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Floral Resource Provisioning for Possible Enhancement of Biological Control of
Leptoglossus zonatus (Heteroptera: Coreidae) by the egg Parasitoid *Hadronotus*
pennsylvanicus (Hymenoptera: Scelionidae)

A Dissertation submitted in partial satisfaction
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in

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by

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ABSTRACT OF THE DISSERTATION

Floral Resource Provisioning for Possible Enhancement of Biological Control of *Leptoglossus zonatus* (Heteroptera: Coreidae) by the egg Parasitoid *Hadronotus pennsylvanicus* (Hymenoptera: Scelionidae)

by

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Dr. Houston Wilson, Chairperson

The leaffooted bug, *Leptoglossus zonatus* (Dallas) (Heteroptera: Coreidae) is a key pest of almonds and pistachios in California, USA. With limited monitoring strategies and no economic thresholds developed, the use of broad-spectrum insecticides remains the primary control tactic for leaffooted bug. In pursuit of more sustainable management options, a series of experiments were carried out to evaluate if floral resource provisioning could enhance biocontrol of leaffooted bug by the egg parasitoid *Hadronotus pennsylvanicus* (Ashmead) (Hymenoptera: Scelionidae). The first portion of this work identified key life history traits of *H. pennsylvanicus* on eggs of its host *L. zonatus*, and evaluated the role of food deprivation on parasitoid reproductive performance. The second section assessed how diet quality influenced parasitoid nutrient

acquisition and reproductive fitness, and evaluated whether groundcovers could be used to bolster natural enemy populations, and in turn, improve biological control of *L. zonatus* in orchards.

Hadronotus pennsylvanicus exhibited suitable demographic and reproductive traits as a biocontrol agent of *L. zonatus*. However, adult diet had a direct influence on the reproductive fitness of female wasps. When given frequent access to a honey diet, female parasitoids lived significantly longer and parasitized more host eggs compared to starved individuals. Behavioral assays demonstrated that satiated females spent more time interacting with host eggs and tended to less frequently divert from oviposition behavior. The quality of adult diet also influenced the reproductive fitness of female *H. pennsylvanicus*. My data indicated that diets containing high sucrose concentrations increased female longevity and offspring production two-fold in comparison to individuals fed a low sucrose diet. Subsequent laboratory assays demonstrated that buckwheat flowers provided a significantly greater benefit to female longevity than oat, mustard, or cowpea flowers. However, in field trials, flowering groundcovers of any type did not enhance resident *H. pennsylvanicus* populations nor the biological control of *L. zonatus*. This dissertation project highlighted the role of diet on parasitoid reproductive fitness, and provided insight into the prospects and limitations of using floral resources for enhancing biological control of *L. zonatus* by the egg parasitoid *H. pennsylvanicus*.

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Introduction

While the intensification and specialization of modern agriculture has led to significant gains in the agriculture industry, it has also negatively affected natural habitats, biodiversity, and ecosystem services such as biological control (Tscharntke et al. 2005, 2008; Chaplin-Kramer et al. 2011). The communities of natural enemies that support biocontrol services are often compromised in agriculture systems due to the extensive, simplified nature of modern production (i.e. monoculture) and frequent use of pesticides (Fiedler et al. 2008; Crowder and Jabbour 2014; Nilsson et al. 2017). Natural enemies, such as predators and parasitoids, are more susceptible to the deleterious effects of reduced habitat than their associated prey in agricultural landscapes (Tscharntke et al. 2008). The simplified nature of modern cropping systems often lacks many of the resources required to support natural enemies of crop pests, including overwintering habitat, protection from tillage, and refuge from adverse biotic and abiotic conditions (Gillespie et al. 2016). Modern agriculture provides a concentration of abundant food for pests while simultaneously eliminating habitat for their natural enemies, which can reduce the biological control of pests and increases the risk of pest outbreaks (Heimpel and Mills 2017).

The leaffooted bug, *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae), is a polyphagous pest native to the Western Hemisphere (Brailovsky 2014) and is known to attack numerous economically important crops (Marchiori 2002; Rivero and Hernandez 2009; Xiao and Fadamiro 2010; Foresti et al. 2017). In California's Central Valley, *L. zonatus* is primarily a pest of almonds and pistachios (Daane et al. 2005). *Leptoglossus*

zonatus has the potential to cause significant damage to these tree nut crops, including epicarp lesions and kernel necrosis, and can facilitate fungal contamination such as *Botryosphaeria* infection and stigmatomycosis (Michailides 1987, 1989, 2016; Daane et al. 2005; Joyce et al. 2019; Stahl et al. 2020). Over the past 20 years, the amount of almond and pistachio acreage has increased significantly, leading to increased pressure from *L. zonatus* in orchards (Daane et al. 2016; Zalom et al. 2018). Current methods for monitoring *L. zonatus* populations in orchards include beat-tray sampling of the crop canopy and visual assessment of developing nuts to assess feeding damage (Daane et al. 2016; Zalom et al. 2018). These pest monitoring tactics are time and labor intensive while only providing limited information on true pest densities. Furthermore, there are currently no economic thresholds associated with such monitoring efforts. Given the lack of effective monitoring tools, along with grower aversion to feeding damage, broad-spectrum insecticides 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, exploring alternative control tactics may help improve current integrated pest management (IPM) programs for *L. zonatus* in tree nut crops.

In many cases, egg parasitoids have demonstrated potential as effective biocontrol agents in cropping systems given their relative host specificity, efficiency in host searching, and ability to suppress pest outbreaks (Abram et al. 2020). One of the most prominent egg parasitoids of *Leptoglossus spp.* is *Hadronotus* (= *Gryon*) *pennsylvanicus* (Talamas et al. 2021) (Ashmead) (Hymenoptera: Scelionidae) (Masner 1983; Marchiori 2002; Bates and Borden 2004; Maltese et al. 2012). *Hadronotus pennsylvanicus* is a

synovigenic, solitary endoparasitoid of several coreid species and has been of interest for many biocontrol programs for insect pests, including *Anasa tristis* (DeGeer) (Nechols et al. 1989), *Leptoglossus phyllopus* (L.) (Mitchell and Mitchell 1986), *L. australis* Fabricius (Yasuda 1998), and *L. occidentalis* Heidemann (Bates and Borden 2004; Maltese et al. 2012; Roversi et al. 2013). Under laboratory conditions, *H. pennsylvanicus* demonstrates desirable biological and reproductive traits as a prospective biocontrol agent of *A. tristis* (Nechols et al. 1989) and *L. occidentalis* (Sabbatini Peverieri et al. 2012). In these controlled settings, *H. pennsylvanicus* exhibited high fecundity rates, was able to maintain a high female sex ratio in progeny, and was long lived when housed under conditions with continuous reproductive opportunity (Nechols et al. 1989; Sabbatini Peverieri et al. 2012). However, while *H. pennsylvanicus* attacks *L. zonatus* eggs in California (Maltese et al. 2012), parasitism rates in orchards tend to be highly variable, leading to insufficient and unreliable biocontrol in agricultural landscapes.

Like most species, food is vital for the survival of adult parasitoids (Jervis et al. 2008; Wäckers and van Rijn 2005; Benelli et al. 2017). Access to carbohydrate-rich food resources improves both the survival and lifetime fecundity of numerous parasitoid species (Wäckers and van Rijn 2005; Benelli et al. 2017). In nature, adult parasitoids often obtain food from floral (nectar and pollen), extra-floral (nectaries), or non-floral (honeydew and host feeding) sources (Wäckers 2004, 2005, 2008; Heimpel and Jervis 2005). The nutrients obtained from these diets help fuel the bodily maintenance and physiological energy expenditure incurred by adult wasps (Wäckers and van Rijn 2005; Jervis et al. 2008). In many cropping systems, the quantity and quality of these food

resources can vary widely in both time and space. For synovigenic parasitoids, periods of low food availability can reduce the reproductive capacity of females (Jervis et al. 2001; Jervis and Ferns 2004). Under such conditions, inadequate diet can lead to egg limitation in wasps, in which females are unable to produce eggs at a sufficient rate, depleting their egg load before exhausting their supply of available hosts (Rosenheim et al. 2000). Such periods of limited food availability can negatively impact a parasitoid's biocontrol potential (Wäckers and van Rijn 2005; Benelli et al. 2017).

Floral groundcovers have been used as "trap crops" in an effort to control hemipteran pests in orchards (Nielsen et al. 2016; Akotsen-Mensah et al. 2017; Daane et al. 2016). With this strategy, non-crop plants are added to cropping systems to attract, divert, and/or retain a targeted insect pest that would otherwise cause damage to the primary crop (Gurr et al. 2017). In addition to luring pests, in some cases trap crops have been used to support resident natural enemy communities (Wan et al. 2016; Stahl et al. 2021). For instance, Stahl et al. (2021) demonstrated that the use of groundcovers in pistachio orchards could potentially reduce hemipteran abundance while increasing beneficial arthropod communities. Beyond providing a source of nectar to natural enemies, groundcovers may also provide vital refugia (Carmona and Landis 1999; Gillespie et al. 2016) and alternative prey or hosts for natural enemies during periods of low pest abundance (Duyck et al. 2011; Gillespie et al. 2016). While increasing habitat diversity in cropping systems can have a positive influence on biocontrol services, arthropod response to changes in the landscape are often not uniform, and outcomes can be species- and community-specific (Russell 2015; Tschardt et al. 2016; Zhu et al.

2020). Therefore, growers may benefit by carefully selecting and evaluating floral species that are attractive to *L. zonatus* while also providing a dietary resource to natural enemies to synergistically contribute to pest control.

Improved knowledge about the biology, ecology, and behavior of *L. zonatus* and its associated natural enemies would allow for the development of targeted IPM strategies and may bolster biological control in California tree nut crops. Here, I assess the role that diet plays in the reproductive fitness of the biocontrol agent *H. pennsylvanicus*, and evaluate where and under what conditions floral resource provisioning can enhance biological control of *L. zonatus*. The overall aim of this dissertation was to determine the prospects and limitation of biological control of *L. zonatus* by *H. pennsylvanicus* in California orchard landscapes. I aimed to address four key questions about the relationship between floral resource provisioning and biological control.

How effective is *Hadronotus pennsylvanicus* as a prospective biocontrol agent of *Leptoglossus zonatus*?

The life history traits of a candidate natural enemy can often determine its viability as a successful biocontrol agent. While laboratory evaluations have found that *H. pennsylvanicus* is a prospective biological control agent for other coreid hosts (Nechols et al. 1989; Sabbatini Peverieri et al. 2012), little is known about the parasitoid's life parameters when provided host eggs from *L. zonatus*. In Chapter 1, I investigated the primary qualities necessary for a successful biological control agent, including host-specific demographic and reproductive traits, and compared these results

to assays on alternative host species present in the literature. Understanding the efficacy of the egg parasitoid *H. pennsylvanicus* as a prospective biocontrol agent for *L. zonatus* within controlled laboratory conditions provides a framework to evaluate the current state of biological control of the pest in orchard systems.

How does access to diet influence *Hadronotus pennsylvanicus* reproductive performance?

Understanding the influence that diet has on parasitoid reproduction is crucial for the development of biocontrol programs. For many parasitoids, access to carbohydrate-rich food sources has been shown to significantly improve both their survival and lifetime reproductive fitness (Wäckers et al. 2005; Benelli et al. 2017). In Chapter 2, I evaluated the role that food deprivation has on the reproductive fitness and parasitism behavior of female *H. pennsylvanicus*. An improved understanding of the relationship between diet and the reproductive performance of *H. pennsylvanicus* provides insight into how biocontrol may be affected by extended periods of low floral resource availability.

How does diet quality influence reproductive fitness of *Hadronotus pennsylvanicus*?

In field settings, parasitoids obtain food from various floral and extra-floral resources (Wäckers 2004, 2005). The nutrients obtained from these sources can improve the longevity and fecundity, and increase parasitism behavior of parasitoid wasps (Benelli et al. 2017). Examining the role of diet quality on *H. pennsylvanicus* reproduction enables a better understanding of how provisioning floral resources in field settings may improve

biocontrol programs for *L. zonatus*. In Chapter 3, I explored the role of sucrose concentration and pollen content in diet on the reproductive fitness and nutrient acquisition of female *H. pennsylvanicus*. Information generated from this work provides insight into the importance of diet quality for optimizing parasitoid reproductive fitness.

Can floral resource provisions improve biocontrol of *Leptoglossus zonatus* in orchard systems?

An improved understanding of the role groundcovers have on *H. pennsylvanicus* reproductive fitness may provide insight into the relative contribution of floral resources in bolstering parasitoid populations and biocontrol in orchards. In Chapter 4, I conducted a series of laboratory and field experiments to test the role of candidate floral diets on the reproductive fitness of female *H. pennsylvanicus*, and furthermore, evaluate whether the use of such plant species as summer-flowering groundcovers can successfully enhance the seasonal abundance of natural enemies and increase biological control of *L. zonatus* in pistachio orchards.

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Chapter 1: Evaluation of egg parasitoid *Hadronotus pennsylvanicus* as a prospective biocontrol agent of the leaffooted bug *Leptoglossus zonatus*

Abstract

The 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.

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, *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). *Leptoglossus 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 1987, 1989, 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). *Hadronotus 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* Heidemann (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/female, *A. tristis* host; 144.6 eggs/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 (60cm x 60cm x 60cm, 680µm 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. *Hadronotus 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 16:8 L:D photoperiod. Observations and dissections were conducted using a Leica® S9i Stereo Microscope and BioQuip® Micro Dissection Kit. *Hadronotus 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, and the Florida State Collection of Arthropods, Gainesville, Florida.

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: a) water and 25 fresh (<72 h) *L. zonatus* host eggs (“water & host”; n=20), b) honey-water solution and 25 fresh host eggs (“honey-water & host”; n=20), or c) 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 were deprived of host eggs for up to 5 days (Vogt and Nechols 1993; Peverieri et al. 2013). Similarly, no oocyte absorption was observed when wasps were deprived of host eggs for 5 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 1st 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 housed 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 (<72hr 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 μ 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; λ , 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 test 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. 1.1). Water-fed males and females provided host eggs lived an average of 2.54 ± 0.20 and 2.90 ± 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 ± 1.81 days in the absence of host eggs, as opposed to 30.60 ± 3.10 days when provided host eggs. Similarly, males fed a honey-water diet lived 97.30 ± 1.23 days in the absence of host eggs and 43.50 ± 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 (post hoc Tukey test, $df = 5$, $p < 0.01$). Males and females provided host eggs and fed a water diet began dying on 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. 1.2). The highest mean parasitism rates observed, indicated as the number of offspring produced per female per day, was on day 1, averaging 12.25 ± 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 ± 0.10 days with an average post-reproductive period of 20.50 ± 2.98 days (Table 1.1). The total progeny produced by a single female ranged from 4 to 89, averaging 39.70 ± 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 ± 0.02 , suggesting under suitable conditions, a population can multiply 1.26 ± 0.03 times per day with a doubling time (T_d) of 3.68 ± 0.33 days. Under these conditions, the average lifetime fecundity of a female (R_0) was 35.70 ± 4.73 with a generation time (T) of 17.00 ± 1.63 days.

Parasitoid host age viability

Under laboratory conditions, host age did not influence parasitism rates or reproductive output of *H. pennsylvanicus* (Fig. 1.3). 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 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 (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. 1.4). 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 ($\chi^2 = 86.14$, $df = 1$, $p < 0.001$). 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 ± 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 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 5 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 ± 5.55 when provided host eggs every other day) was less than that reported on hosts *A. tristis* (80.7 ± 36 when provided host daily; Nechols et al. 1989) and *L. occidentalis* (144.55 ± 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. *Hadronotus 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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<i>Reproductive parameter</i>	<i>Mean value (\pm S.E.)</i>
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/female)	39.70 \pm 5.55
Total development time (days)	18.65 \pm 0.23
Total aborted progeny (unemerged offspring/female)	5.80 \pm 1.29
Sex ratio (% female offspring)	92.75 \pm 1.52
<i>Demographic parameter</i>	
Intrinsic rate of increase (r_m) (days ⁻¹)	0.22 \pm 0.02
Finite rate of increase (λ)	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/female)	35.70 \pm 4.73
Gross reproductive rate (GRR) (female offspring/female)	35.78 \pm 4.89

Table 1.1 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 16:8 L:D, fed honey-water *ad libitum*

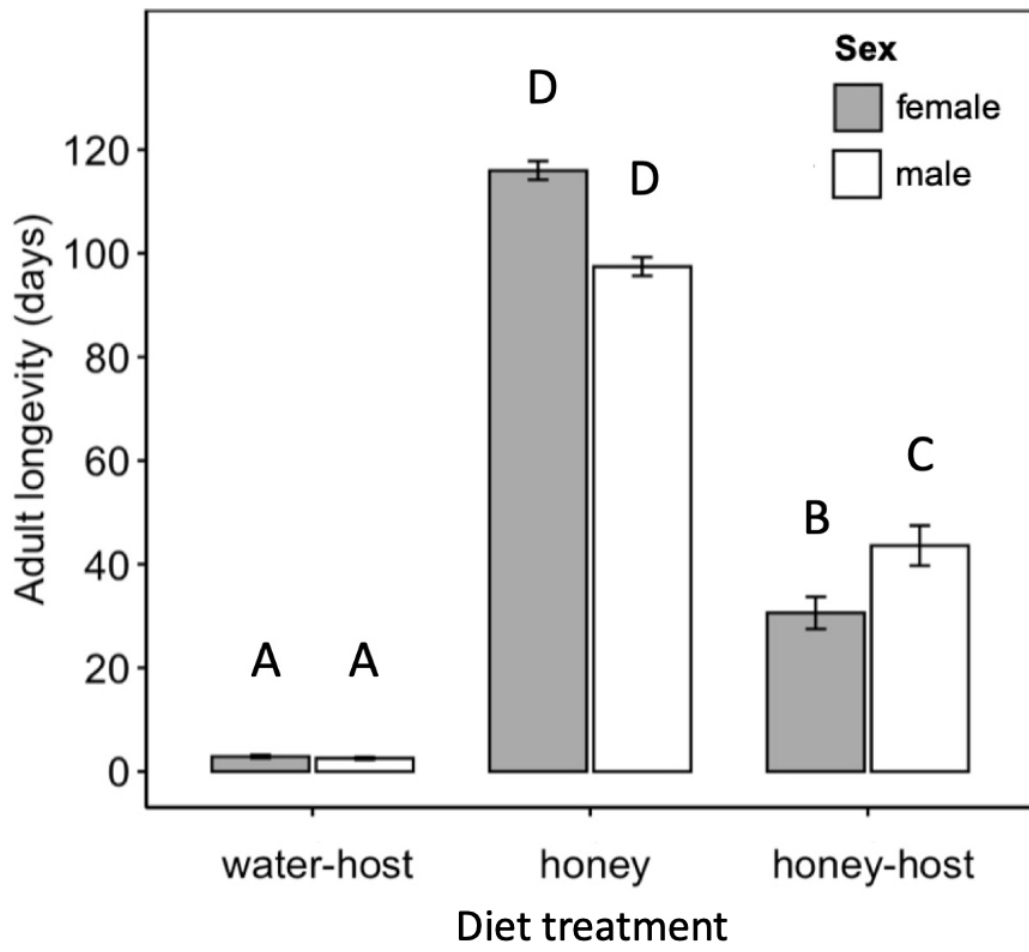


Figure 1.1 Mean longevity (\pm SE) of adult female (grey bars) and male (white bars) *Hadronotus pennsylvanicus* when reared with water and host eggs, honey-water and host eggs, or honey-water only. All bars with different letters are significantly different from one another ($P < 0.05$) (Gamma (link = “identity”) GLM and Tukey post hoc test)

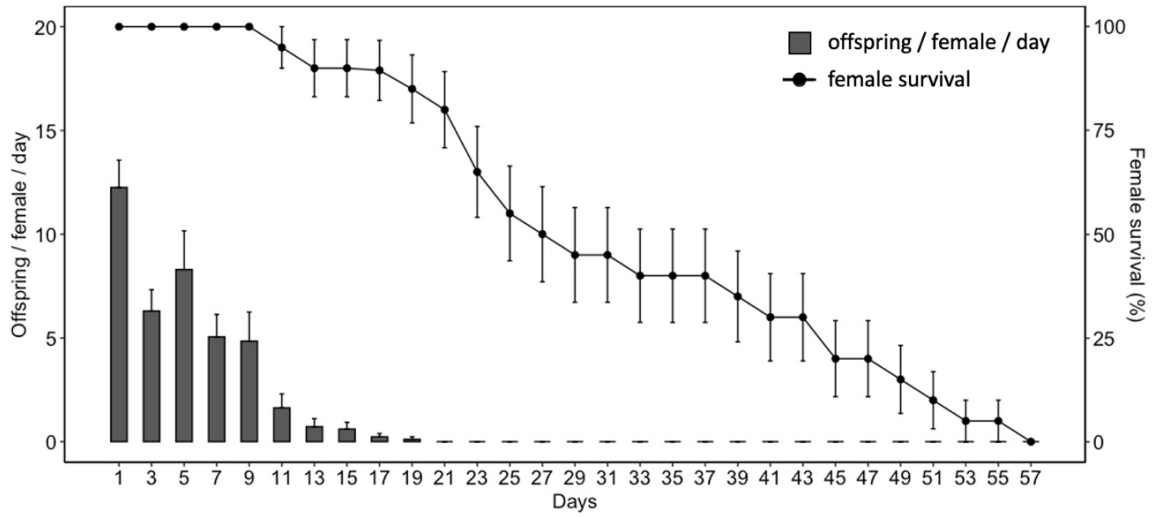


Figure 1.2 Mean age-specific fecundity (grey bars) (+ SE) and female survival rate (black dots) (\pm SE) of *Hadronotus pennsylvanicus* when provided *Leptoglossus zonatus* host eggs and fed honey-water *ad libitum*

