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Studies on Egg Parasitoids of *Homalodisca vitripennis* (Hemiptera: Cicadellidae): Biology, Sex Ratio Dynamics, and Distribution Across Southern California

A Dissertation submitted in partial satisfaction
of the requirements for the degree of

Doctor of Philosophy

in

Entomology

by

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March 2012

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ABSTRACT OF THE DISSERTATION

Studies on Egg Parasitoids of *Homalodisca vitripennis* (Germar): Biology, Sex Ratio Dynamics, and Distribution Across Southern California

by

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Doctor of Philosophy, Graduate Program in Entomology
University of California, Riverside, March 2012
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Various aspects of the biology, sex ratio allocation, and host specificity of several species of egg parasitoids of the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar), were studied. The distribution and prevalence of egg parasitoids of GWSS were investigated at six field sites over two years. Most of the observed parasitism was attributed to two species: *Gonatocerus ashmeadi* Girault and *Gonatocerus walkerjonesi* Triapitsyn, with the former producing most of the parasitism in interior southern California and the latter being predominant at coastal sites.

Biological traits of *Gonatocerus deleoni* Triapitsyn, Logarzo & Virla and *Pseudoligosita plebeia* (Perkins), two potential candidates for biological control of GWSS, were investigated. The average parasitism rate of *Gonatocerus deleoni* on 1–8-day-old eggs was 45.7% but this was significantly affected by the age of the egg, ranging from 1.4% to 69.9%. *G. deleoni* was able to develop in eggs of

GWSS and *Homalodisca liturata* Ball, but was unable to develop on eggs of *Graphocephala atropunctata* (Signoret) or *Erythroneura elegantula* Osborn. When provided honey and water, water alone, or no food or water, *P. plebeia* adult females lived an average of 64.1, 2.3, and 2.0 days, respectively. *Pseudoligosita plebeia* were able to successfully parasitize GWSS eggs (1-8 days old), with higher parasitism in young host eggs (1-3 days old) than in old host eggs (5-7 days old). An increasing trend in offspring production was seen for *P. plebeia* from adult age 2 to 26 days followed by a decreasing trend with offspring produced up to age 75. *Pseudoligosita plebeia* contained fewer mature eggs at younger ages (1 and 3 days old) than at older ages (5, 11, 15, and 31 days old).

I examined whether *G. ashmeadi* produces precise sex ratios under a field setting. My analyses showed field collected *G. ashmeadi* tended to produce less female biased sex ratios with higher variance in male numbers than were shown in laboratory studies. We found significant effects of proportion parasitism and host density on sex ratio, while proportion parasitism had a significant effect on sex ratio variance.

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Introduction

Homalodisca vitripennis (Germar) (Hemiptera: Cicadellidae), known as the glassy-winged sharpshooter (GWSS), is a xylem fluid-feeding leafhopper native to the southeastern U.S. and northeastern Mexico (Triapitsyn and Phillips 2000). It first was detected in California in the city of Irvine in 1990, probably arriving via egg masses on transported nursery stock (Sorensen and Gill 1996). Since its arrival in California, the distribution of GWSS has increased, and it now is established in most of southern California as well as areas in the San Joaquin Valley. In its new range, GWSS has become a significant economic pest of numerous crop and ornamental plants, principally due to its ability to transmit the xylem limited bacteria, *Xylella fastidiosa* Wells et al. (Blua et al. 1999). Strains of *X. fastidiosa* cause diseases of several crops and ornamentals, including Pierce's disease of grapes, oleander leaf scorch, almond leaf scorch, pear leaf scorch, plum leaf scald, citrus variegated chlorosis, phony peach disease, and alfalfa dwarf disease (Raju et al. 1980, Chang et al. 1993, Leu and Su 1993, Purcell and Saunders 1999). Currently, there is no cure available for the diseases caused by *X. fastidiosa*, and the only treatments in use are vector control and removal of infected plant material.

