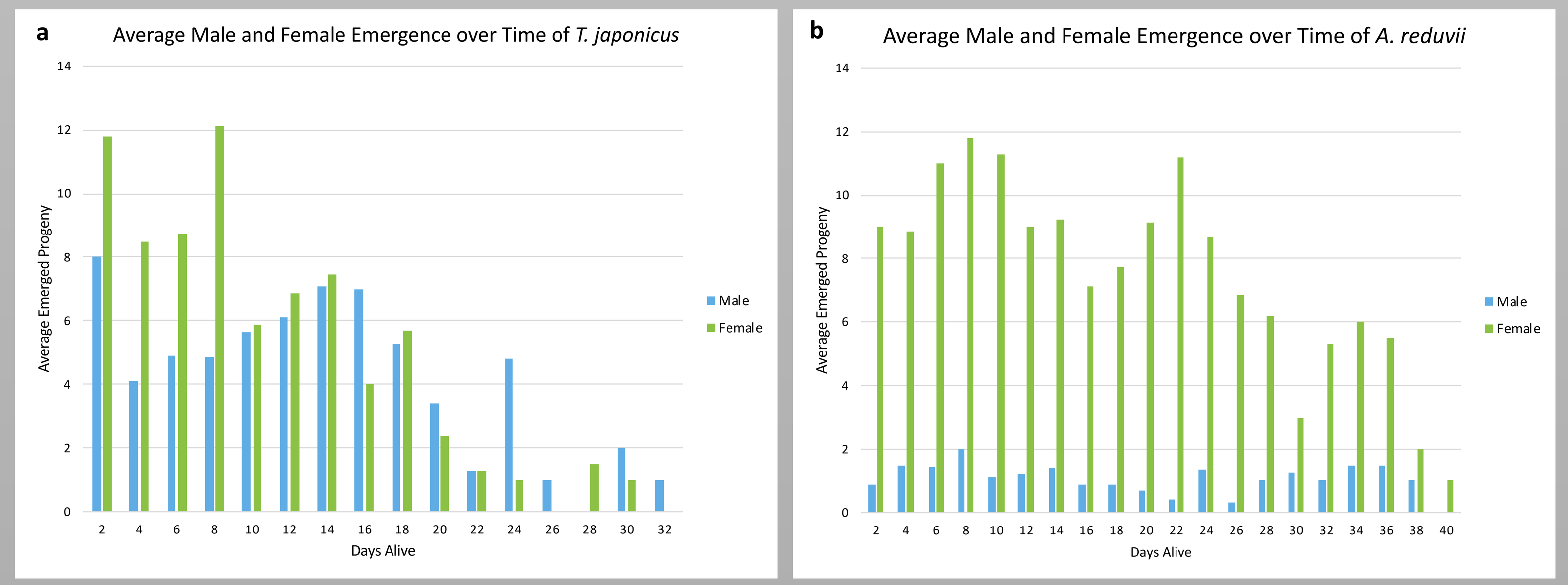

Figure 5a, b. The average male to female ratio of emerged F1 parasitoids from *H. halys* eggs parasitized by a) *T. japonicus* and b) *A. reduvii* F0 females every two days during their lifetime.

Egg fate

Egg masses (n=368) were dissected to determine the fate of each egg and classified as parasitoid emergence, non-egressed parasitoid, or non-parasitized. For *T. japonicus*, progeny successfully emerged from 23.8% of eggs, and 1.4% of eggs contained non-egressed parasitoids. For *A. reduvii*, progeny successfully emerged from 15.2% of eggs, and 0.5% contained non-egressed parasitoids.

DISCUSSION

At the 28°C temperature, *A. reduvii* and *T. japonicus* females lived longer than males. Similar findings were found for *A. bifaciatus* (Stahl et al., 2018). The peak period for successful oviposition was 6-10 days after adult emergence for *A. reduvii* and 2-4 days after adult emergence for *T. japonicus*. One explanation for these differences between species is that *T. japonicus* is more synced to the oviposition timing of the *H. halys* than *A. reduvii*. *Halyomorpha halys* is bivoltine in the Mid-Atlantic states. Due to its specialization on *H. halys, T. japonicus* may more closely track its population dynamics and oviposit in larger numbers during the peak egg load periods of *H. halys* (generalized life cycle in Fig. 6). Contrarily, *A. reduvii* it is a generalist parasitoid and as such, is less likely to track *H. halys* population dynamics.

