



# Evaluation of egg parasitoid *Hadronotus pennsylvanicus* as a prospective biocontrol agent of the leaffooted bug *Leptoglossus zonatus*

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**Abstract** The western leaffooted bug, *Leptoglossus zonatus* (Dallas) (Heteroptera: Coreidae), is a key pest of almonds and pistachios in the USA. With limited monitoring strategies and no economic threshold developed, the use of broad-spectrum insecticides remains the primary control tactic for *L. zonatus*. In pursuit of more sustainable management options, experiments were carried out to assess the biocontrol potential of the egg parasitoid *Hadronotus pennsylvanicus* (Ashmead) (Hymenoptera: Scelionidae) against *L. zonatus*. Biological and demographical attributes of *H. pennsylvanicus* were evaluated under controlled laboratory conditions. Mated females lived on average 116 days. However, female longevity declined by 74% when provided with host eggs, and

by 97% when deprived of a suitable diet. Females produced an average 39.70 offspring, with peak fecundity observed within the first week of their lifespan. The total progeny was on average 92.75% female. Non-reproductive host mortality accounted for > 52% of the total egg mortality observed within the first two weeks, and represented the majority of total host egg mortality thereafter. Age of host eggs did not influence parasitism rates. Under laboratory conditions, *H. pennsylvanicus* exhibits suitable demographic and reproductive traits as a candidate biocontrol agent of *L. zonatus*. Further research is needed to determine how to best manipulate and enhance *H. pennsylvanicus* populations to promote biocontrol of *L. zonatus* under field conditions.

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## Introduction

The western leaffooted bug, *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae), is a polyphagous pest native to the Western Hemisphere (Brailovsky 2014), and known to attack diverse economically important crops (Grimm 1999; Rivero and Hernández 2009; Xiao and Fadamiro 2010; Foresti et al. 2017; Joyce

et al. 2017). Feeding from *L. zonatus* can result in crop damage and reduced yields, as documented for crops such as satsuma mandarin, *Citrus unshiu* (Xiao and Fadamiro 2010), and seed corn, *Zea mays* (Marchiori et al. 2002). In California's Central Valley, USA, *L. zonatus* has been reported as an occasional pest to tree nut crops, such as almond and pistachio (Daane et al. 2005). However, with the increased acreage of these nut crops over the past two decades, the pest is becoming more problematic for growers (Daane et al. 2016; Joyce et al. 2017; Zalom et al. 2018). *L. zonatus* has the potential to cause significant damage to nut crops, including epicarp lesions and kernel necrosis, and can facilitate fungal contaminations including stigmatomycosis and *Botryosphaeria* infection (Michailides et al. 1987; Michailides 1989; Michailides and Morgan 2016; Daane et al. 2005; Joyce et al. 2019; Stahl et al. 2020). Unlike smaller hemipteran pests, damage inflicted by *L. zonatus* can result in kernel damage throughout the growing season (Daane et al. 2016).

Current methods for monitoring *L. zonatus* in orchards include beat-tray sampling of the tree canopy and visual assessment of developing nuts for feeding damage (Daane et al. 2016; Zalom et al. 2018). However, with no existing economic thresholds associated with these monitoring efforts, such tactics can be time and labor intensive while providing limited information on true pest densities or associated crop damage. Given the lack of effective monitoring tools, along with grower aversion to risk from early season feeding damage, broad-spectrum insecticides such as pyrethroids remain the primary control tactic for *L. zonatus* (Haviland et al. 2018; Zalom et al. 2018). With increasing demand for more sustainable pest management options, growers have expressed interest in exploring alternative control tactics to improve current integrated pest management (IPM) programs for *L. zonatus* in tree nut crops.

Egg parasitoids have demonstrated potential as effective biocontrol agents in crop landscapes given their relative host specificity, efficiency in host searching, and ability to suppress pest populations (Abram et al. 2020). One of the most prominent egg parasitoids of *Leptoglossus* spp. is *Hadronotus pennsylvanicus* (previously reported as *Gryon pennsylvanicum*) (Ashmead) (Hymenoptera: Scelionidae) (Masner 1983; Marchiori 2002; Bates and Borden 2004; Maltese et al. 2012). *H. pennsylvanicus* is a