Of the crops affected by *X. fastidiosa*, grapes are of particular concern, as they are one of California's most economically important crops, with a total growing region of 3,280.6 km² and an annual net value of US$4.4 billion (CDFA

2010). Pierce's disease was present in California well before the arrival of GWSS, primarily being vectored to grapes by the blue-green sharpshooter, *Graphocephala atropunctata* (Signoret), in coastal regions, and by the green sharpshooter, *Draeculacephala minerva* Ball, and red-headed sharpshooter, *Xyphon fulgida* Nottingham, in the San Joaquin Valley (Goodwin and Purcell 1992). These cicadellid vectors are found primarily associated with preferred feeding habitats near riparian systems and irrigated areas and thus, Pierce's disease had a concentrated distribution near these areas prior to the introduction of GWSS (Freitag and Frazier 1953). Due to the predicted at-risk areas, Pierce's disease was managed by insecticide applications on surrounding natural vegetation and avoidance of planting near riparian systems (Goodwin and Purcell 1992).

The use of parasitoids to control GWSS is part of the current strategy to reduce Pierce's disease spread and is of particular importance in urban areas and farmland under integrated pest management or organic pest control strategies (Hix et al. 2003). Biological control of citrus pests can be disrupted by insecticide applications (Grafton-Cardwell and Gu 2003), but chemical control of GWSS is of particular importance on citrus because it is one of the most abundant overwintering and reproductive hosts for GWSS in southern California (Blua et al. 1999). To date, most GWSS biological control research has focused on egg parasitoids. *Homalodisca vitripennis* oviposits its eggs as conspicuous egg masses, usually laid on the underside of leaves. Glassy-winged

sharpshooters typically have two broods per year, one in late spring and the second in late summer, with an occasional third brood in early winter. To reduce densities of GWSS, the California Department of Food and Agriculture began a classical biological control program in 2001. Part of their efforts includes releasing several species of parasitoids to augment existing field populations. To date, released species include *Gonatocerus ashmeadi* Girault, *Gonatocerus morrilli* (Howard), *Gonatocerus walkerjonesi* Triapitsyn (previously confused with *Gonatocerus morrilli*), *Gonatocerus triguttatus* Girault, *Gonatocerus fasciatus* Girault, *Gonatocerus morgani* Triapitsyn, and *Anagrus epos* Girault. *Gonatocerus ashmeadi* has been found in California since 1978 (Huber 1988) and likely was an accidental introduction, subsisting on eggs of the native smoketree sharpshooter, *H. liturata* (Ball) (Vickerman et al. 2004). *Gonatocerus walkerjonesi* was recovered from *H. liturata* prior to the introduction of GWSS into California (de León et al. 2006). *Gonatocerus fasciatus* was mass reared and released in the state beginning in 2002 (CDFA 2003). *Gonatocerus triguttatus* was imported from eastern Texas and released in California beginning in 2001 (Irvin and Hoddle 2005). *Ufens principalis* Owen and *Ufens ceratus* Owen are endemic to California and are typically found near plants native to southern California, especially those harboring *H. liturata* (Al-Wahaibi and Morse 2010). All species of *Gonatocerus* found in California to date are solitary parasitoids, with the exception of *G. fasciatus*, which can produce up to two parasitoids per

H. vitripennis egg. *Ufens* species are gregarious and up to nine wasps can emerge from a single GWSS egg (Al-Wahaibi and Morse 2010).

Gonatocerus deleoni Triapitsyn, Logarzo & Virla and *Pseudoligosita plebeia* (Perkins) are two species of parasitoids that have been imported into UCR's Quarantine Facility for evaluation and potential future release in California. *Gonatocerus deleoni* first was reared from sentinel eggs of *Tapajosa rubromarginata* (Signoret) from the Mendoza Province of Argentina and *P. plebeia* first was reared from field collected *H. liturata* eggs from Sonora, Mexico (Triapitsyn and Bernal 2009). While its natural host range is unknown, field studies showed that *G. deleoni* parasitizes only eggs of *T. rubromarginata* in its native range and would not attack eggs of various Cicadellini endemic to that area (Triapitsyn et al. 2008).

While serious non-target effects of introduced parasitoids are rare over the past 45 years, ensuring a high level of host discrimination for introduced biological control agents is still considered highly important (Hoddle and Syrett 2002). Understanding the host specificity of an introduced parasitoid can reduce the likelihood of it attacking and developing on unexpected non-target hosts. *Homalodisca liturata* is the closest related species to GWSS in California, and is placed in the same genus (tribe Proconiini of the subfamily Cicadellinae). *Graphocephala atropunctata* is a more distantly related sharpshooter in the tribe Cicadellini of the Cicadellinae. *Erythroneura elegantula* Osborn is in the tribe

Erythroneurini of the subfamily Typhlocybinae and is even more distantly related to GWSS. Among the Proconiini, only *H. vitripennis*, *H. liturata*, and several seldom collected species in the genus *Cuerna* exist in California.