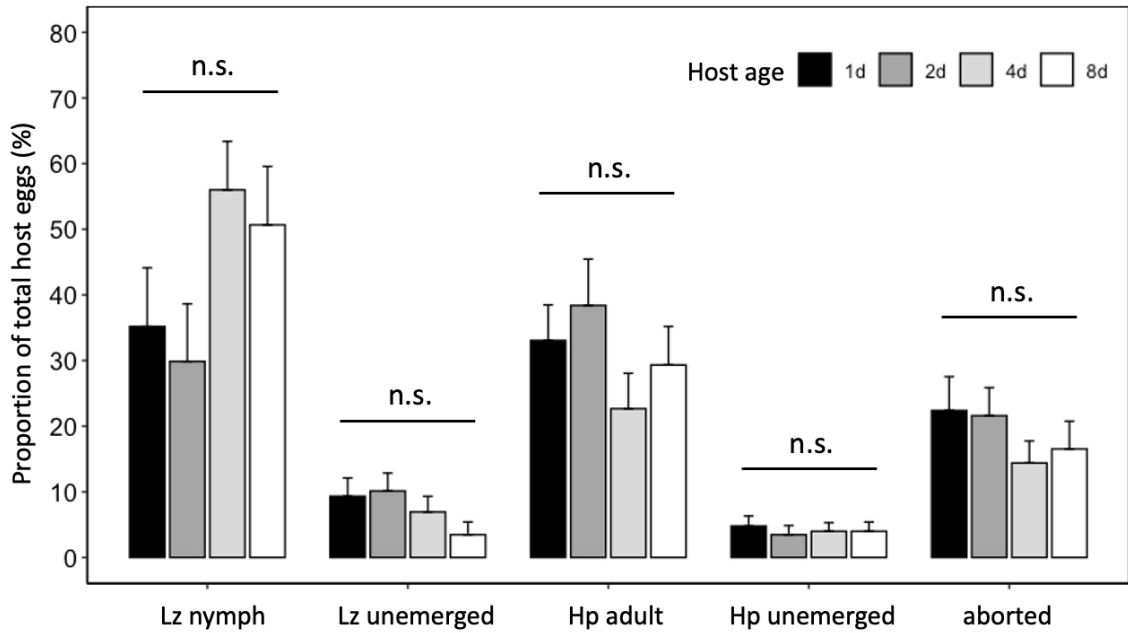


Figure 1.3 Proportion of total host eggs (mean + SE) to yield a *Leptoglossus zonatus* nymph ("Lz nymph"), *Hadronotus pennsylvanicus* adult ("Hp adult"), unemerged *Leptoglossus zonatus* ("Lz unemerged"), unemerged *Hadronotus pennsylvanicus* ("Hp unemerged"), or determined "aborted" for eggs of age 1, 2, 4, and 8 days old (n=15 per treatment). Bars labelled with "n.s." are not significantly different ($p > 0.05$) (Binomial (link = "logit") GLM and Tukey post hoc test)

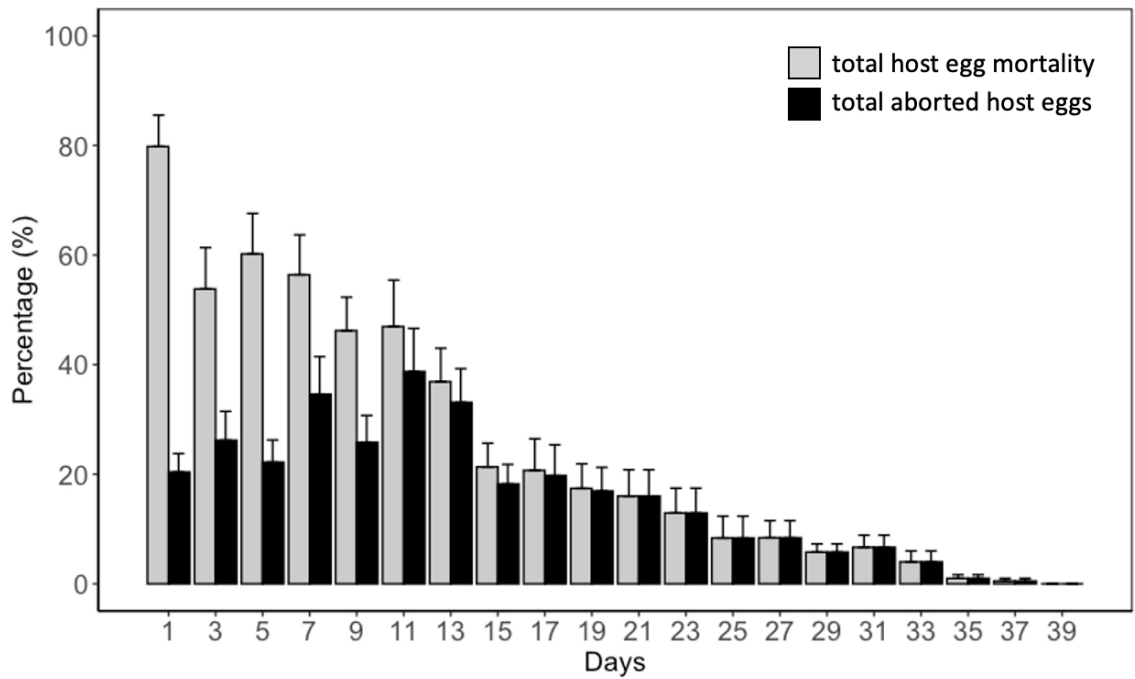


Figure 1.4 Proportion of *Leptoglossus zonatus* host egg mortality (mean + SE) in the presence of *Hadronotus pennsylvanicus*. Eggs that neither yielded a *Leptoglossus zonatus* nymph or adult wasp were determined aborted (black bars), which contributed to the total host egg mortality induced by parasitism (grey bars)

Chapter 2: Food deprivation alters reproductive performance of biocontrol agent

Hadronotus pennsylvanicus

Abstract

Diet can influence parasitoid reproductive performance, and therefore, the efficacy of biocontrol programs. We evaluated the influence of food deprivation on the reproductive fitness and behavior of the egg parasitoid *Hadronotus pennsylvanicus* (Hymenoptera: Scelionidae), a prospective biocontrol agent for *Leptoglossus zonatus* (Heteroptera: Coreidae). Newly emerged female parasitoids were mated and provided host eggs every other day while being provisioned with various honey diet regimes or a consistent supply of water. When given frequent access to a honey diet, female parasitoids lived significantly longer and parasitized more host eggs compared to the water-fed controls. Once depleted of mature eggs, females with frequent access to honey also contributed to greater non-reproductive host mortality. Furthermore, behavioral assays demonstrated that water-fed females spent less time interacting with host eggs and tended to more frequently divert from oviposition behavior. While there was no difference in the average duration until first oviposition between individuals assigned to different diet treatments, increased frequency of honey feeding was associated with more frequent and longer duration of oviposition. The positive effect of honey feeding on the reproductive performance of biocontrol agent *H. pennsylvanicus* suggests that performance of this parasitoid under field conditions could be enhanced through the provision of similar carbohydrate resources, such as flowering summer cover crops.

Introduction

Understanding the influence of diet on parasitoid reproduction is critical for the development of effective biocontrol programs. Access to suitable food sources has shown to significantly improve both the survival and lifetime reproductive fitness of various parasitoids (Wäckers and van Rijn 2005; Benelli et al. 2017). In the field, adult parasitoids obtain food through either floral (nectar and pollen), extra-floral (nectaries), or non-floral (honeydew and host feeding) resources (Wäckers 2004, 2005, 2008; Heimpel and Jervis 2005). These diets provide vital nutrients to fuel bodily maintenance and physiological energy expenditure, enhancing the dispersal capacity and fertility of adult wasps (Wäckers and van Rijn 2005; Jervis et al. 2008). In field settings, the quality and availability of these resources can vary widely in time and space. Periods of insufficient access to sources of carbohydrate-rich food can result in decreased survival, fecundity, and altered behavior of female parasitoids, all of which can negatively impact their biocontrol potential (Wäckers and van Rijn 2005; Benelli et al. 2017).

In order to maximize lifetime reproduction, female parasitoids must balance time limitations, egg limitations, and the costs of oviposition (Rosenheim 1996, 1999, 2008). For synovigenic parasitoids, egg limitation can occur when female egg loads are depleted at a faster rate than they are able to produce. When host resources are abundant, synovigenic parasitoids risk depleting their cache of mature eggs before exhausting their supply of available host eggs (Rosenheim et al. 2000). Furthermore, insufficient access to suitable diet may reduce their ability to replenish egg loads in response to higher host densities, since the availability of a carbohydrate-rich diet has been shown to enhance the

oviposition behavior and parasitism rates for various parasitoids (Benelli et al. 2017). Similarly, feeding frequency has also been shown to be vital for parasitoid survival and reproductive fitness (Benelli et al. 2017). Increased abundance of nutrient rich food sources has been associated with an increase in searching ability, enhanced mobility and sensitivity to olfactory cues, as well as host acceptance (Takasu et al. 1991; Sisterson et al. 2002). In choice assays, satiated wasps preferred a host stimuli over a food resource, whereas starved wasps exhibited no preference, taking more time to initiate searching behavior and to locate a cue (Jacob and Evans 2001; Siekman et al. 2004; Williams et al. 2015). Such patterns in parasitoid behavior may be exacerbated during periods of low food resource availability and extended starvation.

Hadronotus (=Gryon; Talamas et al. 2021) *pennsylvanicus* (Ashmead) (Hymenoptera: Scelionidae) is a generalist synovigenic egg parasitoid, known to attack the leaf-footed plant bug, *Leptoglossus zonatus* (Dallas) (Heteroptera: Coreidae) (Straser et al. 2022) along with several other coreid pests including *L. phyllopus* (L.) (Mitchell and Mitchell 1986), *L. australis* Fabricius (Yasuda 1998), *L. occidentalis* Heidemann (Bates and Borden 2004; Maltese et al. 2012; Roversi et al. 2013), *Anasa tristis* (DeGeer) (Nechols et al. 1989; Cornelius et al. 2016), and *A. armigera* Say (Cornelius et al. 2018). In California, U.S.A., *L. zonatus* has become an increasingly problematic pest of almonds (*Prunus dulcis* L.) and pistachios (*Pistacia vera* L.) (Daane et al. 2016; Joyce et al. 2017; Zalom et al. 2018). Feeding from *L. zonatus* has the potential to cause significant damage to these nut crops and can facilitate such fungal contaminations as stigmatomycosis and Botryosphaeria, all of which lowers crop yield and quality (Michailides 1987, 1989,

2016; Daane et al. 2005; Haviland et al. 2018; Joyce et al. 2019; Stahl et al. 2020). Under laboratory conditions, *H. pennsylvanicus* exhibits suitable demographic and reproductive traits suggesting its potential use as a biocontrol agent for *L. zonatus* (Straser et al. 2022). However, access to a carbohydrate-rich diet is likely critical to maximize lifetime fitness of *H. pennsylvanicus*. Previous studies demonstrated that females deprived of a honey-water diet died shortly after eclosion, resulting in significantly fewer offspring over their short lifetime (Straser et al. 2022; Olson and Nechols 1995; Sabbatini Peverieri et al. 2012; Cornelius et al. 2020). Furthermore, egg limitation appears to be a constraint on lifetime fecundity of female *H. pennsylvanicus* (Straser et al. 2022; Cornelius et al. 2018; Sabbatini Peverieri et al. 2012). Under continuous reproductive opportunity, the proportion of parasitoid-induced aborted eggs increased with female age, ultimately accounting for the majority of total host egg mortality (Straser et al. 2022). Since *H. pennsylvanicus* likely does not feed on their host (Vogt and Nechols 1993), such non-reproductive host mortality may facilitate additional pest control in the absence of mature eggs. However, little is known about the extent of non-reproductive mortality, much less the rate at which *H. pennsylvanicus* are capable of replenishing egg loads when provided sufficient access to a nutritious diet.

Improved knowledge on the role of diet on the reproductive performance of *H. pennsylvanicus* would allow a better understanding of where and under what conditions resource provisioning could be used to improve parasitoid field performance, and by way of that, biocontrol of *L. zonatus*. Here, a series of experiments under controlled laboratory settings were carried out to evaluate the impact of food deprivation on *H. pennsylvanicus*

demographic parameters, reproductive traits, non-reproductive host mortality, and female reproductive behavior. This framework may provide new insight into the role of diet acquisition on the efficacy of biocontrol programs.

Methods

Insect colonies

Laboratory insect colonies were reared from specimens collected in 2017 from Fresno County, California, USA. *Leptoglossus zonatus* were housed in cages (60cm x 60cm x 60cm, 680µm mesh, BugDORM®, Taiwan) that contained a 2.36 L potted juniper (*Juniperus* sp. L.) plant along with a diet of fresh zucchini (*Cucurbita pepo* L.), green beans (*Phaseolus vulgaris* L.) and organic raw sunflower seeds (*Helianthus annuus* L.) that was refreshed weekly. Wooden skewers (25.4 cm x 3 mm) were used as oviposition substrate. *Hadronotus pennsylvanicus* colonies were reared from parasitized sentinel *L. zonatus* egg clusters from pistachio orchard in Parlier, California, USA, and refreshed with wild strain specimens in 2019 and 2020 from pistachio orchards in Fresno County, USA. Parasitoids were housed in 50 ml plastic tubes (Falcon Conical Centrifuge Tubes, Fisher Scientific®) sealed at the open end with 250 µm mesh, and fed honey-water (1:1) (Organic Honey, Wholesome®) *ad libitum*. *Hadronotus pennsylvanicus* was identified to species using Masner (1983) and taxonomic revisions in Talamas et al. (2021). All bioassays were conducted in laboratory conditions of 25°C ± 1, 75 ± 5% RH and 16:8 L:D photoperiod.

Effects of food deprivation on parasitoid demographic & reproductive traits

To investigate the influence frequency of food provisions has on *H. pennsylvanicus* reproductive output, newly emerged (< 24 hr) males and females were housed in mating pairs in 15 ml plastic tubes (Falcon Conical Centrifuge Tubes, Fisher Scientific®) sealed with a 250 µm mesh cap. Each mating pair was provided a honey-water diet (1:1) either once (*x1*), twice (*x2*), three (*x3*) times per week, every other week (*x0.5*), or fed a water diet (*x0*) three times per week (n=15 per treatment). Honey-water was used to standardize the nutrient content of the diet available to wasps throughout the study. A set of 25, fresh (< 72 hr old) *L. zonatus* eggs were provisioned every other day for oviposition until the female died. In order to maintain copulation potential throughout the study, a new male specimen was introduced to the enclosure to replace any expired individuals within 24 hr. Following 24 hr exposure to the parasitoids, *L. zonatus* eggs were removed and observed daily for the emergence of parasitoids or *L. zonatus* nymphs. After 30 days, eggs that did not yield a nymph or adult wasp were dissected to examine for the presence of a dead *L. zonatus*, dead *H. pennsylvanicus*, or determined aborted should neither be discernable.

Effects of food deprivation on non-reproductive host mortality

To test the influence of food accessibility on non-reproductive host mortality, the number of aborted host eggs was compared between diet frequency treatments. To assess the influence parasitoid presence has on host egg abortion, 15 fresh (< 72 hr old) unexposed *L. zonatus* egg clusters containing 25 eggs each were isolated simultaneously

with day 1 replicates of fecundity assays. Similarly, egg clusters were housed in 15 ml plastic tubes closed with a 250 μ m mesh and stored under controlled laboratory conditions for 30 days. Egg clusters were observed for the emergence of *L. zonatus* nymphs and all unemerged eggs remaining were dissected to test for presence of dead nymphs or identified as aborted if contents were indiscernible. Observations from fecundity assay dissections did not account for the presence or absence of a parasitoid meconium. The number and frequency of host-eggs provided to parasitoids were selected based on the maximum parasitism observed within 24 hr, and previous findings that short term host deprivation does not influence parasitism rates (Straser et al. 2022; Vogt and Nechols 1993; Sabbatini Peverieri et al. 2013).

Effects of food deprivation on parasitism behavior

To assess the influence of diet on parasitism behavior, newly emerged (< 24 hr) females were randomly selected and fed either a honey-water diet (1:1) (n=20) or distilled water (n=20). Newly emerged female *H. pennsylvanicus* individuals were housed individually with a newly emerged male for 24 hr prior to observation. After 24 hr, females were removed and placed individually in a 54 x 14mm Petri dish arena containing a set of 25 fresh (< 72 hr old) *L. zonatus* eggs and 3 drops of the designated diet treatment. Parasitoid behavior was recorded using a Dino-Lite Digital Microscope (5MP Edge AM7915MZT, AnMo Electronics Co., Taiwan) for 30 min following the placement of the female in the center of the arena. All behavior assays were conducted between 08:00 - 12:00 during peak parasitism activity (Vogt and Nechols 1991).

Following Wiedemann et al. (2003) behavior was recorded as discrete states: static (remaining motionless or inactive following introduction), walking (host searching, walking or exploring arena), drumming (antennating egg clusters rapidly), oviposition (inserting ovipositor into host-egg for egg laying), marking (scratching ovipositor on egg surface to mark host-egg), grooming (cleaning body parts using legs or mouth), resting (remaining motionless on egg cluster), feeding (drinking from diet droplet). Behavioral observations were analyzed using Behavioral Observation Research Interactive Software (BORIS) (Friard and Gamba 2016) to quantify the number of ovipositions, duration spent on each behavior state, and the frequency of transitions between behavior states.

Data analysis

Data on parasitoid longevity and fecundity were used to calculate biological and reproductive traits of female *H. pennsylvanicus* on host *L. zonatus* under each diet frequency treatment. The effect of diet access frequency on parasitoid longevity, fecundity, and reproductive behavior were evaluated with generalized linear mixed models (GLMM). A Gamma error distribution and an identity link function were used to assess parasitoid longevity, while a Poisson error and log-link function were used to assess ovipositional period, post reproduction period and total progeny produced, and a binomial error distribution used with an identity link function to assess progeny sex ratios. The effect of diet access frequency on number of ovipositions, duration spent in each behavior state, and frequency of behavioral transitions were assessed using a Gaussian distribution and identity link function. 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 test via the “drop1” function. When multilevel variables were found to be significant, means were separated with 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

Effects of food deprivation on parasitoid demographic & reproductive traits

Diet availability significantly influenced the longevity and lifetime fecundity of female *H. pennsylvanicus* ($\chi^2 = 152.27$, $df = 4$, $P < 0.001$ for longevity; $\chi^2 = 35.26$, $df = 4$, $P < 0.001$ for fecundity) (Table 2.1). Similarly, diet availability influenced the duration of ovipositional and post-reproductive periods for females ($\chi^2 = 73.75$, $df = 4$, $P < 0.001$ for ovipositional period; $\chi^2 = 94.90$, $df = 4$, $P < 0.001$ for post-reproductive period). Females fed a honey-water diet two or three times per week lived significantly longer and exhibited greater ovipositional and post-reproductive periods throughout their lifetime than females fed honey-water once per week, every other week, or a water-only diet. When provided host eggs, females fed a honey-water diet twice per week lived 38.80 ± 2.79 days, while females fed three times per week lived 41.60 ± 2.75 days. Water-fed females provided host eggs only lived 2.40 ± 0.31 days. Females fed a honey-water diet three times per week maintained the greatest lifetime fecundity (48.10 ± 7.07 offspring), while females fed honey-water less than twice per week or fed water exhibited fewer

offspring over their lifespan. However, diet availability did not influence the average female sex ratio of offspring over a females lifetime.

Diet availability significantly influenced reproductive and non-reproductive host mortality ($\chi^2 = 35.26$, $df = 4$, $P < 0.001$ for offspring produced per female per day; $\chi^2 = 30.45$, $df = 4$, $P < 0.001$ for induced abortions per female per day) (Fig. 2.1A). All parasitism occurred within first 15 days of the females lifetime, with the highest mean parasitism rates occurring on day 1 of study. On day 1, water-fed females produced fewer offspring (6.70 ± 2.38) than females provided honey-water diet once (13.40 ± 1.69) or three times per week (13.00 ± 1.64) ($z = 2.84$, $P < 0.05$ for $x1$; $z = 2.74$, $P < 0.05$ for $x3$), however was not significantly different than females fed honey-water twice (13.30 ± 2.16) or every other week (12.30 ± 2.50) ($z = 2.66$, $P = 0.06$ for $x1$; $z = 2.29$, $P = 0.15$ for $x0.5$). Fecundity showed relatively consistent declines over the first three weeks for all diet treatments. However, females provided honey-water twice or three times per week maintained higher fecundity rates on days 5 ($x2$, 8.10 ± 1.27 ; $x3$, 11.20 ± 2.04), 7 ($x2$, 3.60 ± 0.92 ; $x3$, 6.70 ± 1.33), and 9 ($x2$, 1.80 ± 0.93 ; $x3$, 5.10 ± 1.96) in comparison to individuals fed once per week, every other week, or a water-only diet.

Effects of food deprivation on non-reproductive host mortality

There was a significantly greater proportion of aborted host eggs observed when *L. zonatus* eggs were exposed to a female wasp than those unexposed ($\chi^2 = 31.62$, $df = 1$, $P < 0.001$). In the absence of a parasitoid, the average proportion of eggs determined aborted was 0.70 ± 0.26 . Diet availability also influenced non-reproductive host mortality

over the course of a females lifetime ($\chi^2 = 146.17$, $df = 4$, $P < 0.001$ for induced egg abortions per female per day) (Fig. 2.1B). Water-fed females exhibited greater rates of induced host abortion (16.20 ± 2.52) on day 1, however this trend declined to 4.57 ± 2.42 on day 3 of the study. When females were fed a honey-water diet, individuals fed twice or three times per week maintained higher rates of induced abortions than individuals fed honey-water once per week or every other week ($z = 4.10$, $P < 0.01$ for x_2 ; $z = 5.52$, $P < 0.01$ for x_3). For honey-fed females provided diet twice and three times per week, female age had a significant influence on the proportion of induced abortions ($z = 6.19$, $P < 0.01$). Here, females fed a honey-water diet three times per week exhibited a greater number of induced abortions than wasps fed diet twice per week from day 11 ($z = 2.92$, $P < 0.01$) through day 23 ($z = 3.42$, $P < 0.01$) of the study.