synovigenic, solitary endoparasitoid of several coreid hosts and has been the focus of many biocontrol programs for agricultural pests, including *Anasa tristis* (DeGeer) (Nechols et al. 1989), *Leptoglossus phyllopus* (L.) (Mitchell and Mitchell 1986), *L. australis* Fabricius (Yasuda 1998), and *L. occidentalis* Heide-mann (Bates and Borden 2004; Maltese et al. 2012; Roversi et al. 2013). Under laboratory conditions, *H. pennsylvanicus* exhibits desirable biological and reproductive traits as a biocontrol agent to *A. tristis* (Nechols et al. 1989) and *L. occidentalis* (Sabbatini Peverieri et al. 2012). In controlled settings, *H. pennsylvanicus* can exhibit a high fecundity rate (80.7 eggs per female, *A. tristis* host; 144.6 eggs per female, *L. occidentalis* host), high sex ratio (78% female, *A. tristis* host; 72% female, *L. occidentalis* host) and long lifespan when under reproductive opportunity (35.3 days, *A. tristis* host; 40.27 days, *L. occidentalis* host) (Nechols et al. 1989; Sabbatini Peverieri et al. 2012). However, little is known about how such demographic and reproductive parameters on *A. tristis* and *L. occidentalis* may translate to successful biocontrol of *L. zonatus* specifically.

The life history traits of a candidate natural enemy determine its prospects as a successful biocontrol agent. In the present study, the reproductive and developmental biology of *H. pennsylvanicus* on *L. zonatus* was investigated under laboratory conditions, and parasitoid viability was assessed on host eggs of varying ages. The results of this study highlight the prospects and limitations of egg parasitoid *H. pennsylvanicus* as a candidate biocontrol agent of agricultural pest *L. zonatus*.

## Methods

### Experimental conditions

Laboratory colonies of *H. pennsylvanicus* and *L. zonatus* were reared from specimens collected in 2017 from Fresno County, California, USA. The *L. zonatus* strain was collected from pomegranates and replenished with wild specimens *ad libitum*. Insects were maintained in cages (60 cm × 60 cm × 60 cm, 680 µm mesh, BugDORM®, Taiwan) and fed a diet of zucchini (*Cucurbita pepo* L.), green beans (*Phaseolus vulgaris* L.) and organic raw sunflower seeds (*Helianthus annuus* L.) replenished weekly.

Enclosures contained a 2.36 l potted juniper (*Juniperus* sp. L.) and wooden skewers were used as oviposition substrate. Newly laid egg clusters were collected daily for parasitoid bioassays. *H. pennsylvanicus* colonies were established from parasitized sentinel *L. zonatus* eggs in pistachio and refreshed with wild strain individuals in 2019 and 2020. Colonies were housed in 50 ml plastic tubes (Falcon Conical Centrifuge Tubes, Fisher Scientific®) closed at the open end with 250 µm mesh to allow a permeable feeding substrate. A diet of honey-water solution (1:1) (Organic Honey, Wholesome®) was provided *ad libitum* to colony. All bioassays were conducted in controlled laboratory conditions of 25 °C ± 1, 75 ± 5% RH and L:D 16:8 photoperiod. Observations and dissections were conducted using a Leica® S9i Stereo Microscope and BioQuip® Micro Dissection Kit. *H. pennsylvanicus* was identified using the key to species in the revision of *Gryon* Haliday by Masner (1983), and specimens were compared to images of the holotype specimen, *Telenomus pennsylvanicus* Ashmead. A recent analysis by Talamas et al. (2021) found *Gryon* to be polyphyletic and resurrected the genus *Hadronotus* Förster for many of the species previously placed in *Gryon*, including *H. pennsylvanicus*. The CO1 barcoding region was sequenced from a colony specimen with DNA extracted non-destructively, amplified and sequenced using the primers and methods of Ramírez-Ahuja et al. (2020), and the sequence was deposited in GenBank (MZ129311). Voucher specimens of *H. pennsylvanicus* were deposited in the Entomology Research Museum at the University of California, Riverside, USA, and the Florida State Collection of Arthropods, Gainesville, Florida, USA.

#### Parasitoid longevity and lifetime fecundity

To measure adult longevity and fecundity of *H. pennsylvanicus*, newly emerged (< 24 h) males and females (n = 120) were housed in mating pairs in 10 ml plastic tubes closed with a 250 µm mesh cap. Tubes with mating pairs were randomly assigned to one of the following treatments: (1) water and 25 fresh (< 72 h) *L. zonatus* host eggs (“water & host”; n = 20), (2) honey-water solution and 25 fresh host eggs (“honey-water & host”; n = 20), or (3) honey-water solution and no host eggs (“honey-water”; n = 20). Diet and host eggs were replenished every