Another important aspect of parasitoid biology is sex ratio allocation. Parasitoids are particularly well suited for sex ratio allocation studies due to their use of haplo-diploid sex determination (Flanders 1965, Godfray 1994). This sex determination scheme enables the ovipositing female to determine the sex of her offspring by either fertilizing an egg from stored sperm from previous matings to produce a daughter or forgoing fertilization to produce a son (Flanders 1939). Species that parasitize hosts or patches of hosts which support the development of multiple offspring typically produce female biased sex ratios. These biased sex ratios are explained by Local Mate Competition theory (LMC) (Hamilton 1967). If a single foundress parasitizes a patch of hosts, her offspring may be more likely to mate with each other than with unrelated conspecifics. LMC suggests that female parasitoids attempt to maximize their reproductive fitness so when a single female visits a patch of hosts, her fitness increases with the number of fertilized daughters from the patch (Green et al. 1982, Hardy 1992, Hardy et al. 1998). Consequently, the foundress should oviposit just enough sons in each patch to ensure her daughters are fertilized. This maximizes female fitness, ensures sufficient males are present to mate with females, and reduces competition between brothers for sib-mating with sisters (Luck et al. 2001).

The development and application of a successful biological control program requires thorough knowledge of the biology of the pests and natural enemies in a given system. Host range and sex ratio production are among the biological aspects of parasitoids investigated in this dissertation. The research described herein was conducted at the University of California, Riverside, on endemic and exotic parasitoids of the glassy-winged sharpshooter, *Homalodisca vitripennis*. Chapter one describes a two-year field study of temporal and spatial parasitoid abundance in two climate zones in southern California (coastal versus interior southern California). Chapters two and three, respectively, are laboratory studies investigating the biological characteristics of two species of parasitoids as potential candidates for biological control of *H. vitripennis*, *Gonatocerus deleoni* and *Pseudoligosita plebia*. Chapter four investigates the sex ratio dynamics of several species of *Gonatocerus*, covering four years of field collected data (2006-2009).

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Chapter 1

Distribution of Several Species of Parasitoids of the Glassy-winged Sharpshooter (Hemiptera: Cicadellidae) in Southern California

Abstract

We investigated the distribution and prevalence of egg parasitoids of the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar 1821), at six field sites over two years. Percentage parasitism often reached 100% from June until October. Most of the observed parasitism was attributed to two species of *Gonatocerus*, *G. ashmeadi* Girault 1915 and *G. walkerjonesi* S. Triapitsyn 2006, with the former producing most of the parasitism in interior southern California and the latter being predominant at coastal sites. This is the first published study that indicates *G. walkerjonesi* may be dominant in parts of California. Other species detected at low levels were *G. novifasciatus* Girault 1911, *G. morgani* S. Triapitsyn 2006, *Ufens principalis* Owen 2005, and *U. ceratus* Owen 2005.

Introduction

The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar 1821), is native to the southeastern U.S., but entered California in the late 1980s (Sorensen & Gill 1996). It is a highly mobile vector of the bacteria *Xylella fastidiosa* Wells et al. 1987, strains of which cause scorch diseases on numerous types of plants, including oleander and grapes (Purcell & Saunders 1999).