Effects of food deprivation on parasitism behavior

Diet influenced the duration parasitoids spend in behavioral states (Fig. 2.2). During the 30 min observation period, honey-fed females spent on average $74.82 \pm 8.51\%$ of their time interacting with host eggs, whereas water-fed females spend $37.09 \pm 10.16\%$ of their time. Water-fed females spent significantly more time walking ($\chi^2 = 7.56$, $df = 1$, $P < 0.01$) or remaining static ($\chi^2 = 4.11$, $df = 1$, $P < 0.05$) in the arena than honey-water fed females. However, diet did not influence the amount of time females spent grooming or feeding from provided diet. When interacting with host eggs, honey-water fed females spent more time ovipositing in eggs than water-fed individuals ($\chi^2 = 13.18$, $df = 1$, $P < 0.001$). However, there was no significant difference in the duration of

time spent drumming, marking, or resting between honey- and water-fed individuals. While there was no difference in the average duration until females began ovipositing (Fig. 2.3A), 50% of water-fed females failed to oviposit on host eggs, whereas only 10% of honey-water fed females failed to oviposit. Of the individuals that exhibited oviposition behavior, honey-water fed females oviposited more frequently than females fed a water diet ($\chi^2 = 3.88$, $df = 1$, $P < 0.05$) (Fig. 2.3B. Females fed honey-water oviposited 2.7 ± 0.44 times within a 30 min period while females fed water diet oviposited 1.50 ± 0.42 times.

Adult diet influenced the frequency of behavioral transitions in female *H. pennsylvanicus* (Fig. 2.4). Females fed a honey-water diet more frequently transitioned from walking to drumming behavior on the host ($\chi^2 = 5.06$, $df = 1$, $P < 0.05$). Here, $50.00 \pm 9.00\%$ of honey-water fed females transitioned from walking to drumming behavior, whereas only $28.10 \pm 6.70\%$ of water fed females made the transition. Once interacting with host eggs, honey-water fed individuals more frequently transitioned from drumming to oviposition ($79.80 \pm 7.20\%$ for honey-water fed, $31.70 \pm 8.20\%$ for water fed) ($\chi^2 = 8.63$, $df = 1$, $P < 0.01$). Furthermore, water-fed females more frequently transitioned from drumming host eggs to walking behavior ($31.30 \pm 8.90\%$ for water fed, $4.30 \pm 2.10\%$ for honey-water fed) ($\chi^2 = 7.65$, $df = 1$, $P < 0.01$), aborting parasitism behavior on the host. There was no significant difference in the frequency of all other behavior state transitions between honey-fed and water-fed females.

Discussion

Access to carbohydrate-rich food sources has shown to bolster the longevity, dispersal, and fecundity of natural enemies, and therefore enhance the efficacy of biocontrol (Wäckers 2004, 2005, 2008). During periods of food deprivation, parasitoids may spend more time foraging, reabsorbing eggs to extend longevity, or maturing eggs more rapidly in order to maximize reproductive fitness (Rosenheim et al. 2008; Olson et al. 2000; Jervis et al. 2001; Jervis and Ferns 2004). The aim of this study was to assess how inadequate access to diet impacts the fitness and reproductive performance of the egg parasitoid *H. pennsylvanicus*, a key biocontrol agent for the tree nut pest *L. zonatus*. Under laboratory conditions, we found support for the theory that food deprivation negatively impacts the longevity, lifetime fecundity, and reproductive behavior of parasitoids (Wäckers and van Rijn 2005; Benelli et al. 2017).

When provided frequent access to a carbohydrate-rich diet, female *H. pennsylvanicus* produced more offspring while maintaining extended ovipositional periods. Alternatively, females fed < 2 times per week exhibited a drastic decline in offspring production early in their lifetime. Given *H. pennsylvanicus* does not show evidence of egg resorption (Vogt and Nechols 1993), this may indicate that females are capable of maturing eggs only when provided sufficient access to diet. Previous studies have demonstrated that consistent access to food, rather than longer feeding intervals, provides greater benefits to adult longevity (Lee and Heimpel 2008). Wu et al. (2008) found parasitoids provided with continuous access to a sugar diet produced significantly more offspring than those provided fewer opportunities to feed on a sugar diet. Theory

suggests that insufficient access to a suitable diet reduces the ability of synovogenic parasitoids to replenish egg loads (Benelli et al. 2017). Our findings suggest that during periods of low food resource availability, *H. pennsylvanicus* may be unable to produce egg loads at a sufficient rate to suppress high pest densities in cropping systems. While we did not observe female egg load, future research that documents egg count over the course of a female lifetime would illuminate the link between egg maturation and diet.

The sex ratio of progeny of parasitoids can be influenced by several biotic and abiotic factors, such as parental age, host quality, female population density, and environmental temperature (King 1987). While Berndt et al. (2005) found that food deprivation in female wasps can lead to male-biased progeny, parasitoids respond to external pressures differently (Benelli et al. 2017). However, we found that frequent access to honey-water did not influence the female sex ratio of *H. pennsylvanicus* progeny over the lifetime of a female. Instead, *H. pennsylvanicus* appears to maintain consistently high female-skewed progeny despite ovipositing female age and host age (Sabbatini Peverieri et al. 2013; Straser et al. 2022). These outcomes vary between host species (Nechols et al. 1989; Sabbatini Peverieri et al. 2012). Previous studies have found that such trends in sex ratio are due to sperm depletion in females despite being held under conditions of continuous copulation opportunity (Sabbatini Peverieri et al. 2012). However, little is known on how sperm depletion may influence the reproductive performance of egg parasitoids, and this requires further evaluation. While parasitoid fecundity is ultimately dependent on host availability, maintaining a high female-skewed

sex ratio in progeny despite food availability would likely benefit parasitoid population growth rates in field settings.

Egg parasitoids provide biocontrol by killing the host egg. Oviposition of a parasitoid egg into the host egg results in host egg mortality and reproduction of the parasitoid (i.e. reproductive mortality). However, non-reproductive host mortality also plays an important, and often understated role in biocontrol programs for insect pests (Abram et al. 2019). Previous studies have found that when *H. pennsylvanicus* was provided honey-water *ad libitum*, non-reproductive host mortality accounted for > 50% of the total host mortality observed within the first 2 weeks of a females life (Straser et al. 2022). Furthermore, researchers found that the proportion of parasitoid-induced aborted eggs increased with female *H. pennsylvanicus* age, ultimately accounting for the majority of total host egg mortality (Straser et al. 2022). In this study, we found that the frequency in which diet was provided influenced parasitoid-induced host abortion rates over the lifetime of a female wasp. Though there was large variation between diet treatment groups, initial feeding following parasitoid emergence appeared to impact the proportion of non-reproductive host egg mortality thereafter. Starved females exhibited their highest rates of induced host abortions on day 1 of the study, whereas wasps fed ≥ 2 times per week maintained higher rates of induced abortions as successful production of progeny began to decline. Our findings may suggest that during extended periods of food deprivation, *H. pennsylvanicus* females may maintain parasitism activity despite reaching potential egg maturation limits. Such trends in parasitoid induced abortions may help elucidate the role that non-reproductive host mortality may play in applied biocontrol

programs for *L. zonatus*. However, further research is needed to better understand how diet constraints may impact the parasitism rates by *H. pennsylvanicus* females, and furthermore, the relative proportion of insect-induced host egg abortions resulting in the suppression of *L. zonatus* in field settings.

Female parasitoids primarily allocate energy to locate a host or forage for food (Benelli et al. 2017). If their diet does not fulfill their nutritional requirements, they may not be able to locate food or hosts (Wäckers and van Rijn 2005). While in general, starved parasitoids tend to show preference in locating a food resource rather than a host, these trends can often vary and outcomes may be species specific (Lewis and Takasu 1990; Takasu and Lewis 1995; Wäckers 1994; Lightle et al. 2010; Varennes et al. 2016). Previous studies have demonstrated that food deprivation can enhance the oviposition behavior of egg parasitoids, resulting in increased female reproductive fitness (Takano and Takasu 2019). For instance, Takano et al. (2019) found that starved females of egg parasitoid *Paratelenomus saccharalis* (Dodd) (Hymenoptera: Platygasteridae) oviposited quicker and more frequently on host eggs than satiated females. These findings would suggest parasitoids would exhibit preference to reproduce as soon as possible when under conditions of potentially high mortality for adults. However, our results contradict with these findings. We found that honey-fed females spent more time interacting with host eggs, and more commonly engaged in parasitism behaviors. Furthermore, of the water-fed *H. pennsylvanicus* females that engaged in drumming behavior, an average 31% of those individuals abandoned the host eggs prior to oviposition and did not complete parasitism. These findings would suggest that *H. pennsylvanicus* may seek suitable

carbohydrate-rich diets over opportunities for reproduction. However, it is important to note that *H. pennsylvanicus* parasitism behavior can vary between host species. Cornelius et al. (2020) found that when provided continuous access to honey diet, *H. pennsylvanicus* maintained increased rates of parasitism on *A. tristis* egg masses, however diet did not impact parasitism of *A. armigera* egg masses. Female wasps also spend the same amount of time probing egg masses of the two host species, however they spend significantly more time drilling *A. tristis* eggs than *A. armigera* eggs (Cornelius et al. 2020). Little is known about how individual variation within species and between-species variation may influence parasitoid behavior in responses to food deprivation.

Under field conditions, the availability of carbohydrate-rich resources can vary widely. Parasitoids may experience periods with insufficient access to a suitable diet, which can negatively impact their reproductive fitness and the efficacy of biocontrol programs (Wäckers and van Rijn 2005). A nuanced understanding of the influence of food deprivation on host parasitism may lead to improved strategies to enhance biocontrol within integrated pest management programs, such as the optimization of floral resource provisioning (Landis et al. 2000). The spatial and temporal arrangement of habitat resources necessary to provide reliable biocontrol services in a given agroecosystem is contingent on a variety of ecological and agronomic factors, including natural enemy feeding frequency requirements (Wäckers and van Rijn 2005), as demonstrated here. Findings from this study suggest that floral resource provisioning in tree nut orchards to enhance *H. pennsylvanicus* populations should be designed to maximize and increase parasitoid feeding frequency. Here, there remains a need to better

understand the phenology of both pest and parasitoid in relation to the floral provisions, how *H. pennsylvanicus* populations may respond to extended periods of low food resource availability, and furthermore, when and where resource provisioning may bolster biocontrol services in applied settings. For the latter, it will be important to characterize potential energetic tradeoffs between foraging and host location efforts and behavior under different resource availability scenarios.

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<i>Reproductive parameters</i>	<i>x0</i>	<i>x0.5</i>	<i>x1</i>	<i>x2</i>	<i>x3</i>
Adult female longevity (days)	2.40 ± 0.31 a	8.20 ± 0.95 b	9.20 ± 0.70 b	38.80 ± 2.79 c	41.60 ± 2.75 c
Ovipositional period (days)	1.20 ± 0.33 a	4.40 ± 0.60 b	3.20 ± 0.55 b	10.40 ± 1.19 c	11.20 ± 1.09 c
Post-reproductive period (days)	1.20 ± 0.29 a	3.80 ± 0.92 b	6.00 ± 1.03 b	28.40 ± 3.38 c	30.40 ± 2.96 c
Total progeny (offspring/female)	8.90 ± 2.49 a	22.10 ± 3.57 b	17.00 ± 1.98 b	36.10 ± 5.52 bc	48.10 ± 7.07 c
Total female progeny	7.60 ± 1.98 a	20.40 ± 3.42 b	14.60 ± 1.74 b	31.20 ± 5.05 bc	42.10 ± 5.93 c
Total male progeny	1.30 ± 0.72 a	1.70 ± 0.47 ab	2.40 ± 0.67 abc	4.90 ± 0.87 bc	6.00 ± 1.80 c
Sex ratio (% female offspring)	89.57 ± 3.95 a	94.10 ± 1.34 a	86.10 ± 4.32 a	85.30 ± 1.75 a	88.60 ± 2.18 a

Table 2.1 Reproductive parameters (mean \pm SE) of *Hadronotus pennsylvanicus* reared on *Leptoglossus zonatus* eggs every other day at 25°C \pm 1, 75 \pm 5% RH and 16:8 L:D, fed a honey-water diet once (x1), twice (x2), three times (x3) per week, once every other week (x0.5), or fed a water diet (x0). Parameters with different letters indicate significant differences across the different treatments (P < 0.05; GLM and Tukey post hoc test)

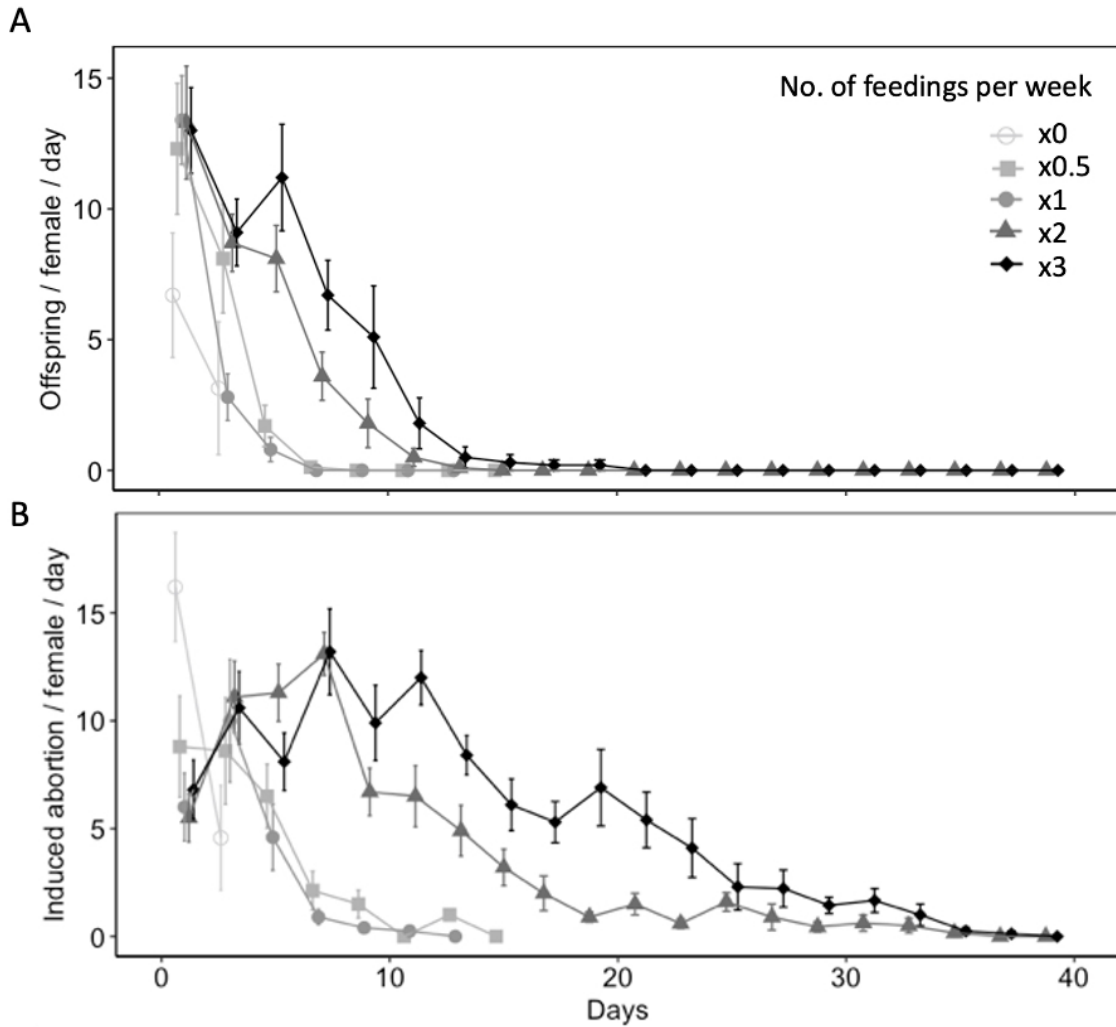


Figure 2.1 Age specific fecundity (A) and induced egg abortions on host (B) (mean \pm SE) of *Hadronotus pennsylvanicus* when provided *Leptoglossus zonatus* host eggs and fed honey-water diet once (x1), twice (x2), or three (x3) times per week, every other week (x0.5), or fed a water-only diet (x0)

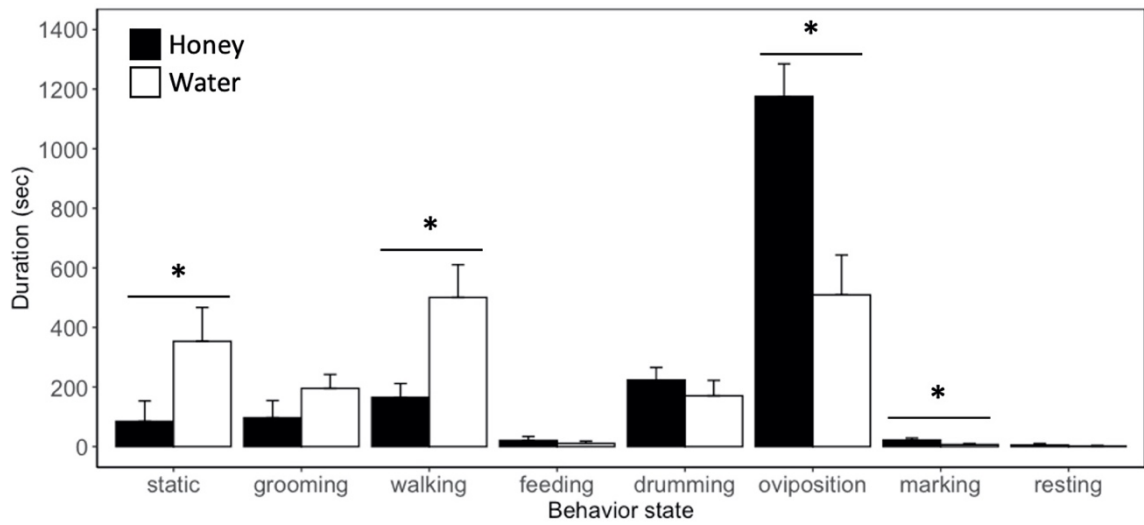


Figure 2.2 Duration (mean + SE) spent within each behavior state in 30 min period by *Hadronotus pennsylvanicus* female when provided *Leptoglossus zonatus* host eggs and fed a honey-water (honey) or water diet (water). Statistical significance between treatments are indicated by asterisks ($P < 0.05$; GLM and likelihood ratio chi-squared test)

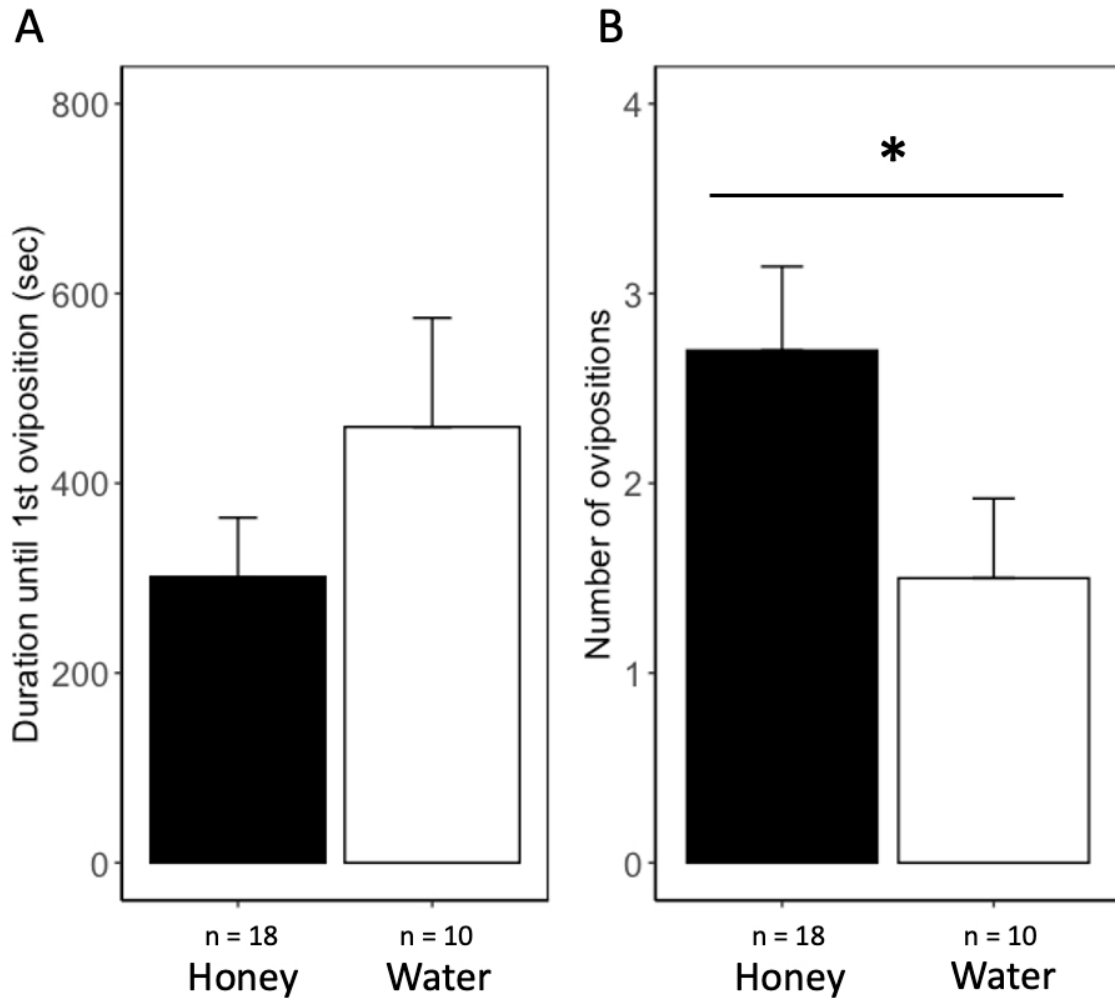


Figure 2.3 Duration until the first oviposition on host (A) and the number of ovipositions (B) (mean + SE) within 30 min period by *Hadronotus pennsylvanicus* female when provided *Leptoglossus zonatus* host eggs and fed a honey-water (honey) or water-only diet (water). Statistical significance between treatments indicated by asterisks ($P < 0.05$) (GLM and likelihood ratio chi-squared test)