other day until the female wasp expired. A new male specimen was introduced to the tube to replace any expired males to maintain copulation potential throughout the study. Data collected on male survival only consisted of the initial individuals provided at the start of each mating pair. Following 24 h exposure to the parasitoids, host eggs were removed, stored under laboratory conditions and observed daily for the emergence of a parasitoid or *L. zonatus* nymph. Eggs that neither hatched nor yielded a parasitoid after 30 days were dissected to examine for the presence of a dead nymph, dead parasitoid, or were determined aborted if neither occurred. The number of eggs and frequency of host-egg exposure to parasitoids were selected based on maximum parasitism observed within 24 h, and previous findings that parasitism rates were unaffected when wasps was deprived of host eggs for up to five days (Vogt and Nechols 1993; Peverieri et al. 2013). Similarly, no oocyte absorption was observed when wasps were deprived of host eggs for five days (Vogt and Nechols 1993).

#### Parasitoid host age viability

Given that host egg age may influence parasitism behavior and oviposition, we assessed the effect of egg age on *H. pennsylvanicus* parasitism rates. Newly laid (< 24 h) egg clusters were collected from *L. zonatus* colonies and stored under controlled laboratory conditions. Egg clusters were standardized to contain 25 eggs and prepared for ages 1, 2, 4, or 8 day treatments (n = 15 for each treatment). Age treatments were selected based on the observed 10.9 day average duration for development from oviposition to first instar under similar environmental conditions (Daane et al. 2019). Egg cluster treatments were exposed individually to a randomly selected, fed, < 24 h old mated female wasp. Adult females were house for 24 h with multiple < 24 h old males prior to being supplied a host egg cluster. Egg clusters were removed after 24 h exposure to a female wasp and maintained under laboratory conditions. Clusters were observed daily for the emergence of either parasitoid or *L. zonatus* nymphs. All remaining eggs after 30 days were dissected to determine presence of a dead nymph, dead parasitoid, or identified as aborted if neither occurred. Observations from dissections did not account for the presence or absence of a parasitoid meconium.

## Non-reproductive host mortality

To evaluate the potential role non-reproductive host mortality has in *L. zonatus* biocontrol, we compared the proportion of aborted eggs to the total host egg mortality observed from lifetime fecundity assays. To evaluate the influence parasitoid presence has on non-reproductive host mortality, 20 fresh (< 72 h old) unexposed *L. zonatus* egg clusters containing 25 eggs were isolated congruently with day 1 replicates from female fecundity assays. Egg clusters were housed in 10 ml plastic tubes closed with a 250  $\mu$ m mesh and stored under controlled laboratory conditions for 30 days. Egg clusters were observed for the emergence of *L. zonatus* nymphs and unemerged eggs remaining were dissected to determine presence of dead nymphs or identified as aborted if contents were indiscernible following dissection.

## Life table, demographic parameters and statistical analysis

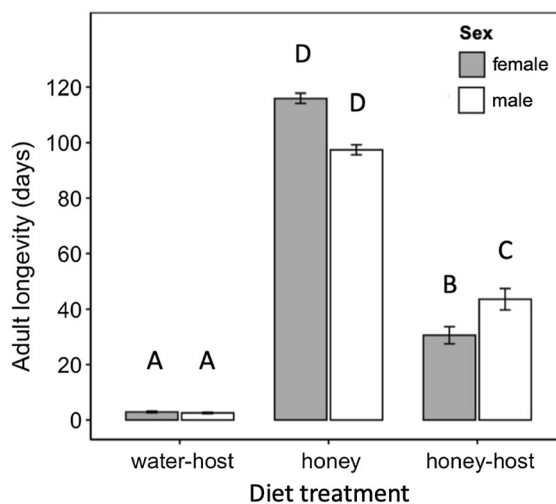
Observations on age-specific survival, longevity and fecundity were used to calculate life table parameters for *H. pennsylvanicus* ( $r_m$ , intrinsic rate of increase;  $\lambda$ , finite rate of increase;  $T$ , mean generation time;  $T_d$ , doubling time;  $R_0$ , net reproductive rate;  $GRR$ , gross reproductive rate) (see Sabbatini Peverieri et al. 2012). The effect of diet and host presence on adult longevity was assessed using a generalized linear mixed model (GLMM) with a gamma distribution and identity link function, testing for an interaction between “sex” and “treatment” with treatments as fixed effects. Influence of host egg age on parasitism viability was evaluated using a GLMM with binomial distribution and logit link function. The influence of female age on progeny sex ratio was assessed using a GLMM with binomial distribution and logit link function. The effect of host egg exposure to a parasitoid on proportion of aborted eggs was evaluated using a GLMM with binomial distribution and logit link function with “host presence” as a fixed effect. Replicates were included as a random effect in all statistical models. Analyses were carried out with R version 3.6.1 (R Core Team 2019). GLMM analyses were conducted using the “glmer” function in the “lme4” package (Bates et al. 2015). Fixed effects were evaluated through model comparisons using likelihood ratio tests via the “drop1” function. When multilevel variables were found to be

significant, means were separated using a post-hoc Tukey test using the “glht” function in the “multcomp” package (Hothorn et al. 2008). All data are presented as mean  $\pm$  SE.

