Journal of Economic Entomology, 116(4), 2023, 1261–1267
https://doi.org/10.1093/jee/toad097
Advance Access Publication Date: 25 May 2023
Research

ENTOMOLOGICAL SOCIETY OF AMERICA
SHARING INSECT SCIENCE GLOBALLY
OXFORD

Horticultural Entomology

Reproductive biology of overwintering leaffooted bug *Leptoglossus zonatus* (Hemiptera: Coreidae) in California

Judith M. Stahl[1], **Kent M. Daane**[*]

Department of Environmental Science, Policy, and Management, University of California, Berkeley, CA 94720-3114, USA [1]Present address: CSIRO, Health and Biosecurity, Clunies Ross, GPO Box 1700, Acton, ACT 2601, Australia *Corresponding author, mail: kmdaane@berkeley.edu

Subject Editor: Joseph Munyaneza

Received on 16 January 2023; revised on 24 April 2023; accepted on 11 May 2023

Leptoglossus zonatus (Dallas) (Hemiptera: Coreidae) is a polyphagous insect pest attacking a wide variety of crops. In California's Central Valley, it is now the dominant leaffooted bug on almonds, pistachios, and pomegranates. *Leptoglossus zonatus* pest status depends largely on overwintering adult survival and reproductive potential, which determines its population size in spring and early summer when nut crops are particularly susceptible to bug damage. Here, we investigated the overwintering reproductive biology of *L. zonatus* in laboratory and field experiments to gain information about its ovary development, time of mating, and the impact of low temperatures on egg hatch. With dissections of laboratory-reared *L. zonatus*, we established a baseline for ovarian development and determined that the size of the spermathecal reservoir is larger in mated than in unmated females. Dissections and behavioral experiments of field-collected material provided evidence of mating events before dispersal from overwintering sites. Laboratory trials showed that temperature significantly impacted *L. zonatus* egg hatch. *Leptoglossus zonatus* reproductive biology presented provides valuable information on its population dynamics and dispersal from overwintering sites, and will contribute to the development of monitoring and management tools.

Key words: Heteroptera, mating, ovary, spermatheca

Introduction

Leptoglossus zonatus (Dallas, 1852) (Hemiptera: Heteroptera: Coreidae) is a polyphagous insect pest attacking a wide variety of crops, including tree nuts (Daane et al. 2019), corn (Sawazaki et al. 1989), pomegranate (Raga et al. 1995), passion fruit (Rodrigues Netto and Guilhem 1996), and satsuma mandarin (Henne et al. 2003). Of the more than 60 recorded *Leptoglossus* species, *L. zonatus* has the most widespread geographical distribution reported (Joyce et al. 2021), which includes South, Central, and North Americas (Heidemann 1910, Allen 1969). In the Southeastern United States, *L. zonatus* was first reported around 2,000 (Henne et al. 2003, Buss et al. 2005) and in California's Central Valley it is now the dominant leaffooted bug in the affected crops such as almond, pistachio, and pomegranate (Joyce et al. 2017). As part of a group of "large bugs" that otherwise consists of several pentatomid species (Daane et al. 2016, Stahl et al. 2021), *L. zonatus* feeding by adults and to a lesser extent nymphs damages developing fruits and nuts (Stahl et al. 2020). They can cause nut drop, external and internal lesions, and deformities (Stahl et al. 2020), lowering harvest quantity and quality, and have been implicated in the movement of pathogens such as *Botryosphaeira* fungi in pistachio (Michailides and Morgan 2016).

Leptoglossus zonatus pest status depends largely on overwintering adult survival and reproductive potential, which determines its population size in spring and early summer when nut crops are particularly susceptible to bug damage. In California's nut producing areas in the San Joaquin Valley, *L. zonatus* has 3 to 4 overlapping generations (Daane et al. 2019). Adults are known to move among host plants throughout the season, before migrating to their overwintering sites, such as palm trees, piles of debris around orchards, or citrus orchards (Daane et al. 2016), often in large aggregates that are believed to form, in part, due to an aggregation pheromone (Franco-Archundia et al. 2018, Taszakowski et al. 2023). The similarly male-produced sex pheromone (Inoue et al. 2019) seems to be utilized in spring, as the insects can regularly be seen in mating pairs during that time. It is not known if mating also occurs before, during, or after overwintering as has been discussed for other insects that overwinter in aggregations (Susset et al. 2018).

Mating and egg fertilization are necessary for successful *L. zonatus* reproduction. While the females can lay unfertilized eggs, no nymphs develop (JMS, personal observation). For *L. zonatus* reared in the laboratory under summer conditions, the pre-mating period ranges between 14 and 49 days, with an average of 32 days (Matrangolo and Waquil 1994). During mating, sperm is transferred from the males to the females and stored in the female's spermatheca, a specialized organ in many invertebrates (Pascini and Martins 2017). For *L. zonatus*, the spermatheca has been described by Souza et al. (2016) and contains a reservoir to store the sperm as well as an expanded duct connected to the common oviduct that is then connected to the ovaries where eggs develop and mature, however, there is no information about ovary development for *L. zonatus*. In the congeneric *Leptoglossus occidentalis* (Heidemann), sperm stored in the spermatheca is transported into the common oviduct to fertilize the mature eggs before oviposition (Chiang 2010). Oviposition of fertilized *L. zonatus* eggs can start 5 days after mating occurs (Matrangolo and Waquil 1994). In other heteropteran species multiple matings can stimulate oviposition (e.g., Rodrigues et al. 2008), while this is unknown for *L. zonatus*.