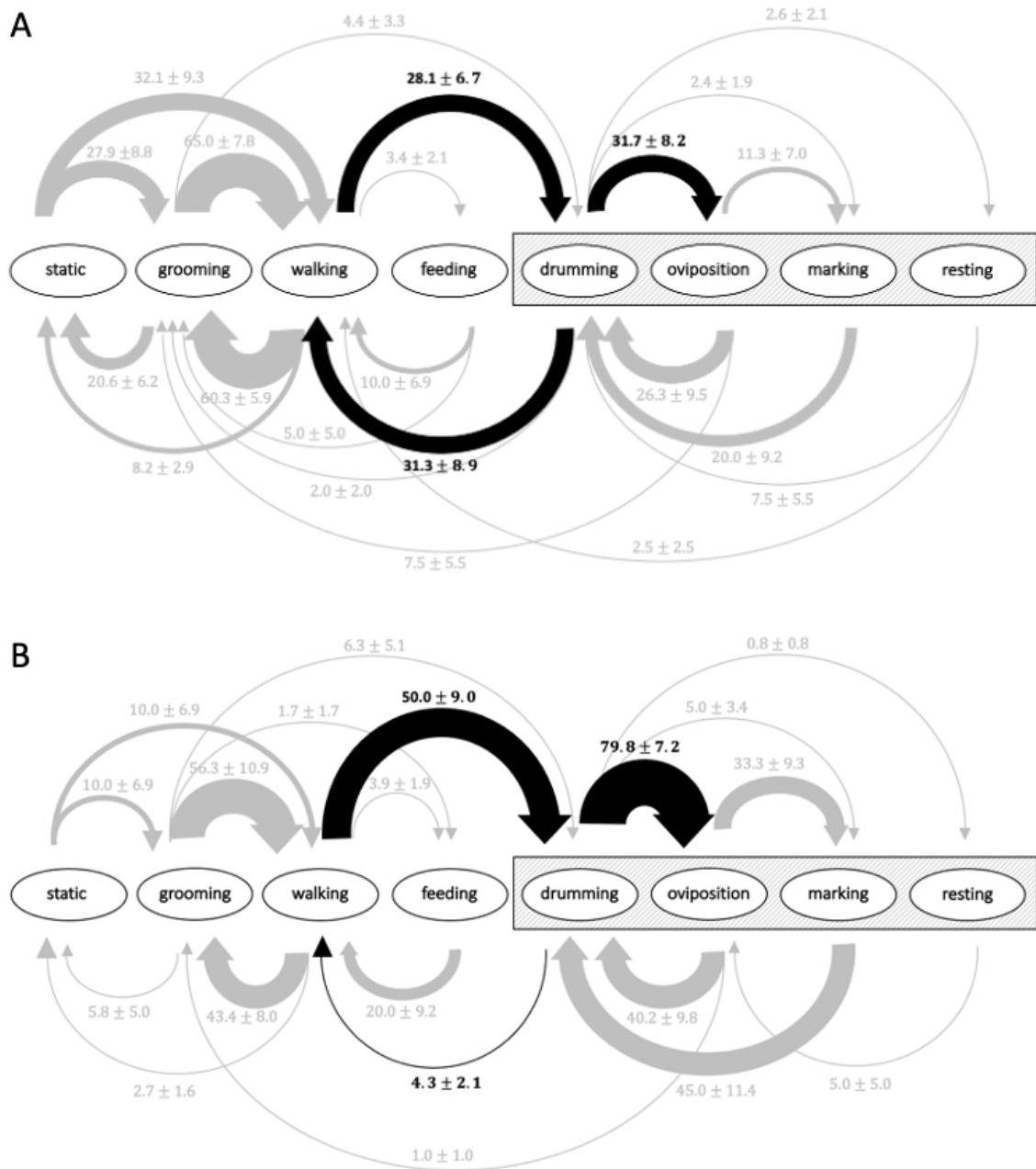


Figure 2.4 Ethogram of the proportion of transitions between behavioral states (mean ± SE) within a 30 min period by *Hadronotus pennsylvanicus* female when provided with *Leptoglossus zonatus* host eggs and fed a water (A) or honey-water diet (B). Grey box highlights behaviors in which parasitoid is interacting with host eggs. Line thickness indicates relative frequency of transition. Black arrows highlight behaviors where a statistical difference was observed between the two treatments ($P < 0.05$; GLM and likelihood ratio chi-squared test)

Chapter 3: Diet quality influences nutrient retention and reproductive fitness of the egg parasitoid *Hadronotus pennsylvanicus*

Abstract

Diet can have a direct influence on the reproductive success of parasitoid wasps. For synovigenic parasitoids, the nutrients obtained from floral resources, such as nectar and pollen, play a vital role in fueling bodily functions and physiological energy expenditure incurred from reproduction. Insufficient access to nutrient-rich diets can lead to lower rates of reproductive fitness, therefore reducing the efficacy of biocontrol. Here, a study was conducted to evaluate the influence of diet quality on nutrient retention and reproductive fitness of the egg parasitoid *Hadronotus pennsylvanicus* (Hymenoptera: Scelionidae), a prospective biocontrol agent for the leaf-footed bug *Leptoglossus zonatus* (Heteroptera: Coreidae). Newly emerged parasitoid females were provided host eggs every other day accompanied by diets of varying sucrose concentrations (source of carbohydrates) and pollen (source of lipid and proteins). The sucrose concentration in the diet, regardless of pollen content, significantly increased the survival and lifetime fecundity of female *H. pennsylvanicus*. While wasps fed high sucrose diets depleted bodily sugars, glycogen, and lipids at a slower rate than wasps fed low sucrose diets, there was no effect on bodily protein levels. Given these findings, further research is now needed to identify floral resources that are compatible, attractive, and nutritionally-sufficient for optimal *H. pennsylvanicus* reproductive fitness, which could lead to enhanced parasitism of *L. zonatus* in field settings.

Introduction

Access to nutrient-rich diets is vital for the survival of adult parasitoids (Jervis et al. 2008; Wäckers and van Rijn 2005; Benelli et al. 2017). In nature, parasitoids obtain food through various floral (nectar and pollen), extra-floral (nectaries), and non-floral (honeydew and host feeding) resources (Wäckers 2004; Heimpel and Jervis 2005; Wäckers 2005; Wäckers et al., 2008). The nutrients obtained from these sources can improve adult parasitoid longevity, enhance female fecundity, and increase parasitism behavior, and in turn lead to increased parasitism rates and pest control (Siekmann et al. 2001; Tena et al. 2015; Benelli et al. 2017). For synovigenic parasitoids, periods of low food resource availability may limit the reproductive capacity of females (Jervis et al. 2001; Jervis and Ferns 2004). Under these conditions, inadequate diet may lead to egg limitation, in which females are unable to produce eggs at a sufficient rate, ultimately depleting their egg load before exhausting their supply of available host eggs, and resulting in less effective biological control (Rosenheim et al. 2000). Therefore, the retention and allocation of these nutrients plays a vital role in sustaining the physiological demands incurred during bodily function and reproduction in adult wasps (Jervis et al. 2008; Ellers et al. 2011; Benelli et al. 2017).

Floral resources provide numerous benefits to parasitoid reproductive fitness (Wäckers and van Rijn 2005). The nectar and pollen provided by floral resources contain sugars and amino acids to attract and nourish parasitoid wasps (Wäckers 2004; Zhang et al. 2004; Heil 2011; Jones et al. 2016). Ingested nutrients contribute to energy production in adult wasps, enabling the storage of glycogen (Olson et al. 2000; Giron and Casas

2003), providing greater retention, and thereby slower depletion of lipid and protein reserves (Rivero and Casas 1999; Jervis et al. 2008), and increasing the rate of egg maturation for reproduction (Wheeler 1996). Furthermore, adult nutrition has been shown to improved search ability, mobility, and olfactory cue sensitivity in parasitoid wasps (Takasu and Hirose 1991; Sisterson and Averill 2002; Benelli et al. 2017). Together, the nutrients obtained from floral diets are vital for the survival and reproductive success of parasitoid wasps (Wäckers and van Rijn 2005). However in agricultural landscapes, the quality and accessibility of nutrient-rich food resources can vary widely, potentially hindering the acquisition and retention of the nutrients necessary for maximizing parasitoids' fitness, and thereby their biocontrol potential (Segoli and Rosenheim 2013; Russell 2015; Benelli et al. 2017; Kishinevsky et al. 2018).

Hadronotus (=Gryon) *pennsylvanicus* (Talamas et al. 2021) (Ashmead) (Hymenoptera: Scelionidae) is a generalist synovigenic egg parasitoid of several coreiid pests (Mitchell and Mitchell 1986; Yasuda 1998; Bates and Borden 2004; Maltese et al. 2012; Roversi et al. 2013), including the western leaffooted bug, *Leptoglossus zonatus* (Dallas) (Heteroptera: Coreidae) (Straser et al. 2022). Leaffooted bugs have become an increasingly problematic pests for tree nut crops, such as almond and pistachio, in California, U.S.A. (Daane et al. 2016; Joyce et al. 2017; Zalom et al. 2018). Feeding by *L. zonatus* can result in significant reductions in both crop quality and yield (Michailides and Morgan 2016; Daane et al. 2005; Haviland et al. 2018; Joyce et al. 2019). While *H. pennsylvanicus* exhibits desirable reproductive traits as a prospective biocontrol agent of *L. zonatus* (Straser et al. 2022), the parasitoid has been shown to be highly dependent on

carbohydrate-rich diets in order to maintain reproductive fitness (Olson and Nechols 1995; Sabbatini Peverieri et al. 2012; Cornelius et al. 2020). In laboratory assessments, egg limitation appears to be a limiting factor on offspring production by female *H. pennsylvanicus* (Sabbatini Peverieri et al. 2012; Cornelius et al. 2018). However there is limited information on the nutritional requirements necessary to maximize the lifetime fecundity of this wasp.

As such, understanding the role of diet quality on the reproductive fitness of *H. pennsylvanicus* should enable a better understanding of how providing appropriate food resources in field settings can improve biocontrol programs for *L. zonatus*. In the present study, a series of controlled laboratory experiments assessed the nutrient composition and reproductive fitness of female *H. pennsylvanicus* under varying diet quality regimes. Our specific objectives were to determine the impact of sucrose and pollen diets on female *H. pennsylvanicus* longevity, lifetime fecundity, and bodily nutrient composition. This framework may provide new insight into the importance of diet quality in optimizing parasitoid reproductive fitness, and therefore, biocontrol programs.

Methods

Insect rearing

Colonies of *H. pennsylvanicus* and *L. zonatus* were reared from specimens collected in 2017 from Fresno County, California, U.S.A. The *L. zonatus* strain was collected from pomegranates and augmented with wild specimens *ad libitum*. Insects were maintained in cages (60 cm x 60 cm x 60 cm, 680 μ m mesh, BugDORM®),

Talchung, Taiwan) containing a 2.36 l potted juniper (*Juniperus sp. L.*) and provided a diet of zucchini (*Cucurbita pepo L.*), green beans (*Phaseolus vulgaris L.*) and organic raw sunflower seeds (*Helianthus annuus L.*) once per week. All diet remained in cages for the entire week so insects had continuous access to food. Egg clusters were collected from wooden skewers (25.4 cm x 3 mm) provided within the enclosures as oviposition substrate. *Hadronotus pennsylvanicus* colonies were initially reared from parasitized sentinel *L. zonatus* egg clusters that had been exposed in a pistachio orchard in Fresno County, U.S.A. This colony was subsequently replenished with additional wild strain specimens in 2019 and 2020 obtained from additional pistachio orchards in the same area. Here, wooden skewers (25.4 cm x 3 mm) were placed inside the leaf-footed bug cages and used as oviposition substrate. Freshly oviposited (< 48 hr) *L. zonatus* egg clusters were removed from the cages and placed in the pistachio canopy using Elmer's Wood Glue (Elmer's Products, Westerville, Ohio, U.S.A.). After 72 hr, all sentinel eggs were collected from the orchard and stored under controlled laboratory conditions. Parasitoids were reared in 50 ml plastic tubes (Falcon Conical Centrifuge Tubes, Fisher Scientific®) sealed at the open end with 250 µm mesh, and fed a honey-water diet (1:1) (Organic Honey, Wholesome®) *ad libitum*. *Hadronotus pennsylvanicus* was identified to species using Masner (1983) and taxonomic revisions in Talamas et al (2021). Colonies were held and bioassays were conducted under controlled laboratory conditions of 25 ±1°C, 75 ±5% RH and 16:8 L:D photoperiod using artificial, fluorescent lights.

Longevity and fecundity

To assess the influence that diet quality on *H. pennsylvanicus* reproductive fitness, newly emerged (< 24 hr) male and female wasps were housed in mating pairs in 15 ml plastic tubes (Falcon Conical Centrifuge Tubes, Fisher Scientific®) sealed with 250 µm mesh. Pairs were provided one of eight diet treatments varying in sucrose concentration and availability of pollen (n = 10 per treatment) (Table 3.1). The sugar concentration in floral nectar can vary widely. However most nectar typically ranges between 50-75% (w/v) sucrose concentrations (Chalcoff et al. 2005). Therefore, to test the influence of nectar sucrose concentration on female reproductive fitness, wasps were provided a diet containing pollen with either a low (25% w/v) or high (75% w/v) sucrose solution, or water without sucrose (water, 0% w/v). Similarly, 50% (w/v) sucrose diet without pollen was provided to assess a pollen-starved diet. Honey-water (1:1) with and without pollen was used in assessments as a common laboratory diet. The pollen used in bioassays contained honeybee-collected pollen of mixed origin, consistent with the generalist floral diet of parasitoids (Zemenick et al. 2018). For treatments that contained pollen, grains were diluted (20 mg/ml) into each designated diet treatment (Geng et al. 2006). Negative and positive control treatments were administered by providing wasps with either water without pollen provisions or a 50% (w/v) sucrose solution with pollen provisions, respectively. In each treatment, a set of 25 fresh (< 72 hr old) *L. zonatus* eggs were provided every other day for oviposition until the female wasp died. To maintain copulation potential throughout the experiment, a new male was introduced to the enclosure to replace any dead individuals. Following 24 hr exposure to the parasitoids,

host eggs were removed and observed daily for the emergence of parasitoids or *L. zonatus* nymphs. Data were recorded on female longevity and lifetime fecundity. The abundance and frequency of host-egg exposure to parasitoids were selected based on observations of maximum parasitism potential within a 24 hr period, and previous findings reporting that the duration of oviposition did not influence parasitism rates nor oocyte resorption when *H. pennsylvanicus* was deprived of host eggs for up to 5 d (Sabbatini Peverieri et al. 2013; Vogt and Nechols 1993).

Nutrient composition

To test the role of diet quality on nutrient acquisition and retention, newly emerged female wasps were housed in 50 ml plastic tubes (Falcon Conical Centrifuge Tubes, Fisher Scientific®) sealed with a 250 µm mesh cap and provided a randomly selected treatment diet (Table 3.1) every other day. A subsample of approximately 10 wasps was removed from each treatment vial on days 1, 3, 5, 7, 10, 15, and 20 and stored at -20°C (Table 3.2). Whole body sugar, lipid, glycogen, and protein content of each specimen was measured using anthrone and vanillin assays (Van Handel 1985a, b). Nutrient assays were conducted with 250 µl reaction volumes to account for the small size of *H. pennsylvanicus* (McIntosh et al. 2020). Individuals were crushed with a pestle in 50 µl of lysis buffer (Sigma Aldrich, Burlington, MA, USA) within a 2 ml microcentrifuge tube (Fisher Scientific, Waltham, MA, USA).

Protein content

To assess protein content, 25 μ l aliquots of the 50 μ l buffer solution from each sample were removed and pipetted into wells of a 96-well plate (Costar 3590, Corning Inc., Corning, NY, USA). Next, 225 μ l of Bradford reagent (Sigma Aldrich, Burlington, MA, USA) was added to each well. After incubating for 20 min at room temperature, we used a spectrophotometer (SpectraMax ABS Plus Microplate Reader, San Jose, CA, U.S.A with SoftMax Pro 7 version 7.1.0) at 595 nm.

Glycogen content

To measure glycogen content, 13 μ l of 20% sodium sulfate (Sigma Aldrich, Burlington, MA, USA) was added to each tube containing a crushed specimen in the remaining 25 μ l of buffer solution. Next, 113 μ l of chloroform:methanol (1:2) (Fisher Scientific, Waltham, MA, USA) was added, then the samples were vortexed (Digital Vortex Mixer, Fisher Scientific, Waltham, MA, USA) and then centrifuged (Sorvall Legend Micro 17R Centrifuge, Fisher Scientific, Waltham, MA, USA) at 13,000 rpm for 2 min. The supernatant of each sample was divided in half (~60 μ l of solution) into 2 ml microcentrifuge tubes (held for sugar and lipid assays), and remaining solvent was allowed to evaporate off the pellet. Once dried, 240 μ l of anthrone reagent (Sigma Aldrich, Burlington, MA, USA) was added with the pellet. Anthrone reagent was mixed by adding anthrone powder into 70% sulfuric acid (Fisher Scientific, Waltham, MA, USA) at a density of 1.42 g/L. The samples were vortexed then heated in a dry bath

(Isotemp Digital Dry Bath, Fisher Scientific, Waltham, MA, USA) at 90°C for 3 min. Once cooled, samples were then pipetted into a 96-well plate and read at 630 nm.

Sugar content

To assess sugar content, one tube containing half of the supernatant of the sample (~60 µl of solution isolated during glycogen processing) was evaporated at 90°C using a dry bath for approximately 2 min until ~10 µl of solution remained. Next, 240 µl of anthrone reagent was added to the samples and they were heated at 90°C for 3 min. Once cooled, samples were pipetted onto a 96-well plate and read on a spectrophotometer at 630 nm.

Lipid content

To assess lipid content, the remaining tube containing half of supernatant (~60 µl of solution isolated during glycogen processing) was heated for approximately 7 min at 90°C using a dry bath to evaporate the samples nearly to dryness. Next, 10 µl of 98% sulfuric acid was added to each sample and the samples were heated at 90°C in a dry bath for 30 sec. Once cooled, 240 µl of vanillin reagent was added to the samples and they were incubated for 20 min at room temperature before pipetting into a 96-well plate. Lipid samples were read on a spectrophotometer at 525 nm.

Spectrophotometer wavelengths uses for assessing light absorption, and therefore amount of product (protein, glycogen, sugar, or lipids) within each sample was selected in accordance with Foray et al. (2012). Standard calibrations were made for each reagent

to evaluate the absorbance readings from each wasp. All standard calibrations included three replicates of 1, 5, 10, 15, and 20 μg ($1\mu\text{g} = 1\mu\text{l}$) of reagent and control. Standards were subjected to the same procedures as samples to generate standard curves. For sugar and glycogen standards, anthrone reagent was added for a final volume of 250 μl , heated at 90°C in a dry bath for 5 min, and cooled prior being read on the spectrophotometer. Lipid standards were heat until complete evaporation, sulfuric acid was added and reacted with vanillin before being read on the spectrophotometer. Protein standards were reacted with 225 μl Bradford reagent before being read on the spectrophotometer. Standards were evaluated following Foray et al. (2012), in which bovine serum (Spectrum Chemical Mfg. Corp., New Brunswick, NJ, USA), rabbit liver (Sigma, St. Louis, MO, USA), dextrose (Fisher Scientific, Waltham, MA, USA), and soybean oil (Sigma Aldrich, Burlington, MA, USA) were used for protein, glycogen, sugar, and lipid calibrations, respectively. For each plate reading, three blank wells were filled with 250 μl of Bradford (protein assay), anthrone (glycogen and sugar assay), or vanillin reagent (lipids assay) and used as controls.

Data analysis

Analyses were carried out with R version 4.0.0 (R Core Team 2019). The effect of diet quality on *H. pennsylvanicus* reproductive fitness and nutrient retention was evaluated using generalized linear mixed models (GLMM). Data were non-normal, and so a Poisson error distribution and log-link function were used to test the influence of diet on female longevity, lifetime fecundity, and nutrient content. GLMM analyses were

conducted using the “glmer” function in the “lme4” package (Bates et al. 2015). Fixed effects were evaluated through model summaries, or through model comparisons using likelihood ratio test via the “drop1” function. When multilevel variables were found to be significant, means were separated with a post hoc Tukey test using the “glht” function in the “multcomp” package (Hothorn et al. 2008). The relationship between nutrient mass (μg , x-axis) and absorbance (y-axis) from standard calibrations were fitted to linear models to evaluate absorbance readings to nutrient values. For proteins, the slope was 0.027 (0.21 – 0.60 abs.; $P < 0.01$, $r^2 = 0.98$). For lipids, the slope was 0.009 (0.04 – 0.16 abs.; $P < 0.01$, $r^2 = 0.96$). For sugars, the slope was 0.038 (0.01 – 0.78 abs.; $P < 0.01$, $r^2 = 0.97$). For glycogen, the slope was 0.034 (0.09 – 0.69 abs.; $P < 0.01$, $r^2 = 0.99$). Blank wells' values from each plate reading were averaged and subtracted from each sample value. These adjusted values were used for all statistical comparisons for whole body nutrient content between diet treatments. The absorbance reading from each sample was divided by the slope of the calibration to estimate nutrient content. Sugar and lipid readings were then multiplied by two, because the supernatant of each sample had been divided in half during laboratory processing (see “Sugar content” and “Lipid content”). All data are presented as mean \pm SE.

Results

Effects of diet on longevity and fecundity

When provided diets with pollen, sucrose concentration significantly influenced female longevity ($X^2 = 40.75$, $df = 4$, $P < 0.001$) (Fig. 3.1A). Diets containing $\geq 50\%$

(w/v) sucrose increased female longevity in comparison to wasps fed 25% (w/v) sucrose diet ($z = 4.89$, $P < 0.001$), however there was no significant difference in female longevity across the various diets that contained $\geq 50\%$ (w/v) sucrose. Females lived an average of 6.40 ± 1.74 days when fed a 25% (w/v) sucrose diet, while individuals provided a 50% (w/v) sucrose diet lived 18.80 ± 4.41 days. Females provided a honey diet with pollen on average exhibited the greatest longevity (34.80 ± 6.31 days), whereas water-fed females died shortly after eclosion, living on average 1.80 ± 0.33 days. There was no significant difference between water-fed and 25% (w/v) sucrose-fed individuals. The presence of pollen in diet did not influence female longevity (Fig. 3.1B).