## Results

### Parasitoid longevity and lifetime fecundity

Diet significantly influenced the longevity of adult *H. pennsylvanicus* ( $\chi^2 = 16.05$ ,  $df = 2$ ,  $p < 0.001$ ) (Fig. 1). Water-fed males and females provided host eggs lived an average of  $2.54 \pm 0.20$  and  $2.90 \pm 0.30$  days, respectively. For honey-water fed females, there was a 74% reduction in longevity when individuals were provided host eggs. Honey-water fed females lived an average  $116.00 \pm 1.81$  days in the absence of host eggs, as opposed to  $30.60 \pm 3.10$  days when provided host eggs. Similarly, males fed a honey-water diet lived  $97.30 \pm 1.23$  days in the absence of host eggs and  $43.50 \pm 2.12$  days when provided host eggs, a 55% reduction in longevity. Females lived significantly fewer days than males only when provided with honey-water diet and host eggs (Tukey test,  $df = 5$ ,  $p < 0.01$ ). Males and females provided host eggs and fed a water diet began dying on



**Fig. 1** Mean longevity ( $\pm$  SE) of adult female (grey bars) and male (white bars) *Hadronotus pennsylvanicus* when reared with water and host eggs, honey-water and host eggs, or honey-water only. All bars indicating different letters are significantly different from one another ( $p < 0.05$ ) GLM and Tukey post-hoc tests)

day 1 of the study, with complete mortality reached on day 3 and day 6, respectively. When fed honey-water and provided host eggs, females started dying on day 9, reaching 50% mortality by day 25, and surviving up to 55 days. Males began dying after 21 days, reaching 50% mortality after day 43, and surviving up to 61 days. When fed honey-water in the absence of host eggs, males started dying after 85 days, living up to 105 days, while females began dying after 105 days and surviving up to 135 days. When honey fed females were provided a continuous supply of host eggs, 79% of all parasitism occurred within the first week of the female's lifetime (Fig. 2). The highest mean parasitism rates observed, indicated as the number of offspring produced per female per day, was on day 1, averaging  $12.25 \pm 1.32$  progeny, equating to 49% of total eggs available. Fecundity showed relatively consistent declines over the first three weeks, with a < 50% reduction in parasitism by day 5, and a complete absence of progeny after day 19.

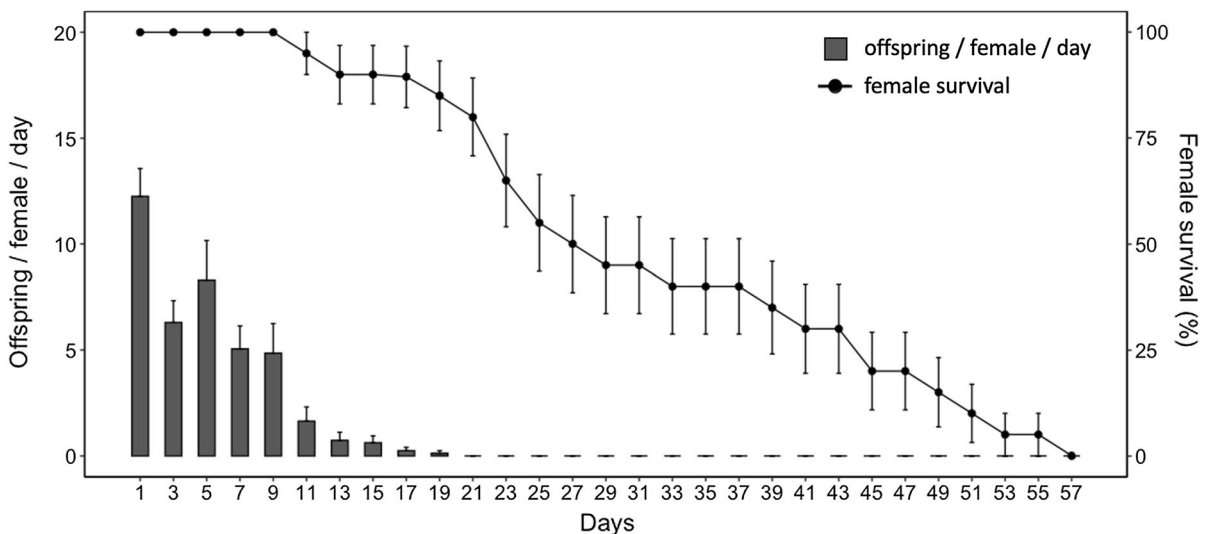
#### Life table and demographic parameters