The period of *L. zonatus* egg development, from oviposition to hatch of the first instar, leaves the egg vulnerable to various abiotic and biotic factors. For example, egg parasitoids like *Hadronotus pennsilvanicus* (Ashmead) (Hymenoptera: Scelionidae) (Straser et al. 2022) or insecticide applications (Jackai and Ndlovu 1988) and unfavorable weather conditions such as cold or hot temperatures (Dreyer and Baumgärtner 1996) can impact hatching. *Leptoglossus zonatus* eggs can hatch even under high field temperatures found in California's San Joaquin Valley (Daane et al. 2019), and while the cold tolerance of adult *L. zonatus* has been studied (Tollerup 2019), the impact of low temperatures on egg hatch is still unknown. In this study, we investigate the overwintering reproductive biology of *L. zonatus* to gain information about its ovary development, possible mating during overwintering, and the impact of low temperatures on egg hatch.

Material and Methods

Rearing

The *L. zonatus* colony was established in 2015 from individuals field-collected in Fresno County, CA, USA. They were maintained in gauze cages ("BugDorm-2120 Insect Rearing Tent 60 x 60 x 60 cm", MegaView Science Co. Ltd., Taichung, Taiwan) and fed with a combination of corn (*Zea mays* L. [Poales: Poaceae]), beans (*Phaseolus vulgaris* L. [Fabales: Fabaceae]), carrots (*Daucus carota* subsp. *sativus* Schübl. & Martens [Apiales: Apiaceae]), and Italian squash (*Cucurbita pepo* L. [Cucurbitales: Cucurbitaceae]) that was replaced twice a week. Additionally, cages were equipped with potted Mediterranean cypress (*Cupressus sempervirens* L. [Pinales: Cupressaceae]) and bamboo skewers (Best Brands Consumer Products, Inc., New York, NY) placed vertically into Styrofoam boards for oviposition. Insects used in all laboratory trials were taken from this colony.

Ovarian Development

To obtain unmated adult females, fifth instars were collected from the laboratory colony and individually isolated in 473 ml (16 oz) deli containers (Anchor Packing, model D16CXL, St. Louis, MO) with an organdy lid for ventilation and provisioned with washed green beans and carrots replaced as needed. The insects were held at 26 °C, 40–50% relative humidity, and 16:8 L:D photoperiod, hereafter referred to as rearing conditions. After molting into adults, subsamples of females were dissected immediately and then daily up to 34 d with the exception that no dissections were made of adult females aged 16–18 d and additional dissections were made of females aged 42–78 d. For dissections, females were placed in 70% ethanol until dead, and immediately dissected using No. 2 insect pins, a razor blade, and forceps. Ovarian development was ranked in 3 categories: immature, intermediate, and mature. Ovaries were defined as immature when no oocytes or clear differentiation and vitellary constriction were visible; as intermediate when they showed vitellary constriction and oocytes; as mature when at least 1 oocyte was located in the lateral oviduct.

In a similar manner, field-collected *L. zonatus* were dissected. Adults were collected from 2 sites in Fresno County, California, USA, one site had both citrus and pomegranates and the other only pomegranates. Collections were made from March to April 2019, September to October 2019, and February to April 2020. Depending on the number of individuals found, up to 20 females from each collection were dissected the same day they were collected. Ovaries were classified as immature, intermediate, and mature as described previously.

Spermatheca

Unmated and mated female *L. zonatus* from the laboratory colony were dissected to evaluate the use of spermatheca measurements to identify mated or unmated females. For mated females, mating couples were carefully transferred from the rearing cages to individual containers and dissected after coupling was completed. Unmated females were obtained as described previously in the ovarian development trial. Upon dissection, spermathecal reservoir and duct lengths and widths were measured with the scaled ocular of the stereomicroscope (Nikon SMZ800, Nikon Instruments, Inc., Tokyo, Japan). Pronotum width, as a measurement of insect body size, was measured similarly.

In a similar manner, *L. zonatus* females were field-collected weekly from the 2 sites in Fresno County from September to October 2019, January to February 2020, and January to April 2021. They were immediately dissected or stored at 8 °C for a maximum of 2 d until dissection.

Oviposition

Female *L. zonatus* were field-collected from the 2 Fresno Co. sites from January to April 2021 and October 2021 to March 2022. Collected insects were placed individually in 946 ml (32 oz) deli containers (Fabri-Kal Corp., model PK32T-C, Kalamazoo, MI) with an organdy lid for ventilation and provisioned with washed green beans replaced as needed, and held under rearing conditions. Females from the laboratory colony were randomly selected and held under similar conditions to serve as controls. Since production of viable eggs indicates mating had occurred prior to collection, oviposition and number of hatching nymphs were recorded twice a week.

Egg Hatch

The influence of low temperatures on egg hatch was evaluated. *Leptoglossus zonatus* egg strands on wood skewers were collected daily from the rearing cages. Egg strands were kept at rearing conditions until they were 0–1 d (fresh), 2–3 d (medium), or 6–8 d (old eggs) old and split into treatments: 3 temperatures (2, 7, and 14 °C) and 6 storage durations (2, 4, 6, 10, and 14 d). Egg hatch was recorded after removal from cold storage. Eggs stored at 21 °C in