Area-wide management programs emphasizing chemical control are used to manage GWSS populations on citrus in the vicinity of vineyards in the Temecula and Coachella Valleys of southern California and in Kern and Tulare counties of the San Joaquin Valley. To reduce densities of GWSS in other areas of the state, the California Department of Food and Agriculture began a classical biological control program in 2001. Part of their efforts includes releasing several species of parasitoids to augment existing field populations. To date, released species include *Gonatocerus ashmeadi* Girault 1915, *G. morrilli* (Howard 1908), *G. walkerjonesi* S. Triapitsyn 2006 (previously *G. morrilli*), *G. triguttatus* Girault 1916, *G. fasciatus* Girault 1911, *G. morgani* S. Triapitsyn 2006, and *Anagrus epos* Girault 1911. *Gonatocerus ashmeadi* has been found in California since 1978 (Huber 1988) and likely was an accidental introduction, subsisting on eggs of the native smoketree sharpshooter, *H. liturata* (Ball 1901) (Vickerman et al. 2004). The origin of *G. walkerjonesi* is unknown, but it may be native to southern California or possibly originated from accidental introduction from Las Flores, Masaya, Nicaragua, as specimens from both regions were found to be genetically identical (Triapitsyn 2006). *Gonatocerus walkerjonesi* was recovered from *H. liturata* prior to the introduction of GWSS into California (de León et al. 2006). *Gonatocerus fasciatus* was mass reared and released in the state beginning in 2002 (CDFA 2003). *Gonatocerus triguttatus* was imported from eastern Texas and released in California beginning in 2001 (Irvin & Hoddle 2005b). *Ufens principalis* Owen 2005 and *U. ceratus* Owen 2005 are both

endemic to California and are typically found near plants native to southern California, especially those harboring *H. liturata* (Al-Wahaibi & Morse 2010).

Homalodisca vitripennis oviposits its eggs as conspicuous egg masses, usually laid on the underside of leaves. Glassy-winged sharpshooters typically have two broods per year, one in late spring and the second in late summer, with an occasional third brood in early winter. All species of *Gonatocerus* found in California to date are solitary parasitoids, with the exception of *G. fasciatus*, which can produce up to two parasitoids per *H. vitripennis* egg. *Ufens* species are gregarious and up to nine wasps can emerge from a single GWSS egg (Al-Wahaibi & Morse 2010).

The study reported herein was initiated to determine what species of parasitoids attack glassy-winged sharpshooter populations in coastal versus interior areas of southern California.

Materials and Methods

GWSS egg mass collection. Glassy-winged sharpshooter egg masses were collected from Eureka variety lemons (*Citrus limon* Burman Fabricius [Rutaceae]) at six field sites in southern California from February, 2005 through October, 2006; three sites were located 38.6-56.3 km from the coast (hence referred to as “interior” sites) and three sites were located 8.0-12.9 km from the coast (referred to as “coastal” sites). Each collection area was located at the center of the lemon grove. We chose to collect from Eureka variety lemons

based on previous research showing this variety was a favorable host for oviposition by *H. vitripennis* that produces high levels of egg emergence compared to other citrus varieties (Al-Wahaibi 2004, Irvin & Hoddle 2004). Sites were chosen based on sharpshooter counts on yellow sticky cards placed at multiple sites as well as egg mass surveys prior to and during the experiments. The interior sites were located in Riverside (GPS location: 33.970129, 117.339327), Temecula (33.56031, -117.01449), and Corona (33.841272, -117.599201); the coastal sites were located in Irvine (33.729307, -117.784652), Mission Viejo (33.520932, -117.633405), and San Juan Capistrano (33.522099, -117.571784). Sampling did not begin until May 2005 for the Corona and Mission Viejo sites, as these were located as replacements for two sites, one each in the coastal and interior areas, where consistently low numbers of egg masses were found. Sampling at the remaining sites began in February 2005.

Two density estimates of *H. vitripennis* egg masses were gathered during each collection for each site using sampling methods similar to those employed by the sharpshooter biological control staff in the California Department of Agriculture. The first density estimate was gathered by conducting two-minute searches for sharpshooter egg masses, replicated five times, each on a different randomly selected tree at the site. These timed searches consisted of visually inspecting the underside of leaves around the entire perimeter of each tree, counting each sharpshooter egg mass that was intact. Egg masses from which sharpshooters or parasitoids had emerged were ignored. The second density

estimate was gathered by inspecting 30 randomly selected leaves, replicated ten times, each on a different tree. In this case, each emerged and unemerged sharpshooter egg mass was counted. To avoid sampling egg masses that were older than one year, the inspected leaves were chosen from the growing terminal region of branches. If fewer than 10 egg masses were collected during the density estimates, nearby trees were searched for up to an additional 60 minutes or until 10 egg masses in total were collected. Each leaf that contained an unemerged egg mass was removed from the tree and placed in a plastic bag with moist paper towels for transport to the lab for further processing, i.e. to allow any parasitoids to emerge for identification.