Females fed a honey-water diet began oviposition behavior immediately after eclosion. Females exhibited a mean ovipositional period of  $10.10 \pm 0.10$  days with an average post-reproductive period of  $20.50 \pm 2.98$  days (Table 1). The total progeny produced by a single female ranged from 4 to 89,

averaging  $39.70 \pm 5.55$  individuals. The average proportion of female progeny was  $92.75 \pm 1.52\%$  when females were continuously paired with a reproductive male. When housed as a mating pair, the age of the adult female did not significantly influence the sex ratio of progeny ( $\chi^2 = 1.75$ ,  $df = 1$ ,  $p = 0.19$ ). However, the average proportion of female progeny declined slightly over time from 93.60% on day 1 to 86.70% on day 9. When provided a continuous supply of *L. zonatus* host eggs, *H. pennsylvanicus* exhibited an intrinsic rate of increase ( $r_m$ ) of  $0.22 \pm 0.02$ , suggesting, under suitable conditions, a population can multiply  $1.26 \pm 0.03$  times per day with a doubling time ( $T_d$ ) of  $3.68 \pm 0.33$  days. Under these conditions, the average lifetime fecundity of a female ( $R_0$ ) was  $35.70 \pm 4.73$  with a generation time ( $T$ ) of  $17.00 \pm 1.63$  days.

#### Parasitoid host age viability

Under laboratory conditions, host age did not influence parasitism rates or reproductive output of *H. pennsylvanicus* (Fig. 3). The proportion of *L. zonatus* nymphs to emerge from exposed eggs was  $42.93 \pm 6.20\%$ , with unemerged nymphs accounting for  $7.47 \pm 1.50\%$  of the total host eggs. The proportion of *H. pennsylvanicus* adults to emerge was  $30.87 \pm 3.31\%$ . Unemerged wasps accounted for  $4.07 \pm 0.27\%$  of the total host eggs. Host eggs that

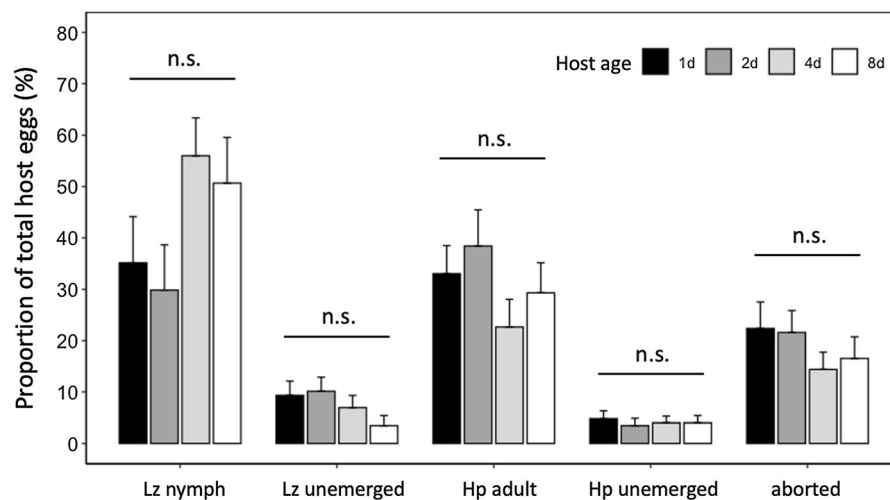


**Fig. 2** Mean age-specific fecundity (grey bars) (+ SE) and female survival rate (black dots) ( $\pm$  SE) of *Hadronotus pennsylvanicus* when provided *Leptoglossus zonatus* host eggs and fed honey-water *ad libitum*



**Table 1** Reproductive and demographic attributes (mean  $\pm$  SE) of *Hadronotus pennsylvanicus* reared on *Leptoglossus zonatus* eggs at 25 °C  $\pm$  1, 75  $\pm$  5% RH and L:D 16:8, fed honey-water *ad libitum*