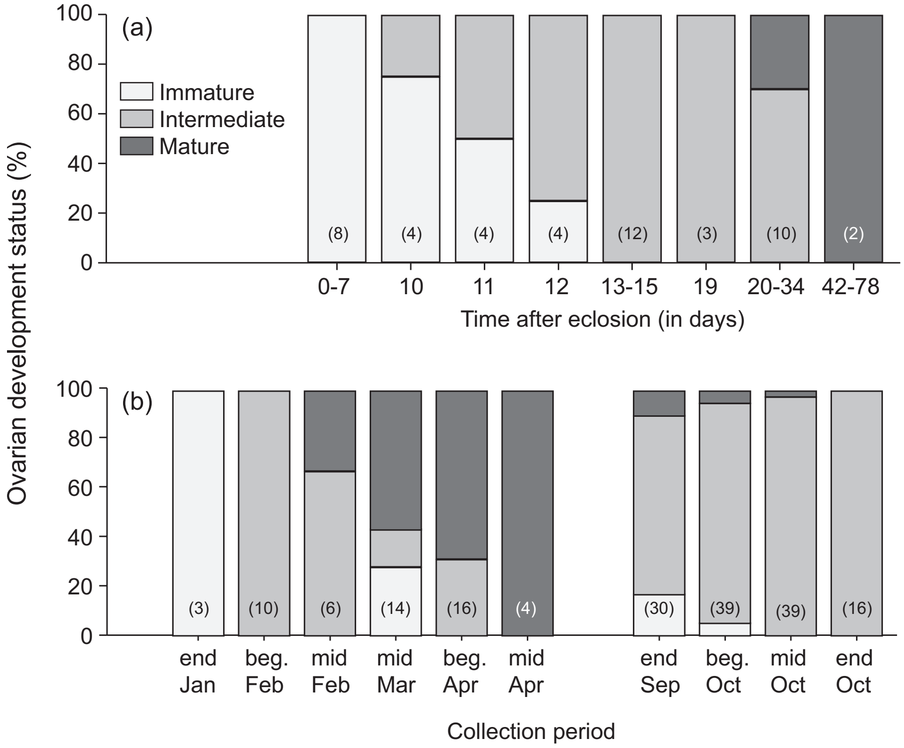

Fig. 1. Ovarian development status (immature, intermediate and mature) of dissected *L. zonatus* for (a) laboratory-reared unmated females, held at 26 °C, and collected at different periods from 1 to 78 d after eclosion and (b) individuals that were field-collected at different overwintering periods from January 2020 to April 2021 and September to October 2021. In each graph, numbers in parentheses in each bar are the numbers of dissected female *L. zonatus*.

the laboratory served as controls. Each treatment combination was replicated thirty times, with 1 replicate being 1 egg.

Statistical Analyses

Results are presented as mean ± SE. Observational data on ovarian development were not statistically compared. A linear model was used to compare spermatheca measurements (reservoir and duct lengths and widths) of unmated and mated laboratory-reared *L. zonatus* as well as spermatheca measurements and pronotum width among collection periods of field-collected *L. zonatus*. The impact of storage temperature on egg hatch was assessed with a generalized linear model (GLM) with temperature (2, 7, 14, and 21 °C) as fixed factor using a binomial error distribution. For 7 °C and 14 °C, egg hatch was additionally assessed with age of eggs (fresh, medium, old) and storage duration (2, 4, 6, 10, 14 d) as fixed factors. Pairwise comparisons of significant variables were conducted with Tukey's multiple comparisons test. All statistics were run with R version 3.6.2 (R Core Team 2020) using RStudio version 1.2.5033 (RStudio Team 2016). Packages used included "car" (Fox et al. 2016)for the GLM, as well as "multcomp" (Hothorn et al. 2017), "multcompView" (Graves et al. 2015), and "emmeans" (Lenth et al. 2018) for Tukey's multiple comparisons.

Results

Ovarian Development

Newly molted adult *L. zonatus* females from the laboratory colony eclosed with immature ovaries. They started showing vitellary constriction and oocytes (intermediate ovaries) 10 d after eclosion at 26 °C. At least 1 oocyte was located in the lateral oviduct (mature) starting 21 d after eclosion (Fig. 1a), but there was a wider variability in the onset of mature ovaries, ranging from 21 to 42 d.

Adult *L. zonatus* that were field-collected at the end of September had a mix of immature, intermediate, and mature ovaries, with a majority being intermediate (Fig. 1b). The proportion of intermediate ovaries increased to 100% by the end of October. Early the following year (end of January), all dissected females had immature ovaries, which quickly changed to intermediate in February (Fig. 1b). The first mature ovaries were observed in mid-February and by mid-April 100% of dissected females had mature ovaries.

Spermatheca

Laboratory-reared mated females had significantly longer and wider sperm reservoirs than unmated females (Fig. 2). Duct length and width were not impacted by mating status (Fig. 2).

Spermatheca measurements of field-collected *L. zonatus* females showed length and width of sperm reservoirs and ducts varied significantly depending on the collection period (Fig. 3). Reservoir length showed an upward trend from fall to spring, but the differences were not significant, whereas reservoir width increased gradually from fall to spring and was significantly larger in March than in the previous October (Fig. 3). Duct length and width fluctuated more, with females collected in January having larger length and width values than in September prior, but values dropping again in spring (Fig. 3). Pronotum width as a proxy of body size was not affected by the field collection period ($\chi^2 = 6.89$; df = 5, 121; $P = 0.229$).

Oviposition

Of the total 250 field-collected *L. zonatus* females, 22% laid eggs under summer (=rearing conditions with 26 °C and long day photoperiod) conditions. However, only 8% produced offspring, showing that many females laid unfertilized eggs. All females producing fertilized eggs started laying them within 49 days of collection from

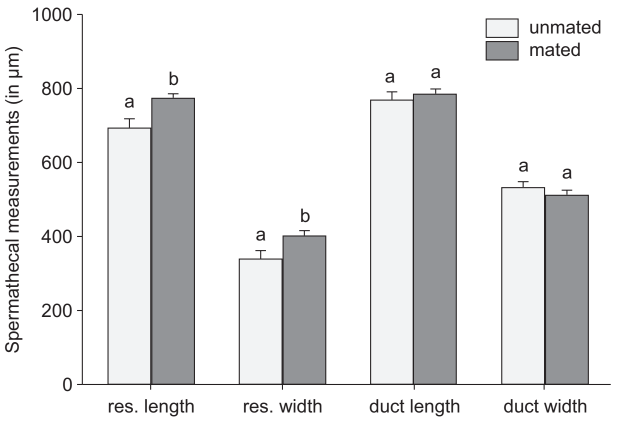

Fig. 2. Spermathecal measurements of reservoir (res.) length and width and duct length and width for laboratory-reared unmated (n = 21) and mated (n = 30) *L. zonatus*. Values were significantly different for reservoir length (χ^2 = 12.12; df = 1, 50; $P < 0.001$) and width (χ^2 = 7.18; df = 1, 50; $P = 0.007$) but were not different for duct length (χ^2 = 0.423; df = 1, 50; $P = 0.515$) and width (χ^2 = 1.13; df = 1, 50; $P = 0.287$). Within each measurement, different letters above the bars indicate a significant difference (Tukey's pairwise comparison, $P < 0.05$).