The concentration of sucrose in adult diet influenced the lifetime fecundity for female wasps ($X^2 = 36.17$, $df = 4$, $P < 0.001$) (Fig. 3.2A). With the exception of 75% (w/v) sucrose-fed individuals, female offspring production was greatest on day 1 of the study for all diet treatments (water, 7.90 ± 2.34 ; 25% (w/v) sucrose, 9.60 ± 2.15 ; 50% (w/v) sucrose, 10.10 ± 2.0 ; 75% (w/v) sucrose, 11.40 ± 2.40 ; honey, 13.00 ± 2.66 offspring) (Fig. 3.2B). However, sucrose concentration did not influence the number of offspring produced on day 1. Females fed a 75% (w/v) sucrose diet produced the most offspring (13.70 ± 1.98) on day 3 of the study. When provided a sucrose rich diet, $> 80\%$ of all parasitism occurred within the first week of the female's lifetime. While fecundity declined with female age, the rate of decline was slower for wasps fed a mid- to high-sucrose diet. By day 5 of the study, sucrose- and honey-fed females exhibited 50% reduction in parasitism. Females fed $\geq 50\%$ (w/v) sucrose diets produced significantly more offspring on day 3 than individuals fed $\leq 25\%$ (w/v) sucrose diet ($z = 2.84$, $P < 0.05$

for 50% (w/v) sucrose; $z = 3.80$, $P < 0.05$ for 75% (w/v) sucrose; $z = 3.23$, $P < 0.05$). There was no significant difference in day 3 fecundity between 25% (w/v) sucrose and water-fed females. While there was no difference in the number of offspring produced on day 5 between diet treatments, females fed a 25% (w/v) sucrose diet produced significantly fewer offspring on day 7 than honey-fed females ($z = -2.32$, $P < 0.05$). The presence of pollen in diet did not affect the lifetime fecundity of female wasps (Fig. 3.3A). Similarly, pollen did not influence daily offspring production or the ovipositional period of female wasps (Fig. 3.3B).

Effects of diet on nutrient composition

Influence of sucrose diet on parasitoid nutrient content

The concentration of sucrose in diet differentially influenced nutrient retention in female *H. pennsylvanicus* (Fig. 3.4). Water-fed females maintained lower levels of sugars ($z = -8.91$, $P < 0.01$) and glycogen ($z = -3.70$, $P < 0.01$) than sucrose-fed wasps. However, providing varying concentrations of sucrose in diet did not affect the lifetime retention of bodily sugars in females. While sugar content generally increased over time, female age did not significantly influence sugar retention over an individual's lifetime.

There was a significant decline in bodily glycogen content with female age when wasps were fed a 25% (w/v) sucrose diet ($z = -4.05$, $P < 0.01$). However, honey-fed females exhibited increased levels of glycogen over their lifetime ($z = 49.21$, $P < 0.01$). Though glycogen content generally increased over time for wasps fed $\geq 50\%$ (w/v) sucrose diets, the concentration of sucrose did not differentially influence lifetime

retention of glycogen for females, although wasps fed $\geq 50\%$ (w/v) sucrose diets did exhibit significant declines in bodily lipids over time ($z = -3.72$, $P < 0.01$ for 50% (w/v) sucrose; $z = -2.12$, $P < 0.05$ for 75% (w/v) sucrose).

In general, bodily lipid content decreased over the lifespan of females. However, female age did not significantly influence lipid content over time when wasps were fed a water, 25% (w/v) sucrose, or honey diet. There was a significant decline in protein content over time for females fed only water ($z = -4.20$, $P < 0.01$) or 25% (w/v) sucrose diet ($z = -6.20$, $P < 0.01$). However the age of females fed a sucrose or honey diet did not affect the lifetime retention of protein in females.

Influence of pollen diet on parasitoid nutrient content

Provisioning pollen in diet did not influence the retention of bodily sugar, glycogen, lipid or protein content over the lifespan of female *H. pennsylvanicus* (Fig. 3.5). Honey-fed wasps provisioned with pollen exhibited significantly greater retention of lipids over time in comparison to individuals not provided pollen ($z = 2.42$, $P < 0.05$). While all diets provisioned with pollen exhibited general declines in lipid levels over time, only wasps fed a 50% (w/v) sucrose diet with pollen demonstrated significant declines in lipid content with female age ($z = -2.53$, $P < 0.05$).

There was a significant decline in protein retention over time for females fed water with ($z = -4.28$, $P < 0.01$) and without pollen ($z = -3.78$, $P < 0.01$). Similarly, wasps fed 50% (w/v) sucrose diet exhibited a decline in protein retention over time ($z = -2.17$, $P < 0.05$), however this trend did not occur when diet was provisioned with pollen.

Discussion

In this study, increasing sucrose concentration in diet, regardless of pollen content, significantly increased the survival and life fecundity of female *H. pennsylvanicus*. Diets containing high ($\geq 50\%$) sucrose doubled female longevity in comparisons to individuals fed a low (25%) sucrose diet. However, there was no significant difference in female longevity when fed $\geq 50\%$ sucrose diets.

Females fed a high sucrose diet also exhibited extended ovipositional periods, potentially enhancing their capacity to produce more offspring over the course of their lifetime. Females fed high sucrose diets exhibited increased fecundity nearly two-fold that of individuals fed a low sucrose diet, and about 3 times the number of offspring compared to water-fed females. There was no significant difference in lifetime fecundity for females fed high sucrose or honey diets. In general, parasitoid reproductive fitness increases with carbohydrate concentration, although there may be limits to these benefits (Ellers et al. 2011). In some cases, the viscosity of highly concentrated sugar diets may decrease ingestion by parasitoids (Benelli et al. 2017). However, given that both longevity and lifetime fecundity did not differ between mid- (50% w/v) and high-sucrose (75% w/v) diets, mid-level sucrose concentration in diet may be sufficient to maintain parasitoid reproductive fitness.

As anticipated, wasps fed diets containing greater sucrose concentrations exhibited greater bodily sugar content over the course of their lifetime. This follows findings from Olson et al. (2000) and Lee et al. (2004), who demonstrated that parasitoid

sugar levels increased with female age for individuals fed sucrose-rich diets. Such trends may suggest females either feed more with age, cumulatively retain sugars over their lifetime, or that their metabolism slows with age and acclimation to the study arena (Lee et al. 2004). There is also the possibility that wasps' cuticle may have become increasingly contaminated with sugar over time when provided frequent contact with the diet. However, further research is needed in order to test these possibilities.

While glycogen levels increased with female age for individuals fed a high sucrose diet, individuals provided with low sucrose diets exhibited declines in glycogen over their lifetimes. These findings suggest that insufficient sucrose acquisition could influence the retention of glycogen in wasps. Regardless of provisioned diet, female *H. pennsylvanicus* exhibited consistent declines in lipid reserves with age. However, these trends suggest a slower rate of decline for individuals fed higher concentrations of sucrose, as observed by Ellers (1996) and Lee et al. (2004). In general, sugars obtained from diet can be used immediately for energy metabolic purposes or be stored for later use when converted to glycogen (Friedman 1985; Rivero and Casas 1999). As bodily sugar is depleted, glycogen reserves are often utilized to generate energy (Rivero and Casas 1999; Giron et al. 2003). Using these sugar and glycogen reserves may enable females to maintain lipid reserves (Giron et al. 2003). Therefore, a slower decline of lipids may be a result of females utilizing the readily available sugars obtained from sucrose-rich diets and thereby reducing their reliance on lipid reserves.

Previous studies have highlighted the potential benefit of pollen on natural enemies, and in turn, biological control (Zhang et al. 2004; Vialatte et al. 2016; Benelli et

al. 2017). Specifically, pollen is known to provide protein and fuel parasitoid egg production (Rivero and Casas 1999). However, while variation in sucrose concentration in diet had direct effect on the sequestration and retention of nutrients, provisioning pollen with diet did not influence female *H. pennsylvanicus* nutrition. To our knowledge, this study may be the first to test the potential nutritional benefits of pollen as a protein source for scelionid parasitoids under laboratory conditions. Here, our findings would suggest that pollen may not represent a primary source of protein for maximizing a parasitoid's reproductive fitness.

Understanding the role of diet quality on the reproductive fitness of *H. pennsylvanicus* may enable a better understanding of how providing suitable food resources in field settings may improve biocontrol of *L. zonatus*. During periods of insufficient access to nutrient-rich diets, resident parasitoid populations may experience lower rates of reproductive fitness, which may result in reduced efficacy of biocontrol programs (Wäckers and van Rijn 2005). Therefore, provisioning floral resources in or around cropping systems may prove crucial in supporting parasitoid populations, and in turn, aid in suppressing pest populations (Fiedler et al. 2008; Hatt et al. 2018). Identifying floral species that are both ecologically compatible and economically viable to integrate into current orchard management practices may benefit biocontrol programs for *L. zonatus*. However, integrating supplemental floral resources into cropping systems can often be complicated, with arthropods likely responding to changes in the environment differently. For instance, previous studies have shown that provisioning floral resources can negatively impact biocontrol programs by unintentionally altering parasitoid behavior

(Foti et al. 2018; Desurmont et al. 2020), indirectly affecting multitrophic interactions (Araj et al. 2009, 2011; Perović et al., 2017), or ultimately be impacted by the biotic complexity of the landscape (Jonsson et al. 2015; Iuliano and Gratton 2020). Therefore, future research investigating the prospects of floral provisioning to enhance biocontrol of *L. zonatus* must consider the multifaceted effects such food resources may have on the pest, its natural enemies, and the greater arthropod community within the landscape.

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<i>Treatment group</i>	<i>Nectar</i>	<i>Pollen</i>
Negative control	0%	-
Positive control	50%	+
Pollen-starved	50%	-
Nectar-starved	0%	+
Low concentration nectar	25%	+
High concentration nectar	75%	+
Honey-water	95%	-
Honey-water & pollen	95%	+

Table 3.1 Experimental diet treatments. ‘Nectar’ values indicate sucrose concentration (w/v). ‘Pollen’ values indicate the presence (+) or absence (-) of pollen in diet. Nectar diets are represented as the percentage of sucrose concentration (w/v) in the solution

<i>Diet treatment</i>	<i>Day</i>	<i>1</i>	<i>3</i>	<i>5</i>	<i>7</i>	<i>10</i>	<i>15</i>	<i>20</i>
Water		10	10	-	-	-	-	-
50% sucrose		10	10	10	8	6	10	12
Honey		10	10	9	8	10	10	12
Water & pollen		10	10	-	-	-	-	-
25% sucrose & pollen		10	10	7	5	-	-	-
50% sucrose & pollen		10	10	7	5	7	10	12
75% sucrose & pollen		10	10	8	8	10	10	12
Honey & pollen		10	10	9	8	10	10	12

Table 3.2 Number of replicates (n) used in nutrient bioassays for each diet treatment. Replicates were based on the number of females available on a given day for each treatment. Sucrose diets are represented as percent sucrose (w/v)

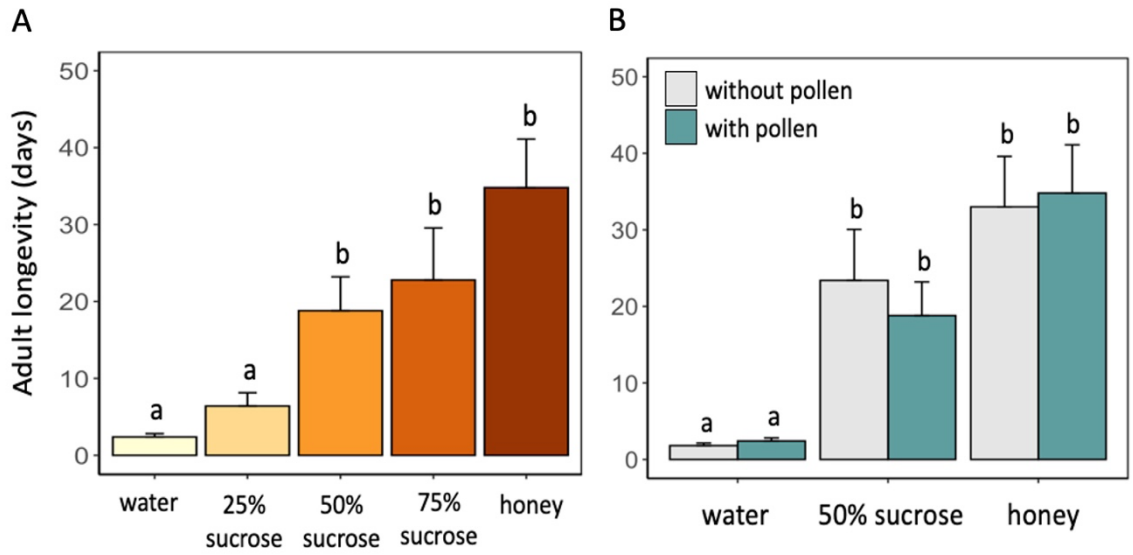


Fig. 3.1 *Hadronotus pennsylvanicus* female longevity (mean \pm SE) when provided *Leptoglossus zonatus* host eggs and fed a diet of escalating sucrose concentrations containing pollen (A) and diets with and without pollen (B)

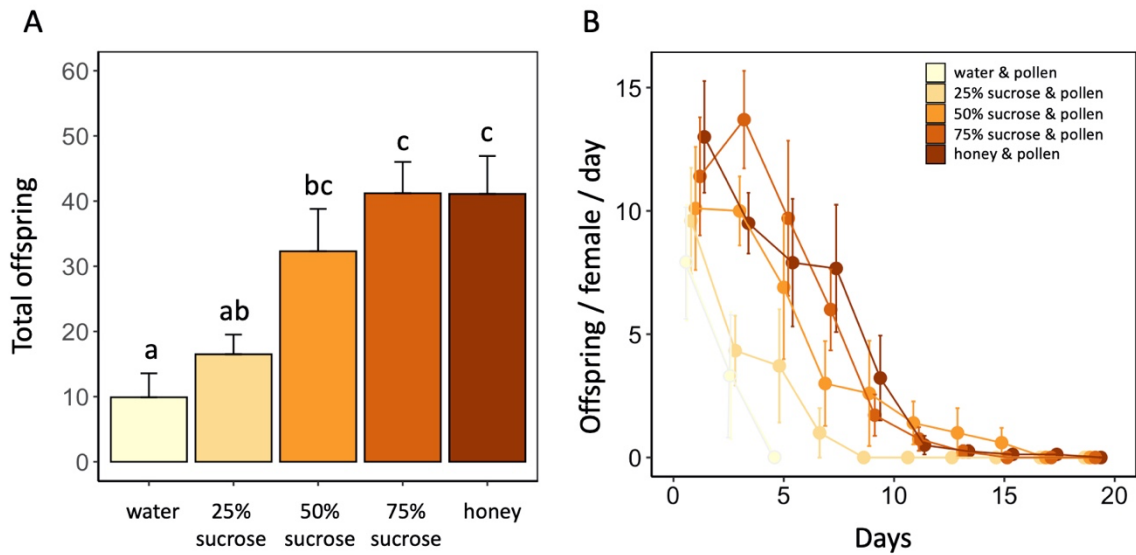


Fig. 3.2 *Hadronotus pennsylvanicus* lifetime fecundity (A) and progeny produced over time (B) (mean \pm SE) when provided *Leptoglossus zonatus* host eggs and fed a diet of escalating sucrose concentrations containing pollen

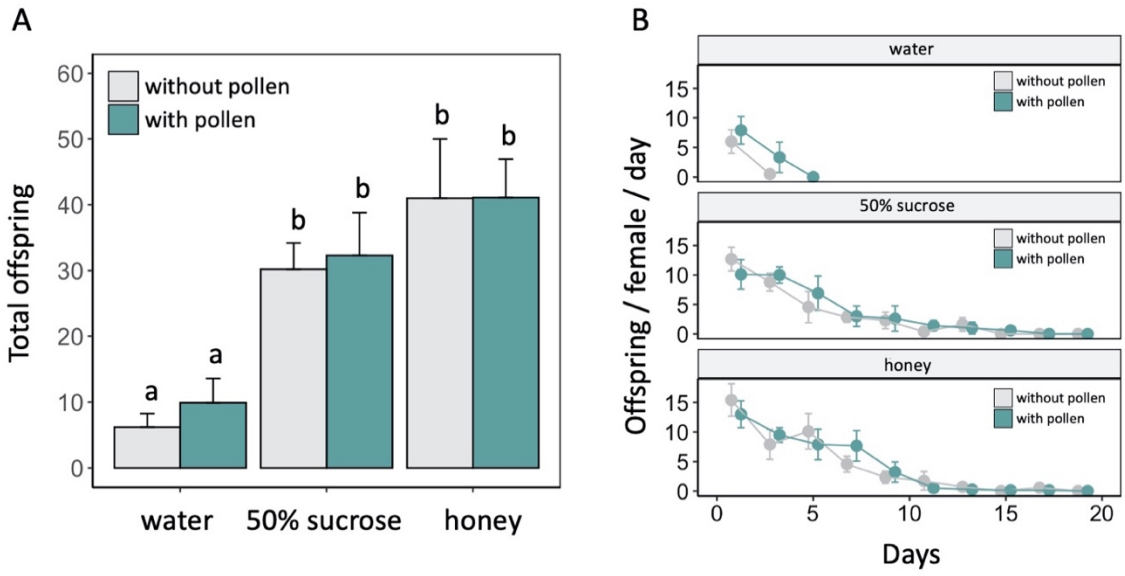


Fig. 3.3 *Hadronotus pennsylvanicus* lifetime fecundity (A) and progeny produced over time (B) (mean \pm SE) when provided with *Leptoglossus zonatus* host eggs and fed diets with and without pollen

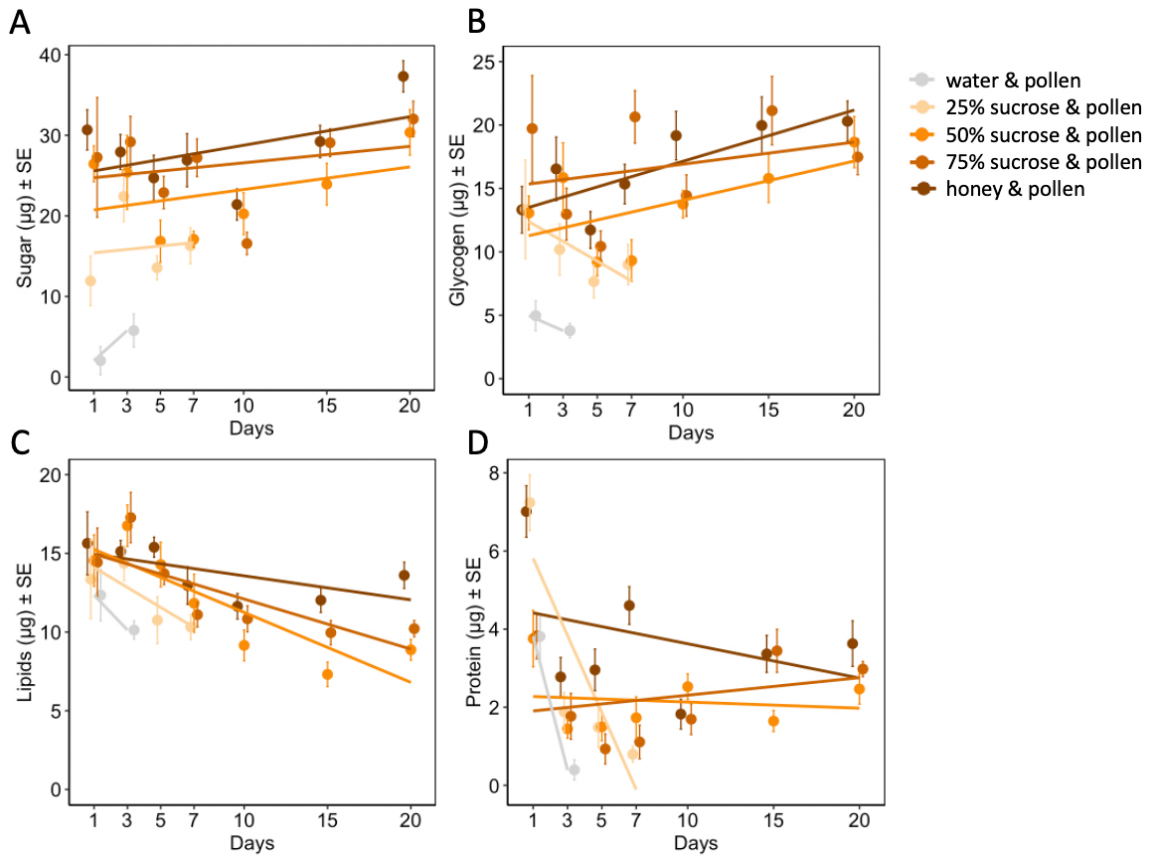


Fig. 3.4 Lifetime nutrient levels of sugar (A), glycogen (B), lipids (C), and protein (D) for female *Hadronotus pennsylvanicus* when fed pollen diets varying in sucrose concentration. Sucrose diets are represented as percent sucrose (w/v)

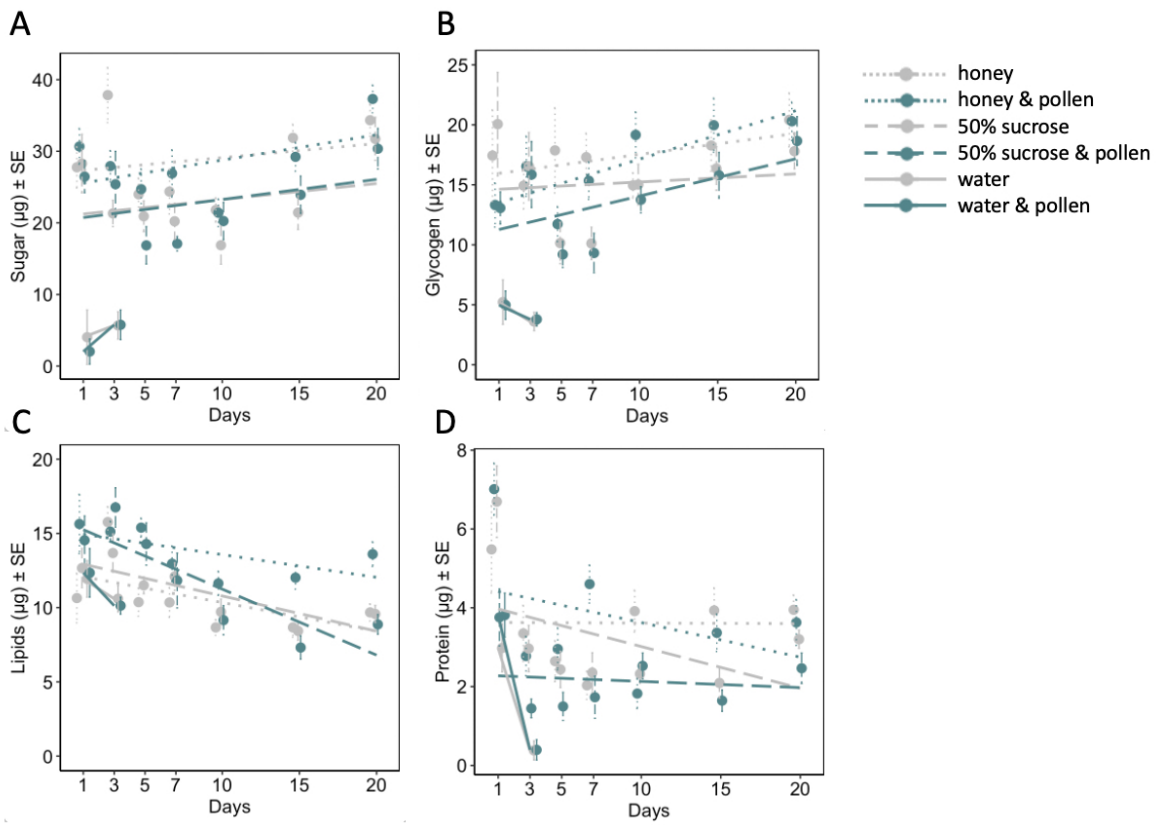


Fig. 3.5 Lifetime nutrient levels of sugar (A), glycogen (B), lipids (C), and protein (D) for female *Hadronotus pennsylvanicus* when fed diets with varying sucrose concentration with and without pollen. Sucrose diets are represented as percent sucrose (w/v)

Chapter 4: Floral provisions enhance a parasitoid's fitness but not biological control of its host, *Leptoglossus zonatus* Dallas (Heteroptera: Coreidae)

Abstract

The diet of adult parasitoid wasps is vital for their survival and reproduction. However, the availability of food resources, such as plant nectar, can vary widely in cropping systems, potentially affecting parasitoid fitness and thereby biocontrol of pests. The egg parasitoid *Hadronotus pennsylvanicus* (Ashmead) (Hymenoptera: Scelionidae) is a potential biocontrol agent of the pistachio pest *Leptoglossus zonatus* (Dallas) (Heteroptera: Coreidae). While *H. pennsylvanicus* is known to attack *L. zonatus* eggs in California, U.S.A., parasitism rates in orchards are highly variable. Floral resource provisioning has the potential to enhance parasitoid longevity and thus improve parasitism rates, leading to reduced pest densities. Here, a combination of field and laboratory studies were used to assess the influence of flowering groundcovers on the reproductive fitness of *H. pennsylvanicus* and the abundance of *L. zonatus*. Evaluated groundcovers included oat (*Avena sativa*), cowpea (*Vigna unguiculata*), mustard (*Brassica hirta*), and buckwheat (*Fagopyrum esculentum*). Under laboratory conditions, buckwheat provided the greatest benefit to female *H. pennsylvanicus* longevity. While all floral diets improved female fecundity, there was no difference in the total offspring produced per female. In field trials, flowering groundcovers did not influence the abundance *H. pennsylvanicus* nor parasitism rates on *L. zonatus*. While the availability of

floral resources can improve the reproductive fitness of *H. pennsylvanicus*, the use of groundcovers in pistachio did not enhance biocontrol of *L. zonatus*.