	Mean value ( $\pm$ S.E.)
Reproductive parameter	
Adult female longevity (days)	30.60 $\pm$ 3.09
Ovipositional period (days)	10.10 $\pm$ 0.10
Post-reproductive period (days)	20.50 $\pm$ 2.98
Total progeny (offspring per female)	39.70 $\pm$ 5.55
Total development time (days)	18.65 $\pm$ 0.23
Total aborted progeny (unemerged offspring per female)	5.80 $\pm$ 1.29
Sex ratio (% female offspring)	92.75 $\pm$ 1.52
Demographic parameter	
Intrinsic rate of increase ( $r_m$ ) (days <sup>-1</sup> )	0.22 $\pm$ 0.02
Finite rate of increase ( $\lambda$ )	1.26 $\pm$ 0.03
Mean generation time ( $T$ ) (days)	17.00 $\pm$ 1.63
Doubling time ( $T_d$ ) (days)	3.68 $\pm$ 0.33
Net reproductive rate ( $R_0$ ) (female offspring per female)	35.70 $\pm$ 4.73
Gross reproductive rate ( $GRR$ ) (female offspring per female)	35.78 $\pm$ 4.89



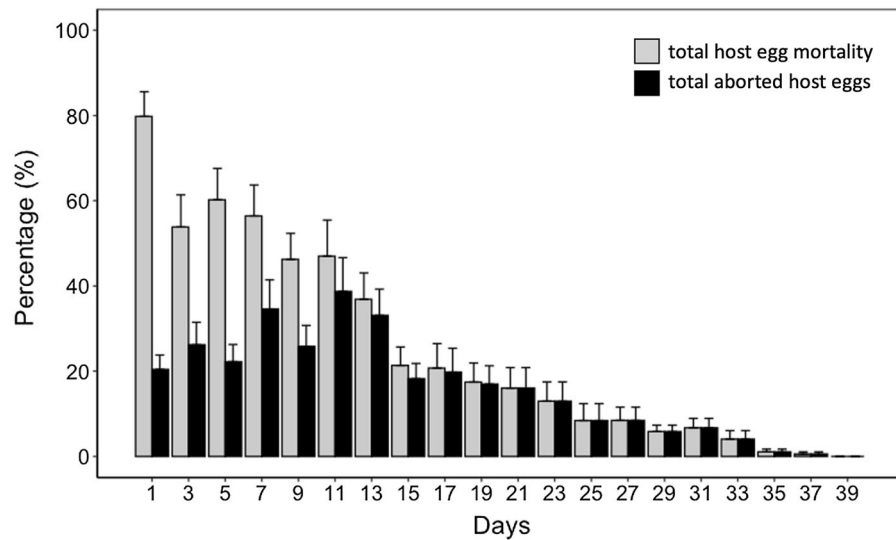
**Fig. 3** Proportion of total host eggs (mean  $\pm$  SE) to yield a *Leptoglossus zonatus* nymph ("Lz nymph"), *Hadronotus pennsylvanicus* adult ("Hp adult"), unemerged *Leptoglossus zonatus* ("Lz unemerged"), unemerged *Hadronotus*

*pennsylvanicus* ("Hp unemerged"), or determined "aborted" for eggs of age 1, 2, 4, and 8 days old ( $n = 15$  per treatment). Bars labelled with "n.s." are not significantly different ( $p > 0.05$ ) GLM and Tukey post-hoc test)

did not yield an *L. zonatus* nymph nor a *H. pennsylvanicus* adult, and therefore identified as aborted, accounted for 18.73  $\pm$  1.94% of the total host eggs. No host feeding behavior was observed throughout the study, concurring with findings from Vogt and Nechols (1993) where authors did not find evidence of *H. pennsylvanicus* feeding on hosts over a cumulative period of 100 h.

#### Non-reproductive host mortality

Total host egg mortality was highest on day 1, averaging 79.80  $\pm$  5.73% (Fig. 4). There was a consistent reduction in total egg mortality over time, with no mortality observed following day 37. The age of ovipositing female significantly influenced the proportion of non-reproductive egg mortality accounting towards the total host egg mortality ( $\chi^2 = 86.14$ ,



**Fig. 4** Proportion of *Leptoglossus zonatus* host egg mortality (mean + SE) in the presence of *Hadronotus pennsylvanicus*. Eggs that neither yield an *Leptoglossus zonatus* nymph or adult

wasp were determined aborted (black bars), which contributed to the total host egg mortality induced by parasitism (grey bars)

df = 1,  $p < 0.001$ ). The proportion of non-reproductive egg mortality increased with female age from  $20.40 \pm 3.37\%$  on day 1 to the maximum observed  $38.74 \pm 7.87\%$  on day 11, accounting for 53% of the total egg mortality observed within the first two weeks of the study. Following day 14, non-reproductive egg mortality accounted for 96% of the total egg mortality. There were significantly more aborted eggs observed in the presence of adult female wasps ( $\chi^2 = 33.25$ , df = 1,  $p < 0.001$ ). In the absence of a female wasp, only  $2.20 \pm 0.61\%$  of eggs were determined aborted.