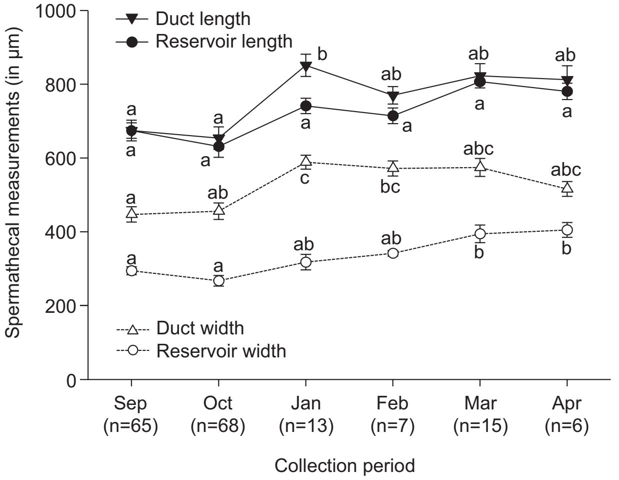

Fig. 3. Spermathecal measurements of reservoir length and width and duct length and width of *L. zonatus* field-collected at different times during the overwintering period. For each measurement, values were significantly different within each collection period for reservoir width (χ^2 = 5.023; df = 5, 149; $P < 0.001$), and duct width (χ^2 = 4.576; df = 5, 145; $P = 0.001$) and length (χ^2 = 5.760; df = 5, 146; $P < 0.001$), but were not different for reservoir length (χ^2 = 2.156; df = 5, 149; $P = 0.062$). Within each measurement, different letters above the bars indicate a significant difference (Tukey's pairwise comparison, $P < 0.05$).

the field. Deprived of a male to mate with, some unmated *L. zonatus* started ovipositing unfertilized eggs from 50 to 150 d after collection.

Offspring were produced consistently from eggs deposited by field-collected *L. zonatus* females in early February 2021 and early March (2022) with 18% of all collected spring females producing offspring (n = 41), after being brought to the laboratory and held under rearing conditions. An average of 11% (0–50% range) of control females (n = 245) randomly taken from the colony at the same times as the field collections produced offspring overall. In 2022, the only year with field sampling throughout the winter, a small proportion of females produced offspring in late November (5%, n = 20) and early January (10%, n = 10).

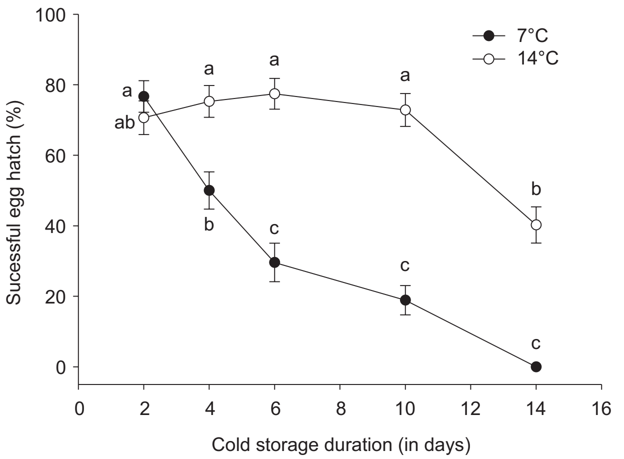

Fig. 4. Percentage *L. zonatus* egg hatch was impacted by different cold storage durations (in days) at temperatures of 7 °C (χ^2 = 28.883; df = 4, 428; $P < 0.001$) and 14 °C (χ^2 = 3.383; df = 4, 458; $P = 0.010$). Within each temperature, different letters indicate significant differences among cold storage periods (Tukey's multiple comparisons).

Egg Hatch

At a constant temperature, there was a significant impact on egg hatch (χ^2 = 808.08; df = 3, 1,614; $P < 0.001$), with no eggs hatching at 2 °C, some nymphs hatching from eggs stored at 7 °C (35.3 ± 2.3%), a significantly greater percentage hatch at 14 °C (67.3 ± 2.2%), and in the control at 21 °C (80.6 ± 6.7%).

When eggs were held for different durations at 7 °C and 14 °C, egg hatch was impacted by storage duration (Fig. 4); however the impact of storage duration was more pronounced at 7 °C where there was a significant impact at 2, 4, and 6 d storage, then at 14 °C where there was only a clear reduction in egg hatch at 14 d storage (Fig. 4). Egg age at the beginning of cold storage presented no clear pattern, while there were differences at 7 °C (χ^2 = 11.531; df = 2, 429; $P < 0.001$) and 14 °C (3.331; df = 2, 459; $P = 0.037$), at 7 °C the older (6–8 d) eggs had the highest egg hatch (fresh: 34.2 ± 3.8%, medium 22.5 ± 3.5%, old 49.3 ± 4.2%), whereas at 14 °C there was less treatment impact (fresh: 64.4 ± 3.9%, medium 75.3 ± 3.5%, old 62.6 ± 3.8%) and medium aged (2–3 d) eggs had a higher egg hatch than the older eggs. Moreover, across both temperatures there was an impact of storage duration (χ^2 = 15.169; df = 4, 880; $P < 0.001$) but not of egg age (χ^2 = 1.282; df = 2, 880; $P = 0.278$) and there was no storage duration × egg age interaction (χ^2 = 1.381; df = 8, 880; $P = 0.201$).