After the leaves containing egg masses were transported to the lab, each was gently washed in a 2% bleach solution, applied with a cotton ball, to prevent mold and fungi from growing during incubation. Leaves were then rinsed with water and placed individually in 100 mm x 15 mm Petri dishes lined with filter paper moistened with a 4% Listerine solution to further prevent mold and fungal growth as well as to keep the leaves hydrated (Irvin and Hoddle 2005a). Petri dishes were sealed with Parafilm (SPI Supplies, West Chester, PA) and kept under constant temperature and light regiments (21–24°C, 40–60% RH, and 16:8 h L:D) for 25 days. Egg masses were checked every two days for parasitoid and sharpshooter emergence; emerged individuals were identified, recorded, and stored in 95% alcohol. After *G. walkerjonesi* was determined to be a distinct species from *G. morrilli* in 2006, all specimens in question were re-examined

and identified by examination of the submedial carinae of the propodeum as described by Triapitsyn (2006). If any eggs failed to show emergence by 25 days, or if it appeared that mold or fungi threatened positive identification of the egg's contents, the egg mass was dissected and its contents were identified and recorded to the best of our ability. Decayed egg contents were sometimes unidentifiable using this methodology and if so, this was noted. Voucher specimens of all species are deposited in the UC Riverside Entomology Museum.

Results

Gonatocerus ashmeadi was the dominant parasitoid encountered at all three interior sites, with 406, 238, and 140 specimens recovered from Riverside, Temecula, and Corona, respectively (Table 1). In aggregate, 64.3%% of all parasitoids recovered in the interior groves were *G. ashmeadi* with *G. walkerjonesi* a distant second at 10.9% (Fig. 1, Table 1). Across the three coastal sites, this trend was reversed with *G. walkerjonesi* and *G. ashmeadi* representing 61.4% and 8.6% of the recovered parasitoids, respectively.

Among the three interior sites, Corona consistently had lower numbers of sharpshooter egg masses, with an average of 3.1 egg masses found per sampling date over the two years of the study (data not shown). Temecula and Riverside sites had 5.5 and 8.3 egg masses found per sampling date, respectively. Egg masses collected from Temecula produced lower rates of

parasitism for most dates, with an overall average of 48.8% parasitized eggs (calculated as total eggs parasitized divided by total eggs parasitized plus eggs producing sharpshooter nymphs). Eggs collected from Riverside and Corona showed 62.5% and 86.6% total observed parasitism, respectively. Parasitism rates were lower for all sites during the first brood of sharpshooters from February to May compared to later months, when parasitism often reached 100% (none reached 100% prior to June over the two years of the study; 18 of 28 did so after June based on samples from the three interior sites). *Gonatocerus novifasciatus* Girault 1911 was recovered occasionally from all three sites from February through April. Among the three interior sites, *Ufens* spp. were only encountered in egg masses collected in Riverside. Out of 76 total *Ufens* spp. recovered, 9 were *U. ceratus*, 44 were *U. principalis*, and the remaining 23 were unidentifiable to species but were clearly in this genus. Mold contaminants obscured our samples collected on 13 May 2005 from Corona.

Among the three coastal sites, egg masses collected from Irvine typically had lower rates of parasitism, with an average of 58.5% of all emerged eggs producing parasitoids. Mission Viejo and San Juan Capistrano had similar parasitism rates, with 84.2% and 86.4% parasitism, respectively. Unlike with interior sites, the pattern of lower parasitism rates early in the year followed by high rates during later months was not as pronounced at the coastal locations (7/21 samples indicated 100% parasitism prior to June; 20/37 did so after June). Irvine had the lowest average number of egg masses found per sampling date,

followed by Mission Viejo and San Juan Capistrano, with means of 4.2, 10.4, and 12.3 egg masses found per date, respectively. *Gonatocerus morgani* was encountered once from Irvine samples in 2005 and became more prevalent the following year at all three coastal sites. *Gonatocerus novifasciatus* were encountered occasionally at all three sites, primarily during spring months.

Discussion

Data from the three interior sites showed lower portions of GWSS eggs were parasitized during the first brood of GWSS followed by higher rates of parasitism during the second brood and this is consistent with data reported by Krugner et al. (2009). The observation that *G. ashmeadi* was the most abundant parasitoid emerging from GWSS eggs (Fig. 1A) is also supported by other GWSS egg mass surveys (Triapitsyn et al. 1998, Krugner et al. 2009, Hoddle 2010).