## Discussion

*Hadronotus pennsylvanicus* is a long-lived species, capable of surviving  $30.60 \pm 3.09$  days under continuous reproductive opportunity in laboratory conditions. Under these conditions, access to a carbohydrate rich diet has shown to be vital for the survival of *H. pennsylvanicus* (Olson and Nechols 1995; Sabbatini Peverieri et al. 2012), and, here, honey-water deprived wasps expired shortly after eclosion. As such, the establishment and maintenance of *H. pennsylvanicus* in agricultural landscapes may be contingent on the availability of suitable carbohydrate food resources such as floral nectar or insect honeydew. Under laboratory conditions, both male and female

parasitoids lived significantly longer in the absence of host eggs. Here, there was 74% reduction in female longevity when wasps were held under continuous reproductive opportunity. This trend was more pronounced in females than males, where only a 55% reduction in longevity was observed. These observations concur with findings reported in Martel et al. (2019) for *Gryon aetherium* Talamas (reported as *G. gonikopalense* Sharma), where a > 50% reduction in female longevity was reported when provided a continuous supply of host eggs. Such reproductive cost on longevity may be attributed to the energy expenditure incurred during egg manufacturing for females under continuous reproductive opportunity (Benelli et al. 2017). While mating status has shown to reduced female parasitoid longevity, little is known of the reproductive costs associated with male longevity and further investigation is required.

Similar to observations found in Nechols et al. (1989) and Sabbatini Peverieri et al. (2012), *H. pennsylvanicus* exhibited a short pre-oviposition time period, with peak female fecundity reached on day 1 following emergence. When reared on *Leptoglossus* sp. hosts, *H. pennsylvanicus* females exhibited an initial spike in parasitism rates, with a second peak after five days of reproductive opportunity (Sabbatini Peverieri et al. 2012). Egg limitation appears to be a limiting factor on lifetime fecundity for female *H.*

*pennsylvanicus* (Sabbatini Peverieri et al. 2012; Cornelius et al. 2018). Here, we observed a rapid decline in fecundity over time, with more than 90% of offspring being produced within the first two weeks of a female's life. The total progeny produced per female *H. pennsylvanicus* ( $39.70 \pm 5.55$  when provided host eggs every other day) was less than that reported on hosts *A. tristis* ( $80.7 \pm 36$  when provided host daily; Nechols et al. 1989) and *L. occidentalis* ( $144.55 \pm 50.66$  when provided host daily; Sabbatini Peverieri et al. 2012). However, both the intrinsic and finite rate of increase of *H. pennsylvanicus* when reared on *L. zonatus* eggs concurred with observations from hosts *L. occidentalis* and *A. tristis* (Nechols et al. 1989; Sabbatini Peverieri et al. 2012). Similarly, observations on mean generation time (17.00 days) were similar to those found in Nechols et al. (1989) and Sabbatini Peverieri et al. (2012). Results in this study suggest population growth attributes of *H. pennsylvanicus* on *L. zonatus* are congruent with those identified from alternative hosts, and similar to trends from other species of *Hadronotus* and *Gryon*, including *H. clavigrallae* Mineo, *H. gonikopalense*, *H. gallardoi* (Bréthes), and *H. philippinense* (Ashmead) (Dasilao and Arakawa 2004; Canto-Silva et al. 2006; Martel et al. 2019; Romeis et al. 2000). Given that these observations show similar trends in life history traits, data obtained on the total progeny produced, and therefore used to calculate estimates to  $R_0$  and  $GRR$ , are likely underestimated given that wasps were only provided eggs every other day for oviposition as opposed to daily, as demonstrated in Nechols et al. (1989) and Sabbatini Peverieri et al. (2012). Here, providing a fewer number of host eggs during the wasps ovipositional period would result in fewer progeny produced over the course of the females lifetime. While these results may help elucidate trends on parasitoid fitness, future research would benefit to assess the reproductive capacity of *H. pennsylvanicus* when provided *L. zonatus* host eggs daily for more accurate comparisons in lifetime fecundity between alternative host species.