Discussion

With dissections of laboratory-reared specimens, we established a baseline for *L. zonatus* ovary development. In accordance with the large variation in pre-mating duration of *L. zonatus* reported by Matrangolo and Waquil (1994), our findings show a range of durations from eclosion to the development of mature ovaries. Since we were dissecting unmated females, the lack of mating could also, after time, have stimulated the resorption of oocytes as has been shown for *Nezara viridula* (L.) (Hemiptera: Pentatomidae) (Fortes et al. 2011). Egg resorption, a common behavior of insects under stress conditions (e.g., Fletcher 1975, Santolamazza Carbone et al. 2008), is also the most likely explanation for the presence of only intermediate ovaries going into the overwintering period and the dominance of immature ovaries in January in field-collected females. *Leptoglossus zonatus* oviposition in Central California starts around mid-April

(Daane et al. 2019), which is when all field-collected females had mature ovaries. Egg resorption might also help explain the long-lived overwintering adults from as early as September and well into April the following year (KMD, personal observation). Overall, this corroborates data collected by Tollerup (2019) who started seeing mature eggs in dissected females beginning late February.

Leptoglossus zonatus overwinters in aggregations, which provides an easy opportunity for mate finding. This opportunity is used by several species (Holmberg et al. 1984, Wells et al. 1990, Susset et al. 2018), but for *L. zonatus* it was assumed that they mate only after leaving their overwintering habitat in spring, as has been shown for *Halyomorpha halys* (Stål) (Nielsen et al. 2017). This assumption is based largely on observations of *L. zonatus* copulating in spring after dispersing from overwintering aggregations, and that the aggregations have other beneficial consequences, such as improved microclimate (Yoder et al. 1992, Klok and Chown 1999) and protection from predators (Holmberg et al. 1984, Vulinec 1990).

In a first attempt to establish if *L. zonatus* mate during their overwintering period, we determined that the size of the spermathecal reservoir is larger in mated than in unmated females. Based on similar results for *Apis mellifera* L. (Hymenoptera: Apidae), this could be due to the thickening of epithelial cells of the reservoir, potentially to protect the sperm until it is needed for fertilization (Poole 1970, Dallai 1975). Translating this knowledge to field-collected specimens, we found that reservoir length increased gradually over the winter, but that only reservoirs of *L. zonatus* in their overwintering habitat in March are significantly longer than those in the previous October. This matches with field observations of caged *L. zonatus* mating starting to occur after overwintering in late March (Daane et al. 2019). However, since the *L. zonatus* females were collected in their overwintering habitat, and the reservoir length increase is independent of *L. zonatus* body size (measured by pronotum width of field-collected specimens) and age (measured on laboratory-reared females), it indicates possible mating events during the winter or before leaving the overwintering site. Sperm can be stored in an insect's spermatheca for weeks to years (Keller 1998), so mating while mates are still close could be a suitable strategy for oviposition starting in April (Daane et al. 2019). Mating in the overwintering site may be relatively rare, while examples are found in the ladybird *Hippodamia undecimnotata* (Schneider) (Susset et al. 2018) and the butterfly *Euploea core corinna* (Macleay) (Kitching et al. 1981), although we could find no examples for other coreids.

Further evidence for some winter mating is presented by a small proportion of overwintering females, field-collected, and isolated under laboratory simulated summer conditions, which produced viable eggs. Afterwards, in the 2 years of collected field data for this experiment, mated females were present in the population starting either in early February (2021) or a month later in early March (2022). Those interannual differences are most likely due to the weather, with January and February being on average 1–1.5 °C warmer in 2021 than in 2022 (CIMIS 2022).

After mated females with mature eggs have oviposited, the embryos have to develop to be able to hatch as nymphs. We found that temperature significantly impacts *L. zonatus* egg hatch, with 2 °C preventing egg hatch, and negative impacts of 7 and 14 °C on hatching. The longer the eggs were exposed to low temperatures, the lower was the egg hatch. Interestingly, the age of the eggs exposed to the low temperatures mattered as well. In California's Central Valley, spring and fall are mild, with temperatures consistently above 7 °C from March onwards and until November for the last 3 years (CIMIS 2022). Thus, temperature does not seem to be the limiting factor for *L. zonatus* oviposition. Like most insect species overwintering as adults, photoperiod could be a main cue to enter and leave the overwintering state for *L. zonatus* (Tauber and Tauber 1976, McDougall et al. 2021).

Data presented here can have various applications. The results on impacts of low temperatures on *L. zonatus* egg hatch can be used for biological control purposes. For example, egg parasitoids can often be easier reared on killed than on fresh host eggs, and those eggs can be used for field studies as sentinel eggs or to mass produce egg parasitoids for field releases (Wong et al. 2021a). For *L. zonatus*, the egg parasitoid *Hadronotus pennsilvanicus*, formerly known as *Gryon pennsilvanicum* (Ashmead), is being considered as a biological control agent (Straser et al. 2022), and studies have looked into mass rearing this species (Sabbatini Peverieri et al. 2013). The impact of even relative low temperatures (7 °C) on egg hatch might partially explain the delay in oviposition with mature ovaries recorded in February and March (Fig. 1) but oviposition reported to begin mid-March and peak in April (Daane et al. 2019).