Introduction

While the specialization and intensification of modern agriculture has led to great net gains in crop productivity, it has in many cases also resulted in negative effects on natural habitats, biodiversity, and ecosystem services such as biological control (Landis et al. 2000; Tschardt et al. 2005, 2008; Chaplin-Kramer et al. 2011). The communities of beneficial insects that support biocontrol, such as parasitoids, are often hindered in agriculture systems that have extensive, simplified nature of production (i.e. monoculture) and/or frequent use of pesticides (Fiedler et al. 2008; Crowder and Jabbour 2014; Nilsson et al. 2017). Some parasitoid species rely on carbohydrate-rich diets from floral and extra-floral resources for survival and reproduction (Jervis et al. 2008; Benelli et al. 2017). The nutrients obtained from such resources improve the longevity, fecundity, and parasitism behavior of many parasitoid species, and in turn can lead to improved biocontrol of insect pests (Wäckers and van Rijn 2005; Tena et al. 2015; Benelli et al. 2017). However, the availability of floral resources can vary widely between cropping systems, potentially hindering parasitoid fitness and their efficacy as biocontrol agents (Segoli and Rosenheim 2013; Russell 2015; Kishinevsky et al. 2018). Under these conditions, pest management programs may rely more heavily on pesticide applications to sufficiently suppress pest populations.

The leaf-footed bug, *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae), is a polyphagous pest of numerous economically important crops (Marchiori 2002; Rivero and Hernandez 2009; Xiao and Fadamiro 2010; Foresti et al. 2017). In California's Central Valley, *L. zonatus* is primarily a pest of almonds and pistachios (Daane et al. 2005) and has the potential to cause significant damage to crop quality and yield (Michailides 1989; Daane et al. 2005; Joyce et al. 2019; Stahl et al. 2020). Over the past 20 years, tree crop acreage has increased significantly and pressure from *L. zonatus* has become more problematic for growers (Daane et al. 2016; Zalom et al. 2018). Current methods for monitoring *L. zonatus* in orchards include beat-tray sampling of the tree canopy and visual assessment of nuts for feeding damage. However, such tactics can be both time and labor intensive while only providing limited insight on true pest densities. Given the lack of effective monitoring tools, along with grower aversion to risk from feeding damage, insecticides remain the primary control tactic for *L. zonatus* (Zalom et al. 2018; Haviland et al. 2018). With increasing consumer and regulatory demand for more sustainable pest management options, growers have expressed interest in exploring non-chemical control tactics to improve integrated pest management (IPM) programs for *L. zonatus*, including conservation biocontrol.

The egg parasitoid *Hadronotus* (=Gryon) *pennsylvanicus* (Ashmead) (Hymenoptera: Scelionidae) is a prospective biocontrol agent of *L. zonatus* (Masner 1983; Marchiori 2002; Maltese et al. 2012; Straser et al. 2022). *Hadronotus pennsylvanicus* is a solitary endoparasitoid of several coreid hosts and has been evaluated for use in many biocontrol programs (Mitchell and Mitchell 1986; Nechols et al. 1989;

Maltese et al. 2012; Sabbatini Peverieri et al. 2012; Roversi et al. 2013). Under laboratory conditions, *H. pennsylvanicus* exhibits desirable reproductive traits as a biocontrol agent because they are long-lived (30.6 ± 3.1 days), highly fecund (39.7 ± 5.6 offspring) and able to maintain a high female sex ratio in progeny (92.8 ± 1.5 % female offspring) (Straser et al. 2022). However, while *H. pennsylvanicus* attacks *L. zonatus* eggs in California (Maltese et al. 2012), parasitism rates in orchards have been shown to be highly variable, leading to insufficient and unreliable biocontrol. As a synovogenic parasitoid, *H. pennsylvanicus* is highly dependent on nutrient-rich diets in order to maintain reproductive fitness (Olson and Nechols 1995; Sabbatini Peverieri et al. 2012; Cornelius et al. 2020), and laboratory assessments have demonstrated that egg limitation appears to be a key constraint on offspring production (Sabbatini Peverieri et al. 2012; Cornelius et al. 2018; Straser et al. 2022). As such, the establishment and maintenance of *H. pennsylvanicus* in orchards may be contingent on the availability of suitable food resources.

Previous studies have evaluated groundcovers as a “trap crop” to control hemipteran pests in orchards (Nielsen et al. 2016; Akotsen-Mensah et al. 2017; Daane et al. 2016). Such plants are used within cropping systems to attract, divert, and/or retain targeted insect pests that would otherwise inflict damage to the main crop (Gurr et al. 2017). However, in addition to luring pests away from crops, in some cases trap crops have been shown to support natural enemy communities (Wan et al. 2016; Stahl et al. 2021). For instance, Stahl et al. (2021) demonstrated that groundcovers in a pistachio orchard could potentially reduce hemipteran abundance while increasing beneficial

arthropod communities. Beyond providing floral and extrafloral nectar to natural enemies, groundcovers also have the potential to provide vital refuges (Carmona and Landis 1999; Gillespie et al. 2016) and provide alternative prey or hosts that natural enemies require during periods of low pest abundance (Duyck et al. 2011; Gillespie et al. 2016). While increasing habitat diversification in agriculture can have a positive influence on the provision of biocontrol services, the response of the arthropod community to changes in cropping systems are not uniform and outcomes can be species- and community-specific (Russell 2015; Tschardt et al. 2016; Zhu et al. 2020). Therefore, evaluation of candidate plants that are attractive to *L. zonatus* while also providing a dietary resource to natural enemies could synergistically contribute to pest control.

Understanding the role of groundcovers on *H. pennsylvanicus* reproductive fitness may provide insight into the relative contribution of floral resources for bolstering parasitoid populations and biocontrol in orchards. In this study, a series of experiments were used to assess the prospects and limitations of using summer flowering groundcovers to enhance biocontrol of *L. zonatus* in pistachio. Laboratory assays were used to assess whether select groundcover plants could enhance the longevity and fecundity of egg parasitoid *H. pennsylvanicus*. After which, field evaluations were conducted to assess whether such groundcovers could enhance natural enemy abundance, improve biocontrol, and ultimately reduce the abundance of *L. zonatus*.

Methods

Laboratory Assays: Influence of floral resources on reproductive fitness

Insect rearing

Laboratory colonies of *H. pennsylvanicus* and *L. zonatus* were reared from specimens collected in 2017 from Fresno County, California. *Leptoglossus zonatus* specimens were collected from pomegranates and replenished with wild specimens *ad libitum*. Colonies were maintained in cages (60 cm x 60 cm x 60 cm, 680 μ m mesh, Bug-DORM®, Taiwan) containing a 2.36 L potted juniper (*Juniperus* sp. L.) and provided a diet of zucchini (*Cucurbita pepo* L.), green beans (*Phaseolus vulgaris* L.), and sunflower seeds (*Helianthus annuus* L.) that was refreshed weekly. Egg clusters were collected on wooden skewers (25.4 cm x 3 mm) that were placed within the enclosures as oviposition substrates. *Hadronotus pennsylvanicus* colonies were reared from parasitized *L. zonatus* eggs collected from a pistachio orchard in Parlier, California, and replenished with wild strain specimens in 2019 and 2020 obtained similarly. Parasitoids were reared in 50 ml plastic tubes (Falcon Conical Centrifuge Tubes, Fisher Scientific®) sealed at the open end with 250 μ m mesh and fed a 1:1 honey-water diet (Organic Honey, Wholesome®) *ad libitum*. *Hadronotus pennsylvanicus* was identified to species using Masner (1983) and taxonomic revisions in Talamas et al. (2021). All colonies were held and insect bioassays were conducted under controlled laboratory conditions of 25°C \pm 1, 75 \pm 5% RH and 16:8 L:D photoperiod using artificial, fluorescent lights.

Parasitoid reproductive fitness

The influence of groundcovers on *H. pennsylvanicus* longevity and fecundity was assessed under controlled laboratory conditions. Parasitoid diet treatments included oat (*Avena sativa* L., Cyperales: Poaceae), cowpea (*Vigna unguiculata* (L.) Walp., Fabales: Fabaceae), white mustard (*Sinapis alba* L., Capparales: Brassicaceae), and buckwheat (*Fagopyrum esculentum* Moench, Polygonales : Polygonaceae), with honey-water (1:1) as a comparative laboratory diet, and water as a control. These plant species were selected because they are readily available for commercial use, attractive to hemipteran insects (Stahl et al. 2021), suited to Mediterranean climates, and have the ability to support natural enemies (Akotsen-Mensah et al. 2017; Fiedler et al. 2008; Zhu et al. 2020). All plants were grown from seed under greenhouse conditions ($27^{\circ}\text{C} \pm 2$, $75 \pm 5\%$ RH and 13:11 L:D using natural and fluorescent light). Plants were propagated in 2.36 L pots containing Miracle-Gro Potting Mix (Scotts Miracle-Gro Products Inc., Marysville, OH) and watered 3 times per week without the use of pesticides or additional fertilizers.

To assess parasitoid longevity, newly emerged (<24 h) male and female *H. pennsylvanicus* were placed in a plastic cylinder enclosure (13 cm x 30 cm) sealed with a 7 cm 250 μm mesh cap. Each enclosure consisted of >5 florets or 10 flowering stems of plant material in a 130 ml plastic vial (40 dram Plastic Vial, Thornton Plastics, Salt Lake City, UT) containing water and sealed with a cotton ball. Honey-water (1:1) and water were used as a positive and negative control, respectively. Each enclosure included 6 females and 3 males, with 5 replicates per treatment. Plant material and water were

replaced every other day until all enclosed specimens died. Enclosures were inspected daily for the presence of dead wasps.

To assess parasitoid fecundity, newly emerged (<24 hr) male and female *H. pennsylvanicus* were housed in mating pairs in 50 ml plastic tubes (Falcon Conical Centrifuge Tubes, Fisher Scientific®) containing either 5 flowers or 3 inflorescences of a given plant treatment. Mating pairs were provided one of 6 diet treatments; oat (n=15), cowpea (n=10), mustard (n=9), buckwheat (n=13), honey-water (n=15), and water (n=23). Water and honey-water treatments were administered using 3 drops on the inside of the enclosure. The number of replications per treatment were based on availability of mature plant material throughout the duration of the study. The stem of each flower was submerged in a container of water and replaced every 3 d to ensure nectar availability (Wade and Wratten 2007). For enclosures not containing floral material (i.e., water and honey-water treatments), a water-base that would normally feed plant stems was provided to account for potential variation in enclosure humidity and temperature. After 24 h of exposure to diet treatments, a set of 25 fresh (<72 h) *L. zonatus* eggs were provided for parasitism. Following 24 h exposure to the parasitoids, host eggs were removed and observed daily for the emergence of parasitoids or *L. zonatus* nymphs. A new male specimen was introduced to the enclosure to replace any expired individuals to maintain copulation potential throughout the experiment. The abundance and frequency of host-egg exposure to parasitoids was selected based on observations of maximum parasitism potential within a 24 h period (Straser et al. 2022).

Field Study: Influence of floral resources on *H. pennsylvanicus* abundance and biological control of *L. zonatus*

Study design

Groundcover plots were established in a 93 ha commercial organic pistachio orchard near Coalinga, CA. This study was conducted using a randomized block design consisting of 5 replicate blocks to evaluate selected groundcovers: oat, cowpea, white mustard, and buckwheat. These groundcovers were evaluated against resident weedy vegetation (control), which included barnyard grass (*Echinochloa crusgalli* L., Cyperales: Poaceae), matted sandmat (*Chamaesyce serpens* (Kunth) Small, Euphorbiales: Euphorbiaceae), southern crabgrass (*Digitaria ciliaris* (Retz.) Koeler, Cyperales, Poaceae), Italian ryegrass (*Festuca perennis* Lam., Cyperales: Poaceae), curly dock (*Rumex crispus* L., Caryophyllales: Polygonaceae), silverleaf nightshade (*Solanum elaeagnifolium* Cav., Solanales: Solanaceae), black nightshade (*S. nigrum* L., Solanales: Solanaceae), and puncturevine (*Tribulus terrestris* L., Sapindales: Zygophyllaceae). Every groundcover type and a control was present in each block. Groundcover seeds were sown as a 1 m wide strip in the row middles in the central 3 rows in each plot in 2020 and 2021. To assess the establishment and retention rate of plant species, the proportion of groundcovers present was recorded biweekly in a 1 m² plot in the center row at 30, 40 and 50 trees from the orchard's edge. For commercial operations, the crop canopy was treated with a pyrethrin on 3 July 2020 and 20 June 2021. Groundcovers were mown on 2 August 2021.

Pest and natural enemy abundance

To assess the seasonal abundance of *L. zonatus*, biweekly sweep samples were taken from groundcovers in all plots (3 replicates of 30 sweeps per plot) using 15” sweep nets. *Leptoglossus zonatus* abundance within the crop canopy was monitored alongside sweep samples using beat sampling (3 sets of 30 beats per plot). Each beat sample consisted of 3 strikes to the tree canopy with a beat stick (0.5” PVC pipe) to allow insects to fall into a sweep net held below. All contents that were collected from sweep and beat samples were placed individually into 3.8 L plastic bags, and stored at 20 °C until the number of *L. zonatus* adults per sample could be recorded. In conjunction with sweep and beat sampling methods, yellow sticky card traps (Seabright Laboratories, Emeryville, CA) were used biweekly to monitor the seasonal abundance of egg predators and parasitoids of *L. zonatus* within the orchard. Each plot consisted of 3 pairs of sticky card traps, each positioned at every 20th tree along the center row of each plot. Canopy traps were hung on the south side of the tree at approximately 2.5 m above the ground, while ground traps were positioned approximately 0.25 m above the ground, attaching traps to bamboo stakes standing upright within the center of the row. Egg predators and parasitoids collected from monitoring traps were stored under laboratory conditions and identified to genus or species. To identify parasitoids, all wasps were removed from the sticky card trap, soaked in Histo-Clear II Cleaner (National Diagnostics, Atlanta, GA) to remove glue, and identified to genus or species using a Leica S9i Stereo Microscope (Leica Microsystems, Wetzlar, Germany).

Biological control

Sentinel egg clusters were used to assess *L. zonatus* parasitism and predation throughout the two growing seasons. While both fresh and freezer-stored *Leptoglossus* spp. sentinel egg clusters have shown equal probability to be located by egg parasitoids, the rate of parasitism has been found to be significantly greater in fresh eggs than in freezer-stored eggs (Sabbatini Peverieri et al. 2013, 2014). Therefore, sentinel egg assays used in this study consisted of *L. zonatus* egg clusters that had been oviposited onto wooden skewer substrates <72 h prior to exposure in the field. Sentinel egg clusters with 10 eggs were attached to a pistachio branch at roughly 2.5 m above the ground at 20, 40 and 60 trees from the orchard's edge using Elmer's Wood Glue (Elmer's Products, Westerville, OH) once per month. After 3 d in the field, sentinel egg clusters were collected, placed individually into petri dishes, and stored under controlled laboratory conditions. Emerged parasitoids were identified to species and their abundance recorded. The number of eggs taken by predators were recorded. Missing eggs without evidence of chewing (evident by remnant egg pieces attached to the skewer base) were recorded as missing. Remaining eggs that neither hatched nor produced a parasitoid were dissected to verify whether they contained a dead parasitoid or dead *L. zonatus*. They were determined non-viable if neither was apparent. To assess the natural proportion of non-viable eggs from clusters, 1 sentinel egg cluster per plot was housed in a 50 ml plastic tube (Falcon Conical Centrifuge Tubes, Fisher Scientific) closed at the open end with a 250 μ m mesh cap and stored in shaded outdoor conditions.

Data analyses

Data analyses were conducted in R version 4.0.0 (R Core Team 2019). The effect of floral resources on *H. pennsylvanicus* reproductive fitness was assessed using generalized linear mixed models (GLMM). A Poisson error distribution and log-link function were used to test the influence of diet on longevity, female ovipositional, and post-reproductive period. A Gaussian error distribution and log-link function were used to evaluate the influence of diet on lifetime fecundity, and a binomial error distribution with logit link function tested differences in progeny sex ratio between diet treatments. The effect of groundcover treatments on seasonal *L. zonatus* and natural enemy abundance was assessed using a GLMM with a Poisson error distribution and log-link function. Similarly, the seasonal rate of biological control of sentinel eggs (number of parasitized and eggs destroyed by predators) between groundcover treatments was assessed using a GLMM with a Poisson error distribution and log-link function. GLMM analyses were conducted using the “glmer” function in the “lme4” package (Bates et al. 2015). Fixed effects were evaluated through model comparisons using a likelihood ratio test with the “drop1” function. When multilevel variables were found to be significant, means were separated with 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

Laboratory assay: Influence of floral resources on reproductive fitness

Adult diet significantly influenced the longevity of female ($X^2 = 3662.8$, $df = 5$, $P < 0.01$) and male ($X^2 = 1500$, $df = 5$, $P < 0.01$) *H. pennsylvanicus* (Fig. 4.1). Females provided a buckwheat diet lived longer (34.2 ± 2.6 d) than those provided a mustard ($z = -3.95$, $P < 0.01$), cowpea ($z = -12.53$, $P < 0.01$), or oat ($z = -21.77$, $P < 0.01$) diet (Fig. 4.1A). However, there was no significant difference in male longevity when provided a buckwheat (23.0 ± 2.3 d) or mustard diet (24.2 ± 2.2 d) (Figure 4.1B). Female and males wasps lived longer when provided a cowpea diet (17.5 ± 0.9 d for females; 13.6 ± 1.0 d for males) than an oat diet ($z = -15.80$, $P < 0.01$ for females; $z = -9.80$, $P < 0.01$ for males). Oat-fed females lived on average 2.4 ± 0.2 d, whereas males lived 2.0 ± 0.2 d. There was no significant difference in longevity when provided water or oat diet for either females or males. Parasitoids survived for the longest duration when provided a honey-water diet (49.6 ± 1.5 d for females, $z = 9.12$, $P < 0.01$; 43.5 ± 2.6 d for males, $z = 9.56$, $P < 0.01$).

Adult diet also influenced the duration of female oviposition ($X^2 = 115.91$, $df = 5$, $P < 0.01$) and post-reproductive ($X^2 = 104.31$, $df = 5$, $P < 0.01$) periods, as well as lifetime fecundity ($X^2 = 32.94$, $df = 5$, $P < 0.01$) (Table 4.1). Females fed a cowpea (5.8 ± 0.9 d), mustard (7.0 ± 1.2 d), buckwheat (7.3 ± 0.9 d), or honey diet (9.8 ± 0.8 d) exhibited longer ovipositional periods than individuals fed an oat diet ($z = -3.08$, $P < 0.05$ for cowpea; $z = -4.01$, $P < 0.01$ for mustard; $z = -4.56$, $P < 0.01$ for buckwheat; $z = -6.43$, $P < 0.01$ for honey). However, there was no significant difference in ovipositional period

between cowpea-, mustard-, buckwheat-, or honey-fed females. Similarly, females fed a buckwheat (9.5 ± 1.3 d) or honey diet (8.1 ± 0.9 d) maintained the longest post-reproductive periods. Water-fed females produced less offspring (8.6 ± 1.9 offspring) than individuals fed a floral diet ($z = -2.27$, $P < 0.05$ for oat; $z = -3.04$, $P < 0.05$ for cowpea; $z = -2.99$, $P < 0.05$ for mustard; $z = -4.96$, $P < 0.01$ for buckwheat) or honey diet ($z = -4.98$, $P < 0.01$) (Figure 4.2). However, there was no significant difference in female fecundity when provided oat (20.2 ± 3.7 offspring), cowpea (26.3 ± 3.9 offspring), mustard (26.7 ± 6.1 offspring), buckwheat (35.1 ± 6.0 offspring), or honey diet (34.0 ± 4.5 offspring). There was also no difference in the sex ratio of progeny between diet treatments.