Over time, the female bias in sex ratio showed a decline in *T. japonicus*. However, both parasitoids had female biased progeny (>1:1). This is speculated to be a response to unlimited host availability causing decreased quality of offspring. According to Hamilton's local mate competition theory, it is believed that parasitoids exhibit female biased sex ratios because females contain a limited supply of eggs, and ovipositing less males decreases the likelihood that an emerging female will be inseminated by a member of the same egg clutch.

One measure of the potential success of using these parasitoids to suppress *H. halys* is shown in their percent emergence. *T. japonicus* parasitism rate was 23.8% and *A. reduvii* was 15.2%. These results look promising for these parasitoids as biological control agents of *H. halys*. However, more testing is needed to determine their potential for augmentative biological control release programs.

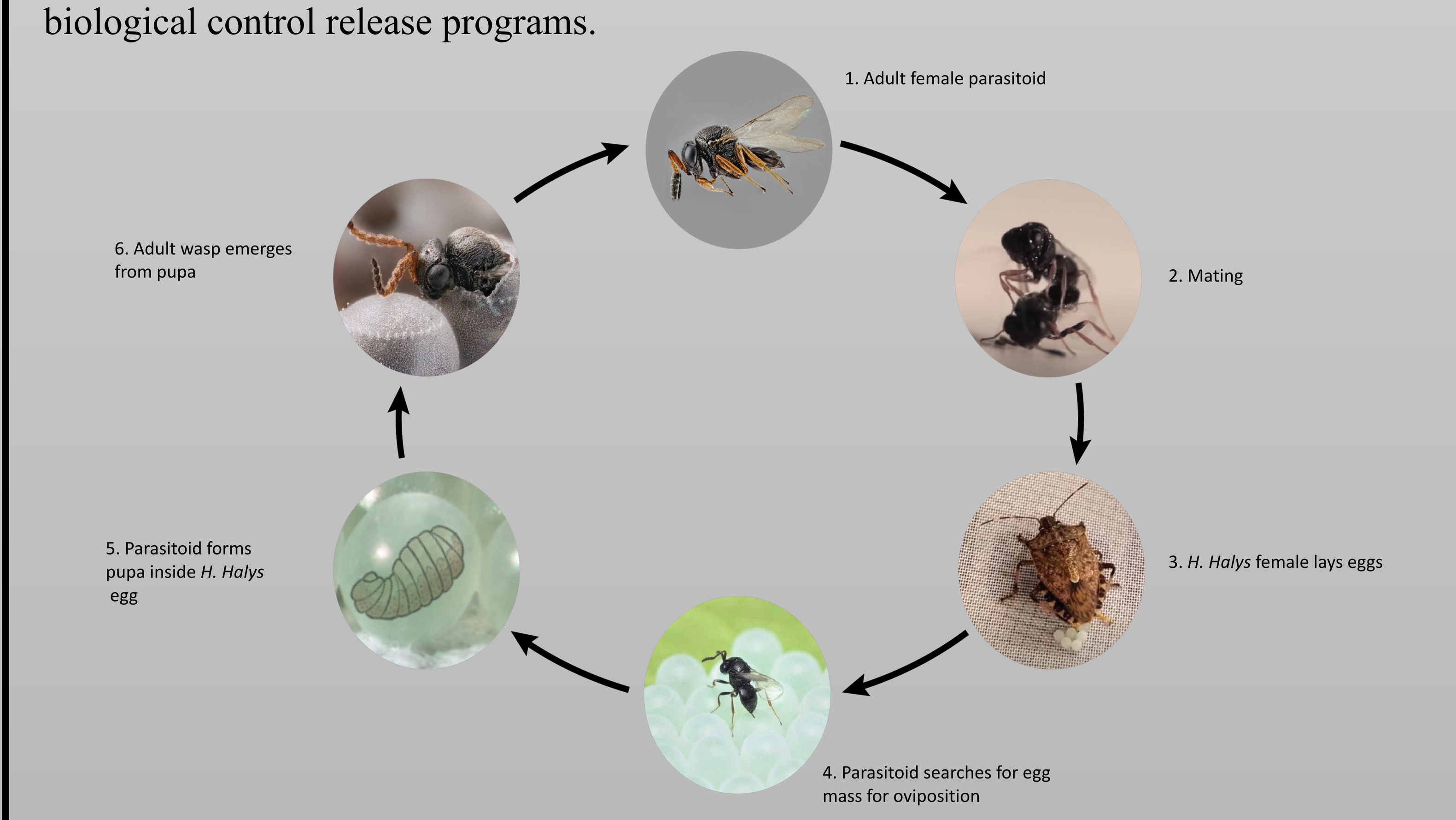

Figure 6. A generalized diagram of a *T. japonicus* parasitoid life cycle

ACKNOWLEDGEMENTS

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