Apart from biological control, other methods are under consideration to improve *L. zonatus* pest management and monitoring. Reducing their migration into orchards after overwintering in spring or using that window for an early-warning monitoring system is a possible approach, which requires attractive cues and trapping mechanisms. Panel traps have been identified as effective for *L. zonatus* (Wilson et al. 2020), and efforts to synthesize a pheromone lure are underway (Millar et al. 2022). If sex pheromones lures are developed for *L. zonatus*, it will be critical to understand if females mated in overwintering aggregations will still respond to pheromone traps in spring after leaving the aggregation – the most critical period to determine their presence in the orchard. Moreover, since a proportion of *L. zonatus* seem to mate before leaving their overwintering sites to forage, in addition to conspecific signals like pheromones, they might also be attracted to host plant cues. Adding host plant cues to pheromone lures has proven to increase trap catches for other insect pests (Gries et al. 1994, Deglow and Borden 1998, Wong et al. 2021b) and could be a viable option. Studying the reproductive biology of insect pest species gives us valuable information on population dynamics and can be a useful way to contribute to the development of monitoring and management tools, something that is still needed for *L. zonatus*.

Acknowledgments

The authors would like to thank Sunny Yang, Garrett Morales, Muey Saechao, Thomas Martin, Michael Lopez, and Thaliana Samarin for assistance with field collections and laboratory setups, growers and managers for the use of their orchards, and Dr. Jose Eduardo Serrao, Dr. Edmilson Souza, and Dr. Kris Tollerup for their advice. This project received funding from the California Pistachio Research Board and is gratefully acknowledged.

Author Contributions

Judith Stahl (Conceptualization-Lead, Data curation-Lead, Formal analysis-Lead, Funding acquisition-Equal, Investigation-Equal, Methodology-Lead, Project administration-Equal, Visualization-Equal, Writing – original draft-Lead), Kent Daane (Conceptualization-Supporting, Funding acquisition-Supporting, Methodology-Supporting, Project administration-Lead, Resources-Equal, Supervision-Lead, Writing – review & editing-Equal)

References

Allen RC. A revision of the genus *Leptoglossus* Guerin (Hemiptera: Coreidae). *Entomol Am*. **1969**:45:35–140.

Buss L, Halbert S, Johnson S. *Leptoglossus zonatus*—a new leaf-footed bug in Florida (Hemiptera: Coreidae). FDACS-DPI Pest Alerts. **2005** [accessed 2022 Dec 8]. http://www.doacs.state.fl.us/pi/enpp/ento/l.zonatus.html and https://www.fdacs.gov/content/download/68507/file/Pest_Alert_-_Leptoglossus_zonatus,_A_New_Leaffooted_Bug_in_Florida.pdf.

Chiang RG. Functional anatomy of the vagina muscles in the adult western conifer seed bug, *Leptoglossus occidentalis* (Heteroptera: Coreidae), and its implication for the egg laying behaviour in insects. *Arthropod Struct Develop*. **2010**:39:261–267. https://doi.org/10.1016/j.asd.2010.02.004

CIMIS (California irrigation management information system). Station 80. **2022** [accessed 2022 Dec 1]. https://cimis.water.ca.gov/WSNReportCriteria.aspx

Daane KM, Yokota GY, Bentley WJ, Weinberger G, Millar JG, Beede RH. In: Ferguson L, Haviland DR, editors. *Stink bugs and leaffooted bugs, Pistachio Production Manual*. Oakland (CA): University of California, Agricul. Natur. Resources Pub. 3545; **2016**. p. 225–238.

Daane KM, Yokota GY, Wilson H. Seasonal dynamics of the leaffooted bug *Leptoglossus zonatus* and its implications for control in almonds and pistachios. *Insects*. **2019**:10(8):255–266. https://doi.org/10.3390/insects10080255

Dallai R. Fine structure of the spermatheca of *Apis mellifera*. *J Insect Physiol*. **1975**:21(1):89–109. https://doi.org/10.1016/0022-1910(75)90072-4

Dallas WS. List of the specimens of hemipterous insects in the collection of the British Museum 2. *Trustees British Mus*. **1852**:2:369–592.

Deglow EK, Borden JH. Green leaf volatiles disrupt and enhance response to aggregation pheromones by the ambrosia beetle, *Gnathotrichus sulcatus* (Coleoptera: Scolytidae). *Can J Res*. **1998**:28:1697–1705. https://doi.org/10.1139/x98-143

Dreyer H, Baumgärtner J. Temperature influence on cohort parameters and demographic characteristics of the two cowpea coreids *Clavigralla tomentosicollis* and *C. shadabi*. *Ent Exp Appl*. **1996**:78:201–213. https://doi.org/10.1111/j.1570-7458.1996.tb00783.x

Fletcher BS. Temperature-regulated changes in the ovaries of overwintering females of the Queensland Fruit Fly, *Dacus tryoni*. *Aust J Zool*. **1975**:23:91–102. https://doi.org/10.1071/ZO9750091

Fortes P, Salvador G, Consoli FL. Ovary development and maturation in *Nezara viridula* (L.) (Hemiptera: Pentatomidae). *Neotropical Entomol*. **2011**:40:89–96. https://doi.org/10.1590/S1519-566X2011000100013

Fox J, Weisberg S, Adler D, Bates D, Baud-Bovy G, Ellison S, Firth D, Friendly M, Gorjanc G, Graves S. Package 'car'. R package version. **2016** [accessed 2022 Feb 26]. https://CRAN.R-project.org/package=car.

Franco-Archundia SL, Gonzaga-Segura AJ, Jiménez-Pérez A, Castrejón-Gómez VR. Behavioral response of *Leptoglossus zonatus* (Heteroptera: Coreidae) to stimuli based on colors and its aggregation pheromone. *Insects*. **2018**:9(3):91–113. https://doi.org/10.3390/insects9030091

Graves SH, Piepho P, Selzer L. Package 'multcompView'—visualizations of paired comparisons. **2015** [accessed 2022 Jun 30]. https://cran.stat.unipd.it/web/packages/multcompView/ multcompView.pdf.