Field Study: Influence of floral resources on biological control of *L. zonatus*

Groundcovers were more prominent early in the growing season (May - June), and plant coverage consistently declined starting in June (Suppl 4.1). Percent coverage for buckwheat ($82.5 \pm 5.7\%$ in Year 1, $42.0 \pm 9.1\%$ in Year 2), cowpea ($72.0 \pm 6.8\%$ in Year 1, $55.0 \pm 9.3\%$ in Year 2), mustard ($50.0 \pm 8.6\%$ in Year 1, $31.9 \pm 8.6\%$ in Year 2), and oat ($91.0 \pm 3.7\%$ in Year 1, $39.3 \pm 7.4\%$ in Year 2) were greatest in May. However the coverage of all groundcover treatments fell below 50% by August in Year 1, and by June in Year 2. Resident weedy groundcover (i.e. control treatment) was readily present throughout the growing season in both Year 1 and Year 2.

Leptoglossus zonatus abundance

In total, 180 *L. zonatus* adults were recorded in the pistachio orchard over the course of this 2-year study. Adult *L. zonatus* were more abundant in the crop canopy than on groundcovers ($z = -6.24, P < 0.01$), with 90% of individuals collected from the crop canopy. In canopy samples, there was a significant difference in total *L. zonatus* abundance across the season ($X^2 = 272.65, df = 18, P < 0.01$), with the greatest counts observed in August – September (Fig. 4.3A). However, there was no difference in *L. zonatus* abundance recorded in the crop canopy between groundcover treatment plots in either year of the study. While *L. zonatus* presence on groundcovers followed similar abundance trends as those observed in the crop canopy, similarly, there were no significant differences in *L. zonatus* abundance on groundcovers between treatment plots (Suppl 4.2).

Natural enemy abundance

In total, 550 *H. pennsylvanicus* individuals were collected from sticky card traps over the course of the study. *Hadronotus pennsylvanicus* were more abundant in the crop canopy than on groundcovers ($z = -12.18, P < 0.01$), with 97% of individuals collected from canopy traps. *Hadronotus pennsylvanicus* abundance in the crop canopy changed throughout successive seasons ($X^2 = 952.39, df = 18, P < 0.01$) (Figure 4.3B). There was a notable increase in *H. pennsylvanicus* abundance in the crop canopy later in the growing season (August) (Fig. 4.3B). During the 2021 growing season, *H. pennsylvanicus* abundance increased early in the growing season (June – July), with a

similar peak in abundance later in the season (August). Despite fluctuations in *H. pennsylvanicus* abundance, there were no significant differences in the number of parasitoids present in the canopy between groundcover treatments. The seasonal abundance of *H. pennsylvanicus* observed within groundcovers followed similar trends to those found in the crop canopy, with populations increasing later in the growing season (Suppl 4.2B). *Hadronotus pennsylvanicus* abundance within groundcovers was greatest early (June) in Year 2 of the study. However, there were no significant differences in *H. pennsylvanicus* abundance in crop canopy or within groundcovers between the groundcover treatments.

Minute pirate bugs *Orius* spp. Wolff (Hemiptera: Anthocoridae), green lacewings *Chrysoperla* spp. Schneider (Neuroptera: Chrysopidae), and ladybird beetles *Hippodamia* spp. Dejean (Coleoptera: Coccinellidae) were the most common predatory insects collected from sampling (1838, 174, and 410 total individuals collected, respectively). *Orius* spp. and *Hippodamia* spp. were most abundant in groundcovers ($z = 25.29$, $P < 0.01$; $z = -12.56$, $P < 0.01$, respectively), while *Chrysoperla* spp. were most abundant in the crop canopy ($z = -4.66$, $P < 0.01$). While seasonal population trends differed between species ($X^2 = 459.86$, $df = 18$, $P < 0.01$ for *Orius* spp.; $X^2 = 92.499$, $df = 18$, $P < 0.01$ for *Chrysoperla* spp.; $X^2 = 45.612$, $df = 18$, $P < 0.01$ for *Hippodamia* spp.), there was no significant difference in seasonal abundance of any species in the crop canopy between groundcover treatments (Suppl 4.3).

Biological control

Parasitism on sentinel *L. zonatus* eggs primarily occurred late in the growing season (September) (Figure 4.4A). A total of 7 of the 762 egg clusters deployed were parasitized over the 2-year study, with *H. pennsylvanicus* accounting for 57% of parasitoids to emerge from eggs, followed by *Ooencyrtus* spp. Ashmead (Encyrtidae), *Anastatus* spp. Motschulsky (Eupelmidae) accounting for the remaining 29% and 14%, respectively. Despite parasitism occurring late in the growing season, there was no significant difference in the number of parasitoids to emerge from sentinel eggs between each sample period, nor between groundcover treatment plots. In the field, the average proportion of eggs that failed to yield a parasitoid or *L. zonatus* nymph (i.e. was determined non-viable) was 6.2 ± 0.6 . Sentinel eggs in the canopies of buckwheat ($8.9 \pm 1.6\%$) exhibited a greater proportion of non-viable eggs than those in plots containing cowpea ($5.6 \pm 1.1\%$; $z = -3.25$, $P < 0.05$), mustard ($5.6 \pm 1.1\%$; $z = -3.25$, $P < 0.05$), and oats ($4.8 \pm 1.1\%$; $z = -4.18$, $P < 0.01$). However, there was no significant difference in the proportion of non-viable eggs between buckwheat and weed plots. The proportion of non-viable eggs from unexposed (control) sentinel eggs ($3.09 \pm 0.62\%$) was significantly less than those placed in the orchard ($z = -5.78$, $P < 0.01$ for buckwheat; $z = 3.44$, $P < 0.01$ for weeds; $z = -3.03$, $P < 0.05$ for cowpea; $z = -3.03$, $P < 0.05$ for mustard).

Groundcover treatment had a significant influence on the number of sentinel eggs taken by predators ($X^2 = 51.61$, $df = 4$, $P < 0.01$) (Figure 4.4B). Plots containing oat groundcovers maintained the greatest total number of predated eggs ($z = -2.95$, $P < 0.05$). However, there was no significant difference in the total number of eggs eaten by a

predator between cowpea, mustard, and weedy control groundcover treatments. Plots containing buckwheat had the fewest total number of eggs eaten by a predator through both years of the study ($z = 3.70, P < 0.01$). While there were seasonal differences in the total number of sentinel eggs eaten by a predator ($X^2 = 369.61, df = 9, P < 0.01$), few dates exhibited consistent differences between groundcover treatments. In Year 1, cowpea maintained greater predation rates than buckwheat ($z = 5.44, P < 0.01$) and mustard ($z = 3.81, P < 0.01$) in May, mustard exhibited greater predation rates than buckwheat ($z = 4.15, P < 0.01$) and cowpea ($z = -2.86, P < 0.05$) in June, and cowpea and oat maintained greater predation rates than the weed plot ($z = -3.37, P < 0.01$ for cowpea; $z = -4.49, P < 0.01$ for oat) in July. In Year 2, buckwheat and mustard maintained greater predation rates than cowpea ($z = -3.47, P < 0.01$ for buckwheat; $z = -4.57, P < 0.01$ for mustard) and weed plots ($z = -3.47, P < 0.01$ for buckwheat; $z = -4.57, P < 0.01$ for mustard) in September. A total of 407 (4.9%) of the sentinel eggs deployed into the orchard were lost without indication of predation, and therefore determined missing.

Discussion

Under laboratory conditions, floral diet had a direct influence on the reproductive fitness of *H. pennsylvanicus*. Of the floral diets evaluated, buckwheat provided the greatest benefit to female longevity. While all diets that contained nectar (eg. mustard, cowpea, and buckwheat) enhanced the ovipositional period of female *H. pennsylvanicus*, there was no significant difference in the lifetime fecundity of female wasps provisioned with any floral diet. These findings likely suggest an egg limitation being met by female

wasps despite variation in diet suitability, concurring with previous studies of *H. pennsylvanicus* reproductive traits (Sabbatini Peverieri et al. 2012; Cornelius et al. 2018; Straser et al. 2022). In general, parasitoid longevity and fecundity increases with the concentration of carbohydrates in adult diet (Benelli et al. 2017), however there may be limits to these benefits (Ellers et al. 2011). Despite floral diet enhancing *H. pennsylvanicus* longevity, the majority of offspring produced by females occurred within the first 10 days of their lifetime. Therefore, the potential benefits gained from nectar feeding on parasitoid longevity may prove immaterial for enhancing biocontrol given the duration *H. pennsylvanicus* is able to successfully reproduce on their host.

Biocontrol programs often rely on the recruitment of natural enemies early in the growing season and the retention of populations through periods of crop vulnerability (Landis et al. 2000; Wäckers and van Rijn 2005). Maintaining floral groundcovers and/or adjacent non-crop habitat may allow *H. pennsylvanicus* to survive periods of decreased host availability and maintain synchronicity with the pest host populations. However, despite floral resources enhancing *H. pennsylvanicus* reproductive fitness under laboratory conditions, deploying the same plant species as groundcovers did not influence the seasonal abundance of the wasp nor parasitism rates on *L. zonatus* egg clusters. The addition of provisioning floral resources to enhance biocontrol in agricultural landscapes can often generate variable outcomes (Tschardt et al. 2016; Wilson et al. 2017). When assessing the influence of buckwheat provisions in squash fields on the biocontrol of squash bug *Anasa tristis* (De Geer) (Hemiptera: Coreidae), Cornelius et al. (2019) found that while *H. pennsylvanicus* accounted for 93% of eggs parasitized, parasitism rates

were not affected by the supplemented floral resources. Similarly, Fair et al. (2017) found that the addition of floral resources in squash fields did not influence *H. pennsylvanicus* population densities throughout the growing season.

Understanding the behavior and phenology of a pest and its natural enemies in relation to the crop systems may be a crucial component in the development of effective biocontrol programs. Cornelius et al. (2016) found that early plantings of squash plants resulted in greater seasonal parasitism rates from *H. pennsylvanicus* than crops sown later in the season. These findings may suggest that *H. pennsylvanicus* populations primarily build up through successive generations emerging from host egg masses, and therefore, early recruitment of the parasitoid may only enhance parasitism rates later in the growing season. Interestingly, year 2 of this study demonstrated that *H. pennsylvanicus* is capable of building in abundance early in the pistachio growing season. Given there may be subsequent impacts on insect communities for years following the deployment of groundcovers, it may take time to adequately observe impacts on biocontrol as natural enemy communities become established locally. Therefore, conservation biocontrol may require more than 2 years of field evaluations to adequately measure a management tactics' impact on pest suppression.

In California, overwintered adult *L. zonatus* typically disperse into pistachio orchards early in the growing season (June) (Daane et al. 2005). As an egg parasitoid, the prospects of biocontrol by *H. pennsylvanicus* may be contingent on whether pest feeding damage can be suppressed prior to this period of crop vulnerability. While *H. pennsylvanicus* and other egg parasitoids, such as *Ooencyrtus* spp., *Anastatus* spp. and

Trissolcus spp. Ashmead (Scelionidae), are known to attack *L. zonatus* eggs (Mitchell and Mitchell 1986; Marchiori 2002; Xiao and Fadamiro 2010; Straser et al. 2022), parasitism rates on sentinel *L. zonatus* host eggs remained low throughout the surveyed seasons. However, while parasitism rates remained low, predation rates fluctuated throughout the season, and ultimately accounted for the majority of biocontrol of *L. zonatus* sentinel eggs. Despite a large degree of variation in trends, groundcovers appeared to influence the predation rates of sentinel eggs over the growing season. Given inconsistencies in seasonal predation rates, along with a lack of correlation between the coverage of groundcover and the number of sentinel eggs eaten by a predator, it is difficult to determine whether predation rates are influenced by the presence of groundcovers or the availability of alternative prey.

Though sentinel egg masses provide a convenient way to assess localized predation and parasitism rates, observations from these egg masses can often misrepresent true rates of biocontrol and inaccurately reflect the composition of natural enemy communities (Mitchell et al. 1999; Jones et al. 2014). One potential explanation of this trend may include the suite of semiochemical cues exploited by egg parasitoids during host searching and oviposition (Colazza et al. 2014). For instance, if particular chemical cues utilized by a foraging parasitoid are compromised in sentinel eggs, these eggs may be more difficult to locate and/or less desirable to oviposit on, resulting in lower rates of parasitism in field settings. Therefore, surveying naturally oviposited *L. zonatus* egg clusters within pistachio may provide a better understanding of the natural enemy community currently attacking *L. zonatus* eggs.

Groundcovers have demonstrated to improve weed control, enhance soil health, and aid in water retention in orchards (Demestihis et al. 2017; Fang et al. 2021), and if there is added benefits to crop protection there may likely be greater adoption from the California nut industries. However, implementing and maintaining effective groundcovers to enhance biocontrol can be complex and highly knowledge intensive. The efficacy of such groundcovers often depends on the spatial and temporal characteristics of the crop and groundcover plants, as well as the diversity, abundance, and behavior of insect pests and their natural enemies (Shelton and Badenes-Perez 2006). For growers, the adoption of such management tactics aimed to enhance biocontrol may be contingent on its economic viability, it's reliability in reducing crop damage, and ultimately, whether such tactics successfully increase revenue and profitability (Chaplin-Kramer et al. 2019). It is also important to consider that the establishment and maintenance of flowering groundcovers requires additional labor, fuel and material costs for growers, all of which is a recur expense given groundcovers must be re-sown each year. Therefore, understanding the potential prospects and limitations of conservation biocontrol within a given crop system must be thoroughly evaluated prior to its implantation.

Findings from this study would suggest that the use of summer flowering groundcovers aimed to improve biocontrol is likely not a viable solution to sufficiently suppress *L. zonatus* populations and meet the current production demands of California nut production. While nutrition may be a major influence on the reproductive fitness of *H. pennsylvanicus*, diet may represent only one of the many resources required by *H. pennsylvanicus* in order to persist in agricultural landscapes. For instance, parasitoids

often require additional non-crop resources, such as overwintering habitat and sufficient refuge from the adverse biotic conditions of cropping systems (Gillespie et al. 2016). In the case of California orchards, the quantity and quality of such habitats may prove to be a limiting factor in the biocontrol potential of *H. pennsylvanicus*. Therefore, understanding the overwintering ecology and habitat requirements of *H. pennsylvanicus* in relation to host *L. zonatus* may prove critical for understanding the seasonality and synchronicity of this pest-parasitoid relationship in agricultural systems.

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<i>Reproductive parameters</i>	<i>water</i>	<i>oat</i>	<i>cowpea</i>	<i>mustard</i>	<i>buckwheat</i>	<i>honey-water</i>
Adult female longevity (days)*	1.9 ± 0.1 a	2.4 ± 0.2 a	17.5 ± 0.9 b	28.5 ± 2.2 c	34.2 ± 2.6 d	49.6 ± 1.5 e
Adult male longevity (days)*	1.7 ± 0.1 a	2.0 ± 0.2 a	13.6 ± 1.0 b	24.3 ± 2.2 c	23.0 ± 2.3 c	43.5 ± 2.6 d
Ovipositional period (days)*	0.6 ± 0.1 a	3.0 ± 0.6 b	5.8 ± 0.9 c	7.0 ± 1.2 cd	7.3 ± 0.9 cd	9.8 ± 0.8 d
Post-reproductive period (days)	0.7 ± 0.2 a	1.2 ± 0.4 a	4.0 ± 0.9 b	6.7 ± 1.3 bc	9.5 ± 1.3 c	8.1 ± 0.9 c
Total progeny (offspring/female)	8.6 ± 1.9 a	20.2 ± 3.7 b	26.3 ± 3.9 b	26.7 ± 6.1 b	35.1 ± 6.0 b	34.0 ± 4.5 b
Total female progeny	8.3 ± 1.8 a	18.6 ± 3.6 b	24.1 ± 3.4 b	24.2 ± 5.5 b	30.8 ± 5.1 b	32.1 ± 4.4 b
Total male progeny	0.3 ± 0.1 a	1.6 ± 0.2 ab	2.2 ± 0.6 ab	2.4 ± 0.7 ab	4.3 ± 1.7 b	1.9 ± 0.2 ab
Sex ratio (% female offspring)	97.1 ± 0.7 a	88.9 ± 2.5 a	92.8 ± 1.6 a	92.8 ± 1.5 a	89.5 ± 2.4 a	93.9 ± 0.9 a

Table. 4.1 Reproductive attributes (mean \pm SE) of *Hadronotus pennsylvanicus* reared on *Leptoglossus zonatus* eggs at 25° C \pm 1, 75 \pm 5% RH and 16:8 L:D, fed oat, cowpea, mustard, buckwheat floral provisions, or a water or honey-water diet *ad libitum*. Asterisks (*) indicate where adults were not provided host eggs for oviposition

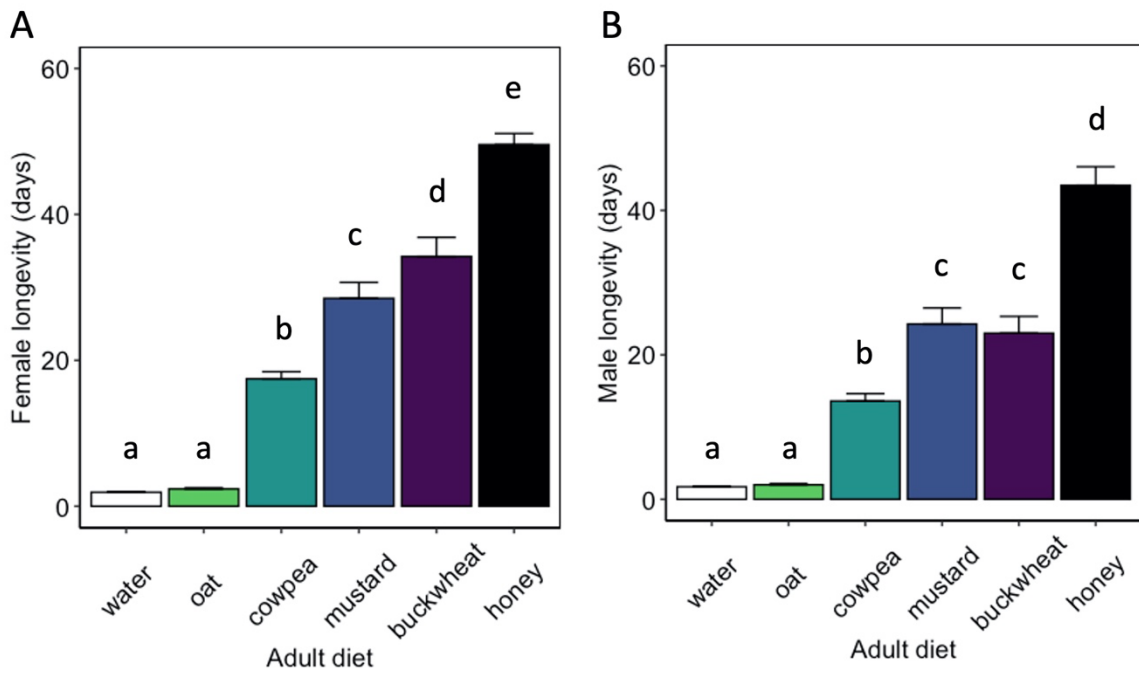


Fig. 4.1 *Hadronotus pennsylvanicus* female (A) and male (B) longevity (mean \pm SE) when fed floral resources, water, or a honey diet

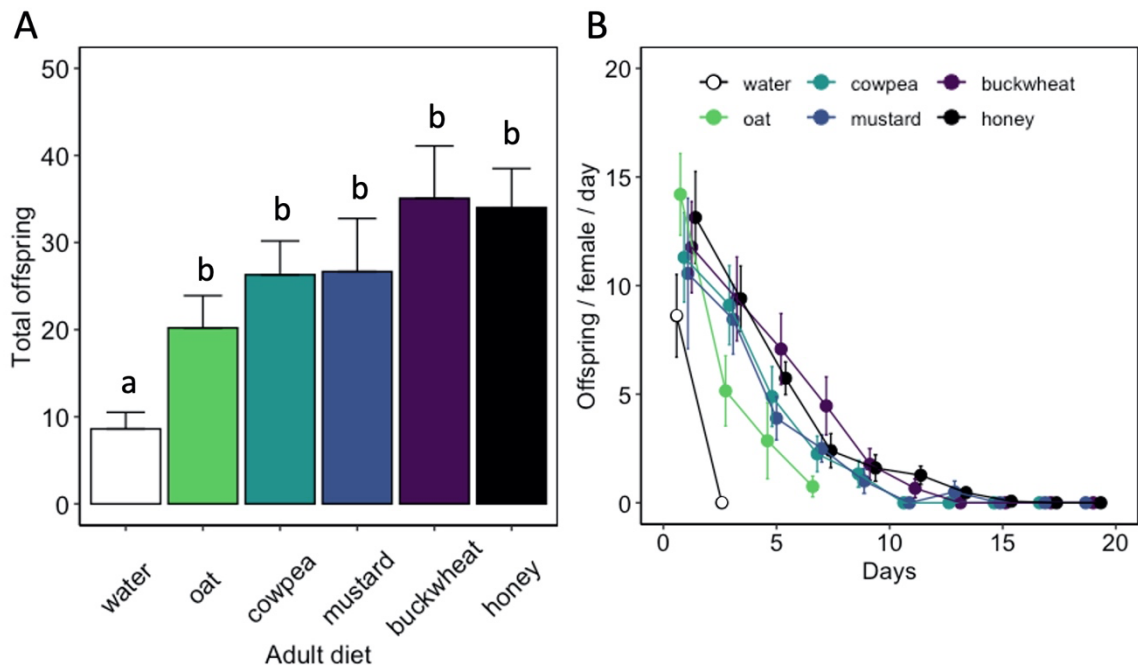


Fig. 4.2 *Hadronotus pennsylvanicus* lifetime fecundity (A) and progeny produced over time (B) (mean \pm SE) when provided *Leptoglossus zonatus* host eggs every other day and fed a floral resource, water, or honey diet