Gonatocerus walkerjonesi was the most abundant parasitoid detected in all three coastal sites (Fig 1B). As far as we know, this finding has not been reported elsewhere. Most of the literature regarding parasitoids of *H. vitripennis* focuses on *G. ashmeadi*, with little research conducted on *G. walkerjonesi* (de León & Morgan 2007). While our results support the notion that *G. ashmeadi* may be a highly effective biological control agent at interior sites, our data from coastal sites indicate *G. walkerjonesi* may be more effective at parasitizing sharpshooter eggs, at least at these three coastal locations. It is not clear what

factors favor the abundance of *G. walkerjonesi* in these areas, but they may include cooler temperatures from the coastal influence. Microclimatic differences also may account for the locally high numbers of *G. walkerjonesi*, as high numbers of *G. ashmeadi* emerged from egg masses collected from trees located on the edges of the same grove we collected from in Mission Viejo (JL, unpublished data). High numbers of *G. walkerjonesi* located in the interior of the grove and high numbers of *G. ashmeadi* located in the exterior margin of the grove was a trend that persisted over the four years that we collected from this site (JL, unpublished data). Due to our findings of dominance in coastal sites, we believe *G. walkerjonesi* may be an effective biological control agent of *H. vitripennis* at some locations within California and warrants further attention and study. *Gonatocerus walkerjonesi* may be of particular interest if *H. vitripennis* populations spread into the cooler climatic regions of central California around or in Napa County.

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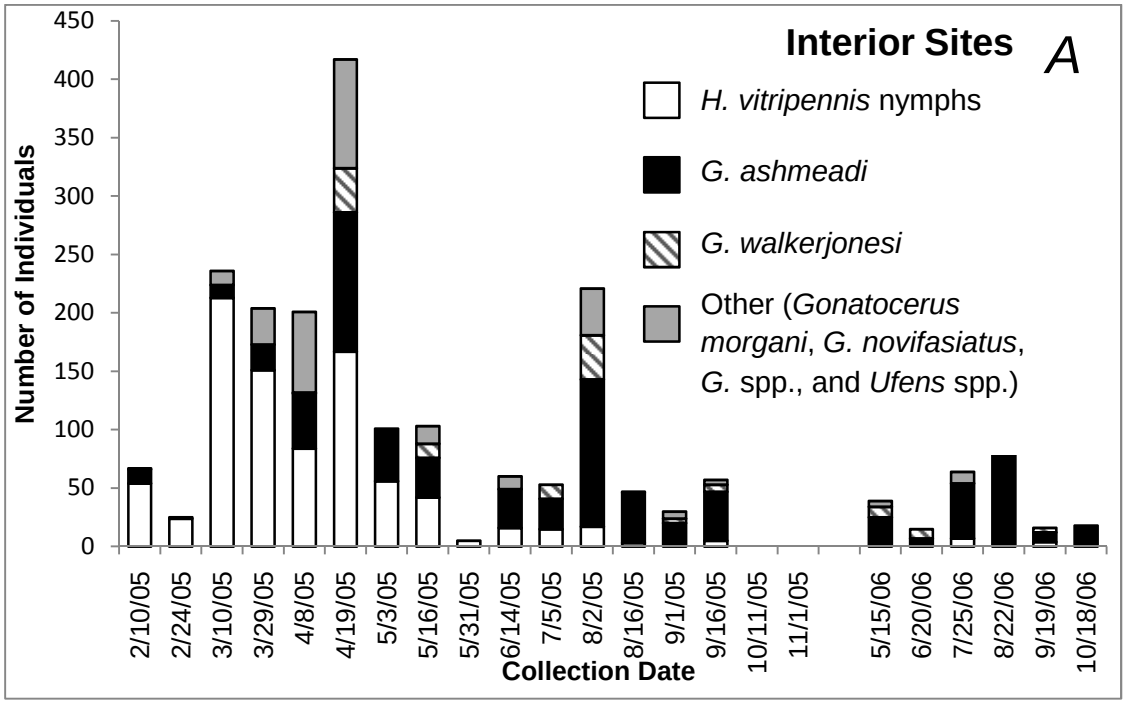
Figure 1.1. Pooled data showing the number of *Gonatocerus ashmeadi*, *G. walkerjonesi*, *H. vitripennis* nymphs, and other parasitoid species (*G. morgani*, *G. novifasciatus*, *G*. spp. [recognizable as this genus but species identify uncertain], and *Ufens* spp.) that emerged from egg masses collected from (A) interior and (B) coastal sites.