Gries G, Gries R, Perez AL, Gonzales LM, Pierce HD, Oehlschlager AC, Rhainds M, Zebeyou M, Kouame B. Ethyl propionate: synergistic kairomone for African palm weevil, *Rhynchophorus phoenicis* L. (Coleoptera: Curculionidae). *J Chem Ecol*. **1994**:20:889–897.https://doi.org/10.1007/BF02059585

Heidemann O. New species of *Leptoglossus* from North America. *Proc Entomol Soc Wash*. **1910**:12:191–197.

Henne DC, Johnson SJ, Bourgeois WJ. Pest status of leaf-footed bugs (Hemiptera: Coreidae) on citrus in Louisiana. *Proc Ann Meet Fla Sta Hortic Soc*. **2003**:116:240–241.

Holmberg RG, Angerilli NP, LaCasse LJ. Overwintering aggregations of *Leiobunum paessleri* in caves and mines (Arachnida, Opiliones). *J Arachnol*. **1984**:12:195–204. https://www.jstor.org/stable/3705173.

Hothorn T, Bretz F, Westfall P, Heiberger RM, Schuetzenmeister A, Scheibe S. Package 'multcomp'. Simultaneous inference in general parametric models. R package version 1.4–8. **2017** [accessed 2022 Feb 26]. http://multcomp.R-forge.R-project.org.

Inoue KM, Vidal DM, Saad EB, Martins CBC, Zarbin PHG. Identification of the alarm and sex pheromones of the leaf-footed bugs *Leptoglossus zonatus*. *J Braz Chem Soc*. **2019**:30:939–947. https://doi.org/10.21577/0103-5053.20180238

Jackai LEN, Ndlovu TM. Screen cage and laboratory evaluation of the efficacy of six foliar insecticides on different stages of the cowpea coreid bug, *Clavigralla tomentosicollis*, Stal. (Hemiptera: Coreidae). *Int J Trop Insect Sci*. **1988**:9(02):223–228. https://doi.org/10.1017/s1742758400006020

Joyce AL, Higbee BS, Haviland DR, Brailovsky H. Genetic variability of two leaffooted bugs, *Leptoglossus clypealis* and *Leptoglossus zonatus* (Hemiptera: Coreidae) in the Central Valley of California. *J Econ Entomol*. **2017**:110(6):2576–2589. https://doi.org/10.1093/jee/tox222

Joyce AL, Parolini H, Brailovsky H. Distribution of two strains of *Leptoglossus zonatus* (Dallas) (Hemiptera: Coreidae) in the Western hemisphere: is *L. zonatus* a potential invasive species in California? *Insects*. **2021**:12(12):1094–1106. https://doi.org/10.3390/insects12121094

Keller L. Queen lifespan and colony characteristics in ants and termites. *Insectes Soc*. **1998**:45(3):235–246. https://doi.org/10.1007/s000400050084

Kitching RL, Zalucki MP. Observations on the ecology of Euploea core corinna (Nymphalidae) with special reference to an overwintering population. *J Lep Soc*. **1981**:35:106–119.

Klok CJ, Chown SL. Assessing the benefits of aggregation: thermal biology and water relations of anomalous Emperor Moth caterpillars. *Funct Ecol*. **1999**:13(3):417–427. https://doi.org/10.1046/j.1365-2435.1999.00324.x

Lenth R, Singmann H, Love J. Emmeans: estimated marginal means, aka least-squares means. R package version 1, no. 1. **2018** [accessed 2022 Jun 30]. https://cran.microsoft.com/snapshot/2018-01-13/web/packages/emmeans/emmeans.pdf.

Matrangolo WJR, Waquil JM. Biologia de *Leptoglossus zonatus* (Dallas, 1852) (Hemiptera: Coreidae) alimentados com milho e sorgo. *Ann Soc Entomol Brasil*. **1994**:23:419–423.

McDougall RN, Ogburn EC, Walgenbach JF, Nielsen AL. Diapause termination in invasive populations of the brown marmorated stink bug (Hemiptera: Pentatomidae) in response to photoperiod. *Environ Entomol*. **2021**:50(6):1400–1406. https://doi.org/10.1093/ee/nvab089

Michailides TJ, Morgan DP. Association of *Botryosphaeria panicle* and shoot blight of pistachio with injuries of fruit caused by Hemiptera insects and birds. *Plant Dis*. **2016**:100(7):1405–1413. https://doi.org/10.1094/PDIS-09-15-1077-RE

Millar JG, Zou Y, Hall DR, Halloran S, Pajares JA, Ponce-Herrero L, Shates T, Wilson H, Daane KM. Identification and synthesis of leptotriene, a unique sesquiterpene hydrocarbon from males of the leaffooted bugs *Leptoglossus zonatus* and *L. occidentalis*. *J Nat Prod*. **2022**:85(8):2062–2070. https://doi.org/10.1021/acs.jnatprod.2c00470

Nielsen AL, Fleischer S, Hamilton GC, Hancock T, Krawczyk G, Lee JC, Ogburn E, Pote JM, Raudenbush A, Rucker A, *et al*. Phenology of brown marmorated stink bug described using female reproductive development. *Ecol Evol*. **2017**:7(17):6680–6690. https://doi.org/10.1002/ece3.3125

Pascini TV, Martins GF. The insect spermatheca: an overview. *Zoology*. **2017**:121:56–71. https://doi.org/10.1016/j.zool.2016.12.001

Poole HK. The wall structure of the honey bee spermatheca with comments about its function. *Ann Entomol Soc Am*. **1970**:63(6):1625–1628. https://doi.org/10.1093/aesa/63.6.1625

R Core Team. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing; **2020** [accessed 2022 Feb 26]. https://www.R-project.org/.