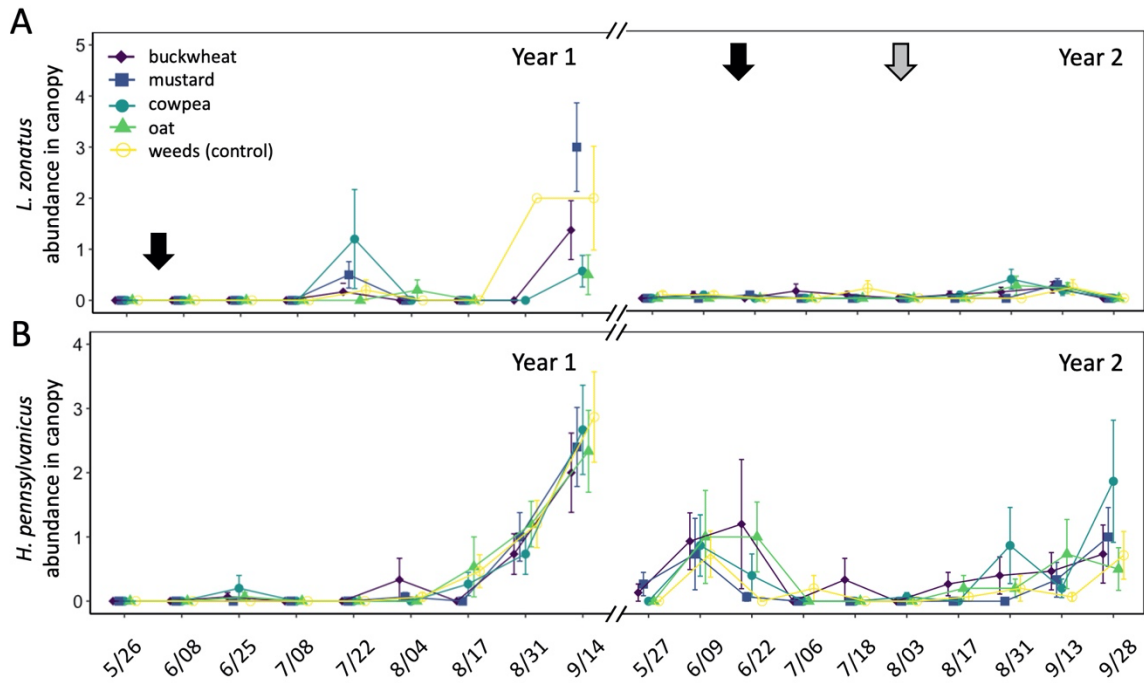


Fig 4.3 Mean abundance (\pm SE) of *Leptoglossus zonatus* (A) and *Hadronotus pennsylvanicus* (B) in the canopy of pistachio containing groundcover treatments. Arrows on the top indicate a treatment with pyrethrin (black arrow; 3 July 2020 & 20 June 2021) and mowing of the groundcover (grey arrow; 2 August 2021)

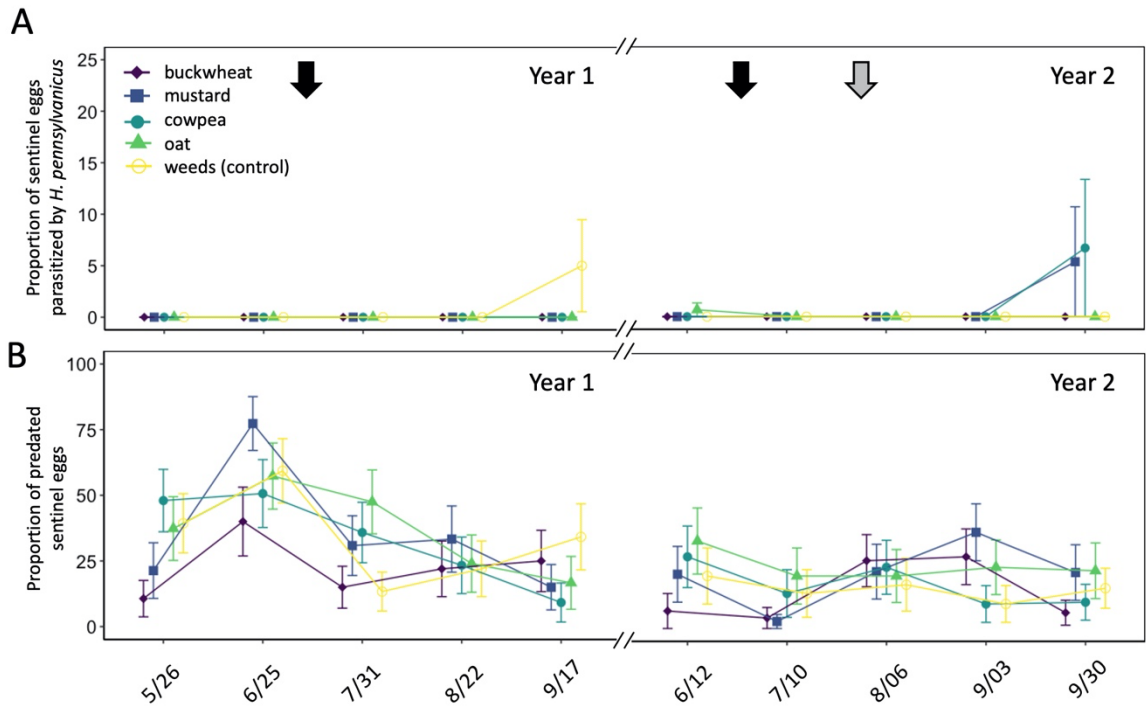
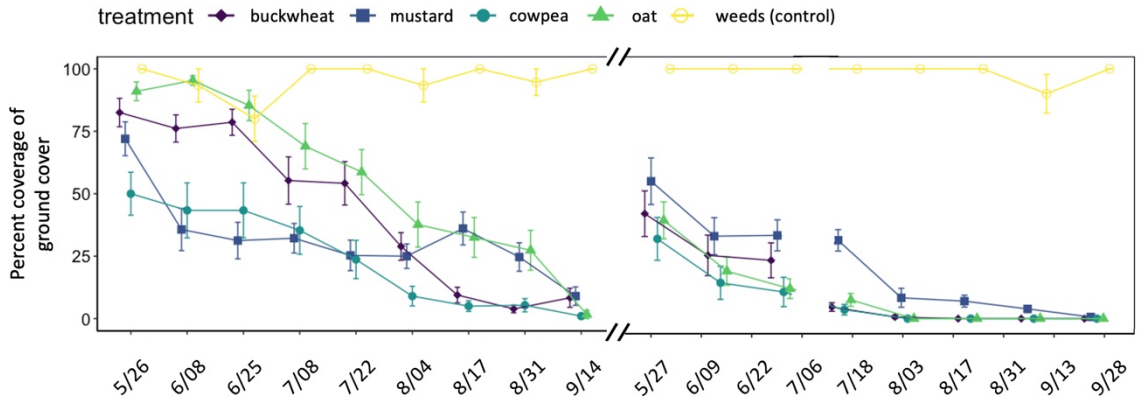
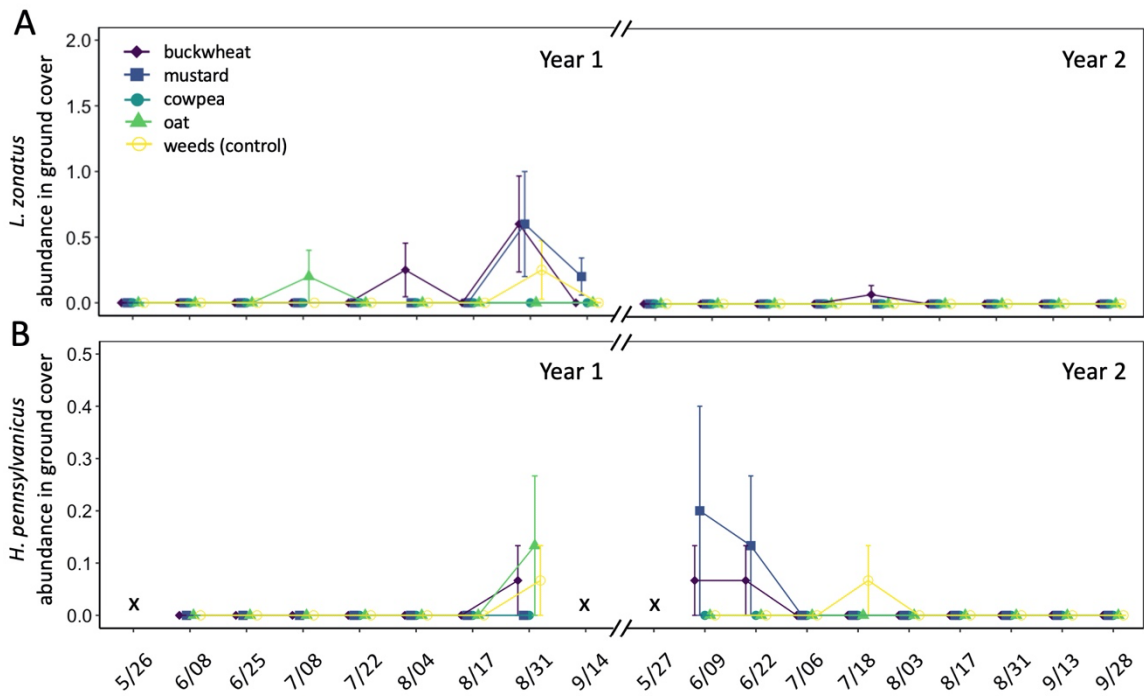


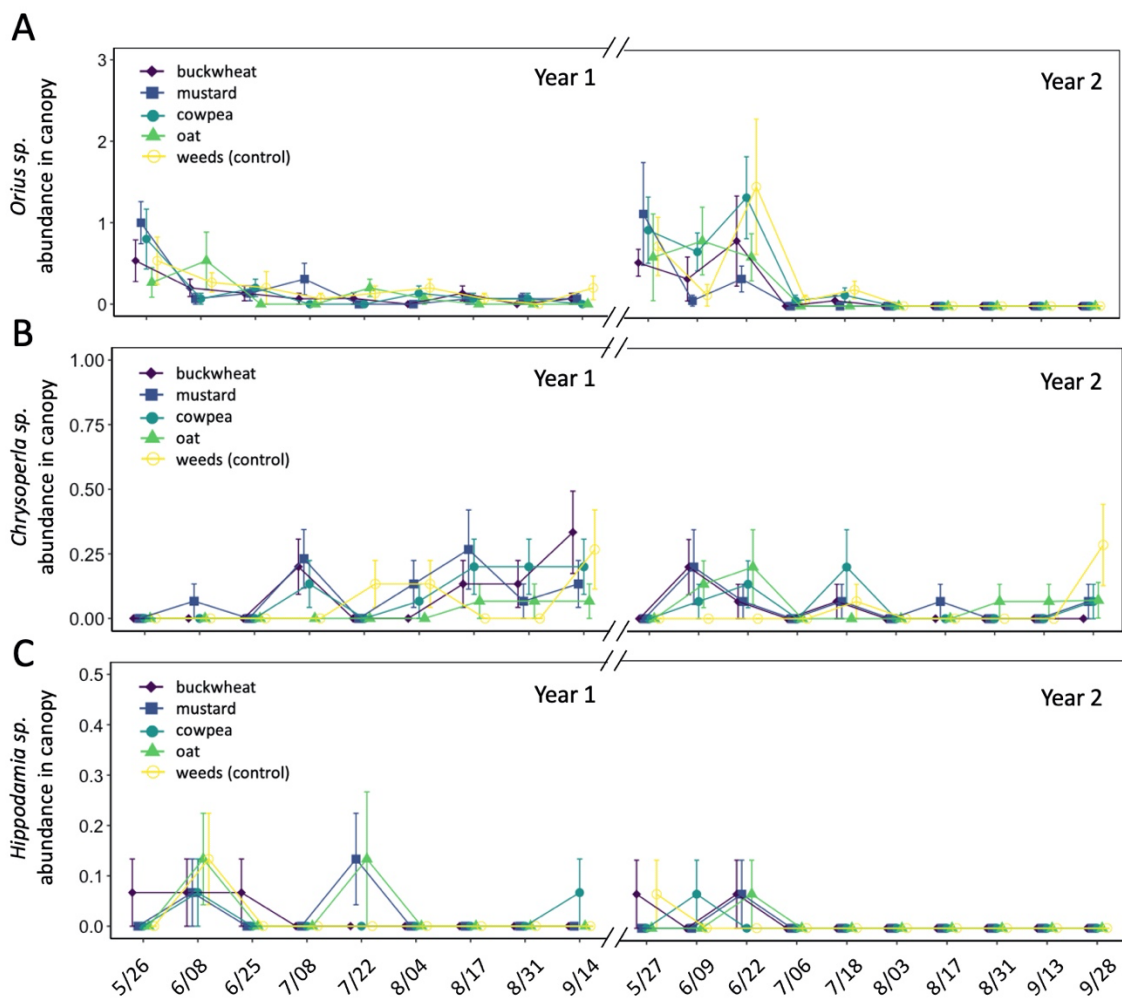
Fig. 4.4 Mean proportion (\pm SE) of sentinel *Leptoglossus zonatus* eggs parasitized by *Hadronotus pennsylvanicus* (A) or predated egg clusters (B) in pistachio canopy within each groundcover treatment. Arrows on the top indicate treatment with a pyrethrin (black arrow; 3 July 2020 & 20 June 2021) and mowing of the groundcover (grey arrow; 2 August 2021)



Suppl 4.1 Seasonal percent coverage (\pm SE) present in 1 m² quadrat for each groundcover plant species within an organic pistachio orchard. Data on the proportion of plant coverage for each groundcover treatment was missing from 6 July 2021



Suppl 4.2 Mean abundance (\pm SE) of *Leptoglossus zonatus* (A) and *Hadronotus pennsylvanicus* (B) within groundcover treatments in organic pistachio



Suppl 4.3 Mean abundance (\pm SE) of *Orius sp.* (A), *Chrysoperla sp.* (B), and *Hippodamia sp.* (C) in pistachio canopy within each groundcover treatments

Conclusions

Main findings

The research presented in this dissertation sought to understand the prospects and limitations of biological control as a tactic to suppress *L. zonatus* populations in California orchards. To accomplish this task, a series of experiments were conducted to evaluate the role of floral resources in enhancing the reproductive fitness of a candidate biocontrol agent *Hadronotus pennsylvanicus* (Ashmead) (Hymenoptera: Scelionidae), and furthermore, assess whether provisioning such floral resources as groundcovers could increase the abundance of this natural enemy, and in turn, enhance biological control of *L. zonatus* in orchards. Findings from this work suggest that *H. pennsylvanicus* exhibits suitable traits as a biocontrol agent of *L. zonatus*. However, while the availability of floral resources did improve the reproductive fitness of *H. pennsylvanicus*, the addition of floral resources as groundcovers did not enhance the biological control of *L. zonatus*. The findings from each of the studies included within this dissertation are summarized to address the key questions proposed within the Introduction:

How effective is *Hadronotus pennsylvanicus* as a prospective biocontrol agent of *Leptoglossus zonatus*?

Under laboratory conditions, *H. pennsylvanicus* was found to be a long-lived species capable of parasitizing a large number of *L. zonatus* host eggs over the first two weeks of their lifespan. Female *H. pennsylvanicus* maintained high rates of female offspring over the course of their lifetime, and continued to parasitize host eggs despite

potential egg load limitations being met. Furthermore, the age of host eggs did not influence parasitism rates by females, suggesting *H. pennsylvanicus* are capable of successfully reproducing from host eggs of different developmental stages under field conditions. Under laboratory conditions, *H. pennsylvanicus* demonstrated desirable biological and reproductive traits that could potentially make it an effective biocontrol agent for *L. zonatus*.

How does access to diet influence *Hadronotus pennsylvanicus* reproductive performance?

Diet can have a direct influence on the reproductive fitness of female *H. pennsylvanicus*. Experiments demonstrated that female parasitoids lived longer and parasitized more host eggs when provided frequent access to a honey diet in comparison to water-fed individuals. Once depleted of mature eggs, females with frequent access to honey diet also contributed to greater rates of non-reproductive host mortality. Access to a carbohydrate-rich diet also influenced the reproductive behavior of female wasps. Here, increased frequency of honey feeding was associated with more frequent and longer durations of oviposition by female wasps. In contrast, water-fed females spent less time interacting with host eggs and tended to more frequently divert from oviposition behavior. This negative effect of food deprivation on the reproductive performance of *H. pennsylvanicus* suggests that under field conditions, parasitism rates could potentially be decreased during periods of low food resource availability.

How does diet quality influence reproductive fitness of *Hadronotus pennsylvanicus*?

Diet quality can affect the reproductive fitness of female *H. pennsylvanicus*. Sucrose concentration in the diet, regardless of pollen content, enhanced the survival and lifetime fecundity of female parasitoids. Diets containing high sucrose concentration increased female longevity and offspring production two-fold in comparison to individuals fed a low sucrose diet. While wasps fed high sucrose diets exhibited greater acquisition of bodily sugars, glycogen, and lipids over their lifetime, there was no effect on bodily protein content. Similarly, provisioning pollen with sucrose diets did not influence female longevity, fecundity, or nutrient acquisition. Given these findings, identifying carbohydrate-rich floral resources that are compatible with cropping systems may enhance the reproductive fitness of *H. pennsylvanicus* under field conditions, which in turn, could lead to enhanced parasitism of *L. zonatus*.

Can floral resource provisions improve biological control of *Leptoglossus zonatus* in orchard systems?

Under laboratory conditions, floral resources influenced the reproductive fitness of female *H. pennsylvanicus*. Experiments evaluated several candidate floral species including oat (*Avena sativa* L., Cyperales: Poaceae), cowpea (*Vigna unguiculata* (L.) Walp., Fabales: Fabaceae), white mustard (*Sinapis alba* L., Capparales: Brassicaceae), and buckwheat (*Fagopyrum esculentum* Moench, Polygonales: Polygonaceous). While access to all floral species improved lifetime fecundity, buckwheat in particular provided

the greatest benefit to female longevity. However, there were no differences in the total numbers of offspring produced per female between the different floral diets. Despite the benefits of floral resources on parasitoid survival, when floral treatments were deployed in pistachio orchards, they did not influence the abundance of *H. pennsylvanicus*, nor enhance biological control of *L. zonatus*.

Limitations and future directions

Effective integrated pest management (IPM) relies on an understanding of the interactions between crops, organisms, and the environment to leverage potential synergies that can promote sustainable pest management. The ability to predict, and ultimately manipulate, the abundance of pests and their natural enemies within cropping systems would allow growers to improve biological control, and in turn reduce the risk of feeding damage on their crop. However, the measure of a natural enemy's impact on biological control must be tailored to the needs of growers for the specific pest problems that they face. For instance, the degree to which a natural enemy is able to suppress pest densities may be less important than the timing and consistency of such control throughout multiple growing seasons (Macfadyen et al. 2014). This approach may push research outcomes beyond simply the preservation of natural enemies, but rather towards how natural enemies can be utilized within current IPM strategies.

For growers, the adoption of tactics to enhance biological control may be contingent on its economic viability, whether it reliably reduces their risk of crop loss, and ultimately, whether such tactics successfully increase revenue and profitability

(Chaplin-Kramer et al. 2019). In the case of floral resource provisioning, the establishment and maintenance of flowering groundcovers requires additional labor, fuel, and material costs for growers, all of which recur annually given plants must be re-sown each year. Therefore, if these efforts are unable to reliably enhance biological control of the target pest, it is all the more important to provide unbiased information on a natural enemy's efficacy to inform growers who otherwise would be risking loss of time and money to maintain groundcovers.

The information generated here provides insight into the importance of diet on *H. pennsylvanicus* reproductive fitness, and its prospects as a biocontrol agent of the leaf-footed bug *L. zonatus*. However, in light of findings from the studies carried out in this dissertation, on-farm floral resource provisioning aiming to improve biological control is likely not a viable solution to sufficiently suppress *L. zonatus* populations and meet the current production demands of California nut production. While these findings may be disappointing, knowledge that provisioning floral resources does not improve pest control is still useful in that it allows researchers to focus on alternative management practices that may have a greater chance of success. To this end, it would be useful to identify why such efforts to improve biological control failed, and furthermore, evaluate whether these conditions could be ameliorated, or whether they are simply not viable for *L. zonatus* suppression in California orchards.

The availability of suitable food resources is a prerequisite for parasitoids to persist in the environment (Wäckers et al. 2005; Benelli et al. 2017). As demonstrated in the work presented here, adult diet can have a significant role in enhancing the survival,

lifetime fecundity, and parasitism behavior of the egg parasitoid *H. pennsylvanicus*. Under laboratory conditions, it would appear that diet could be a major influence dictating *H. pennsylvanicus*' ability to suppress *L. zonatus* populations. However, adult diet likely represents only one of the many factors influencing the prospects of *H. pennsylvanicus* as a biological control agent to *L. zonatus*.

Parasitoids often require additional resources, such as overwintering habitat and refuge from adverse biotic conditions in order for cropping systems to benefit from their biocontrol services (Gillespie et al. 2016). Given scelionid wasps overwinter as adults (Orr 1988), the overwintering ecology of *H. pennsylvanicus* may be an important factor influencing parasitoid-host life history synchrony (Sarhou et al. 2014). Therefore, an improved understanding of the overwinter behavior and dispersal capacity of *H. pennsylvanicus* may provide insight to the seasonality of parasitoid activity, and furthermore, whether wasps are capable of dispersing in-step with host *L. zonatus* throughout the crop landscape.

Lastly, understanding the early season activity of *H. pennsylvanicus* may provide insight to potential preferences to alternative host species, of which may explain delayed parasitism activity of *L. zonatus* in orchards. While *H. pennsylvanicus* is known to attack other coreid hosts (Nechols 1989; Mitchell and Mitchell 1986; Yasuda 1998; Bates and Bordon 2004), little is known regarding host preferences in California agroecosystems. Should *H. pennsylvanicus* exhibit preference for an alternative host, resident parasitoid populations may likely occupy locations more closely associated with the alternative

host, of which would negatively impact biological control programs for *L. zonatus* in pistachio orchards.

Therefore, evaluating the overwintering ecology, dispersal capacity, and potential host preferences of *H. pennsylvanicus* in relation to *L. zonatus* would provide a basis to understanding the seasonality of this pest-parasitoid relationship within and between cropping systems, and furthermore, whether *H. pennsylvanicus* populations can be manipulated to benefit biological services during periods of crop vulnerability.

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