*Hadronotus pennsylvanicus* maintained a greater average proportion of female progeny ( $92.75 \pm 1.52\%$ ) when provided *L. zonatus* eggs than when reared on hosts *L. occidentalis* (72.45%) and *A. tristis* (78%) (Nechols et al. 1989; Sabbatini Peverieri et al. 2012), and higher than *Gryon* spp. on alternative hemipteran hosts (Dasilao and Arakawa, 2004; Canto-

Silva et al. 2006; Martel et al. 2019; Romeis et al. 2000) when reared under similar laboratory conditions. *H. pennsylvanicus* exhibited a slower decline in the proportion of female progeny over time when provided *L. zonatus* eggs in comparison to *L. occidentalis* (Sabbatini Peverieri et al. 2012). Sabbatini Peverieri et al. (2012) accounted for such trends in sex ratio to be due to sperm depletion in females over time despite maintaining copulation opportunity by replacing expired males throughout the female's lifetime. However, the influence of sperm depletion on reproductive output is poorly documented for scelionid wasps, and thus requires further investigation. Furthermore, this would suggest that *H. pennsylvanicus* only mates once after eclosion. Despite observations of copulation occurring shortly after introducing female with male wasps, this study did not specifically document mating behavior following day 1 of the study, and further research is needed to confirm such hypotheses on *H. pennsylvanicus* mating behavior. While parasitoid fecundity ultimately relies on availability of hosts, exhibiting a high female-skewed sex ratio in progeny would likely benefit population growth rates and reproductive capacity in applied settings.

The quality of a host in relation to its age may be highly variable in field settings (Vinson 1998). Egg parasitoids may favor younger hosts given they could potentially provide a longer duration of time for parasitoid offspring to development. This trend was observed for other *Hadronotus* spp., such as *H. gallardoi*, *H. flavipes* (reported as *G. nixonii* Masner), *H. clavigrallae*, and *H. obesus* Masner (Morrill and Almazon 1990; Romeis et al. 2000; Hirose et al. 2003; da Rocha et al. 2006a, b). However, host egg age did not influence parasitism nor reproductive output of *H. pennsylvanicus* on *L. zonatus*. Sabbatini Peverieri et al. (2013) similarly found that parasitoid fitness was not influenced by the age of the host nor when eggs were stored at low temperatures (Sabbatini Peverieri et al. 2014). Successful wasp eclosion was highest early in the ovipositing female's lifetime. However, this trend consistently declined over time. The proportion of aborted eggs increases over time, ultimately accounting for the majority of total host egg mortality following day 11. This may suggest a persistence in parasitism behavior despite a potential egg limitation being reached in ovipositing females. Given non-reproductive egg mortality may play a



significant role in biocontrol programs (Abram et al. 2016, 2019), future research would benefit to document parasitoid egg load under these reproductive conditions to better understand the limits of egg maturation in *H. pennsylvanicus*.

Under laboratory conditions, *H. pennsylvanicus* exhibits suitable biological and reproductive traits suggesting potential for use in biocontrol programs for *L. zonatus*. However, it is important to note that demographic values are valid only when considering stable age populations, and therefore may not accurately reflect populations in applied settings. While *H. pennsylvanicus* and other egg parasitoids, such as *Ooencyrtus* Ashmead (Encyrtidae), *Anastatus* Motschulsky (Eupelmidae) and *Trissolcus* Ashmead (Scelionidae), may help contribute to lowering *L. zonatus* densities (Mitchell and Mitchell, 1986; Marchiori, 2002; Xiao and Fadamiro, 2010; Maltese et al. 2012), resident populations have not provided sufficient control of the pest in orchards. In California, overwintered adult *L. zonatus* disperse early in the season and attack almond (mid-February to March) and pistachio (April to mid-May) resulting in crop damage (Daane et al. 2005; Zalom et al. 2018). Here, future research would benefit by understanding the phenology of both pest and parasitoid populations in relation to tree nut development to identify effective methods for bolstering *H. pennsylvanicus* populations to improve parasitism rates in the field. This information would enable a better understanding of how populations respond to resource availability and if biocontrol can effectively reduce pest pressure at times of crop vulnerability. Improved knowledge of the ecology and behavior of the egg parasitoid *H. pennsylvanicus* in relation to host *L. zonatus* would allow for the development of more targeted, and likely more successful IPM strategies to improve biocontrol of this economically important pest in California orchards.

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**Data availability** Raw data are available upon request.

**Code availability** Analysis code is deposited in Github ([https://github.com/rstraser/Hadronotus\\_biocontrol\\_eval](https://github.com/rstraser/Hadronotus_biocontrol_eval)).

#### Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** As no humans or other mammal subjects were involved in this research, no ethics approvals were required for this study.

**Informed consent** All authors consent to participate in publication of these data of this work.

**Consent to participate** All authors consent to participate in publication of these data.

**Consent for publication** All authors consent to participate in publication of this work.

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