Raga AC, Piza J, Souza Filho MF. Ocorrencia e danos de *Leptoglossus zonatus* (Dallas) (Heteroptera: Coreidae) em Roma, *Punica granatum* L., em Campinas, Sao Paulo. *An Soc Entomol Bras*. **1995**:24:183–185. https://doi.org/10.1590/S0301-80591999000400021

Rodrigues ARS, Serrao JE, Teixeira VW, Torres JB, Teixeira AA. Spermatogenesis, changes in reproductive structures, and time constraint associated with insemination in *Podisus nigrispinus*. *J Insect Physiol*. **2008**:54:1543–1551. https://doi.org/doi.10.1016/j.jinsphys.2008.09.003

Rodrigues Netto SM, Guilhem DJ. Ocorrencias de *Leptoglossus zonatus* (Dallas, 1852) (Hemiptera: Coreidae) em maracujazeiro (*Passiflora edulis f. flavicarpa*). *Arq Inst Biol.* **1996**:63:85–86.

RStudio_Team. *RStudio: integrated development for R*. Boston (MA): RStudio, Inc.; **2016** [accessed 2022 Feb 26]. http://www.rstudio.com/.

Sabbatini Peverieri G, Furlan P, Benassai D, Caradonna S, Strong WB, Roversi PF. Host egg age of *Leptoglossus occidentalis* (Heteroptera, Coreidae) and parasitism by *Gryon pennsylvanicum* (Hymenoptera, Platygastridae). *J Econ Entomol.* **2013**:106:633–640. https://doi.org/10.1603/EC12344

Santolamazza Carbone S, Pestana Nieto M, Cordero Rivera A. Egg resorption behaviour by the solitary egg parasitoid *Anaphes nitens* under natural conditions. *Ent Exp Appl.* **2008**:127:191–198. https://doi.org/10.1111/j.1570-7458.2008.00699.x

Sawazaki E, Rossetto CJ, Fantini GM, Petinelli A Jr. *Leptoglossus zonatus* (Dallas, 1852) nova praga do milho. *Arq Inst Biol.* **1989**:56:22–31.

Souza EA, Lisboa LCO, Araújo VA, Serrão JE. Morphology of the spermathecae of *Leptoglossus zonatus* (Heteroptera: Coreidae). *Ann Entomol Soc Am.* **2016**:109:106–111. https://doi.org/10.1093/aesa/sav097

Stahl JM, Scaccini D, Pozzebon A, Daane KM. Comparing the feeding damage of the invasive brown marmorated stink bug to a native stink bug and leaffooted bug on California pistachios. *Insects.* **2020**:11(10):688–701. https://doi.org/10.3390/insects11100688

Stahl JM, Wilson H, Straser RK, Maccaro JJ, Daane KM. Irrigated trap crops impact key hemipteran pests in organic pistachio orchard. *Arthropod-Plant Interact.* **2021**:15:949–959. https://doi.org/10.1007/s11829-021-09869-7

Straser RK, Daane KM, Talamas E, Wilson H. Evaluation of egg parasitoid *Hadronotus pennsylvanicus* as a prospective biocontrol agent of the leaffooted bug *Leptoglossus zonatus*. *Biocontrol.* **2022**:67(2):123–133. https://doi.org/10.1007/s10526-022-10131-z

Susset EC, Hemptinne JL, Danchin E, Magro A. Overwintering aggregations are part of *Hippodamia undecimnotata*'s (Coleoptera: Coccinellidae) mating system. *PLoS One.* **2018**:13(6):e0197108–e0197123. https://doi.org/10.1371/journal.pone.0197108

Taszakowski A, Masłowski A, Daane KM, Brożek J. Closer view of antennal sensory organs of two *Leptoglossus* species (Insecta: Hemiptera: Coreidae). *Sci Rep.* **2023**. https://doi.org/10.1038/s41598-023-27837-4

Tauber MJ, Tauber CA. Insect seasonality: diapause maintenance, termination, and postdiapause. *Ann Rev Entomol.* **1976**:21:81–107. https://doi.org/10.1146/annurev.en.21.010176.000501

Tollerup KE. Cold tolerance and population dynamics of *Leptoglossus zonatus* (Hemiptera: Coreidae). *Insects.* **2019**:10(10):351–361. https://doi.org/10.3390/insects10100351

Vulinec, K. Collective security: aggregation by insects as a defense. In: Evans DL, Schmidt JO, editors. *Insect defenses: adaptive mechanisms and strategies of prey and predators*. Albany (NY): State University of New York Press; **1990**. p. 251–288.

Wells H, Wells PH, Cook P. The importance of overwinter aggregation for reproductive success of monarch butterflies (*Danaus plexippus* L.). *J Theor Biol.* **1990**:147(1):115–131. https://doi.org/10.1016/s0022-5193(05)80255-3

Wilson H, Maccaro JJ, Daane KM. Optimizing trap characteristics to monitor the leaffooted bug *Leptoglossus zonatus* (Heteroptera: Coreidae) in orchards. *Insects.* **2020**:11(6):358–367. https://doi.org/10.3390/insects11060358

Wong WH, Walz MA, Oscienny AB, Sherwood JL, Abram PK. An effective cold storage method for stockpiling *Halyomorpha halys* (Hemiptera: Pentatomidae) eggs for field surveys and laboratory rearing of *Trissolcus japonicus* (Hymenoptera: Scelionidae). *J Econ Entomol.* **2021a**:114:571–581. https://doi.org/ 10.1093/jee/toaa307

Wong WHL, Gries RM, Abram PK, Alamsetti SK, Gries G. Attraction of brown marmorated stink bugs, *Halyomorpha halys*, to blooming sunflower semiochemicals. *J Chem Ecol.* **2021b**:47(7):614–627. https://doi.org/10.1007/s10886-021-01281-y

Yoder JA, Denlinger DL, Wolda H. Aggregation promotes water conservation during in the tropical fungus beetle, *Stenotarsus rotundus*. *Ent Exp Appl.* **1992**:63:203–205. https://doi.org/10.1111/j.1570-7458.1992.tb01574.x