Interior Sites
A
H. vitripennis nymphs
G. ashmeadi
G. walkerjonesi
Other (Gonatocerus morgani, G. novifasiatus, G. spp., and Ufens spp.)
Number of Individuals
450
400
350
300
250
200
150
100
50
0
2/10/05
2/24/05
3/10/05
3/29/05
4/8/05
4/19/05
5/3/05
5/16/05
5/31/05
6/14/05
7/5/05
8/2/05
8/16/05
9/1/05
9/16/05
10/11/05
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5/15/06
6/20/06
7/25/06
8/22/06
9/19/06
10/18/06
Collection Date

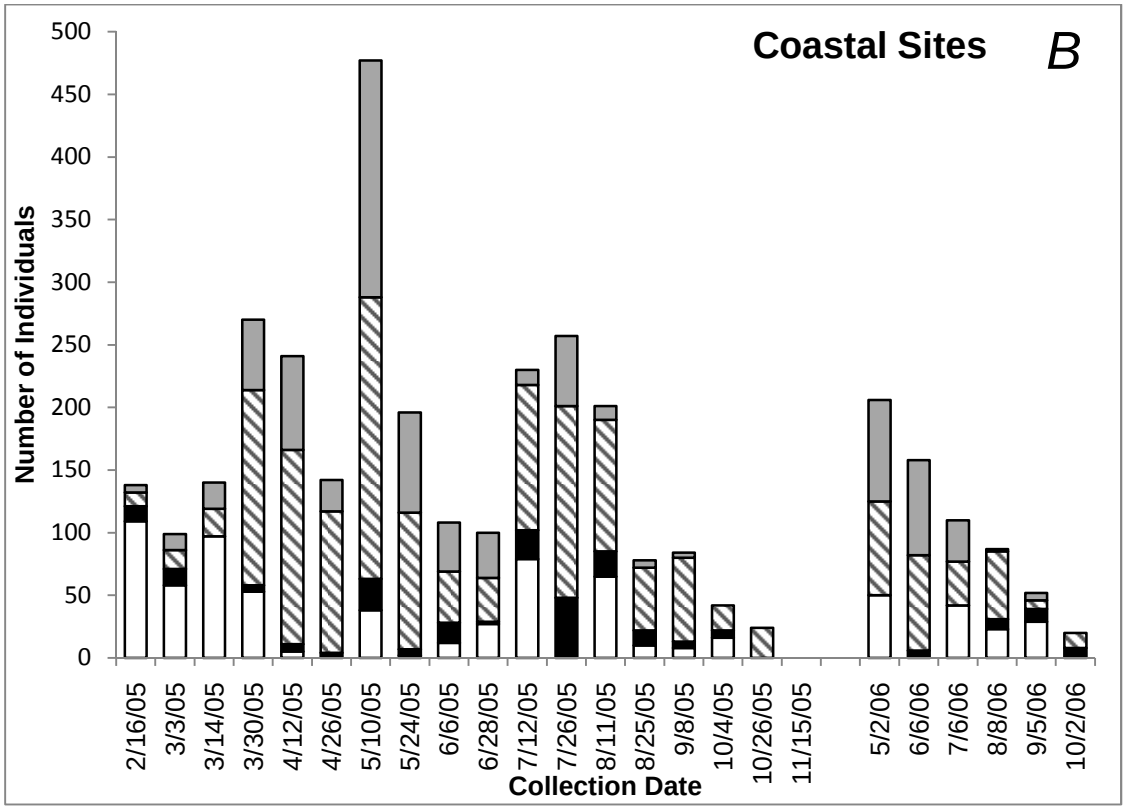
Coastal Sites
B
Number of Individuals
500
450
400
350
300
250
200
150
100
50
0
2/16/05
3/3/05
3/14/05
3/30/05
4/12/05
4/26/05
5/10/05
5/24/05
6/6/05
6/28/05
7/12/05
7/26/05
8/11/05
8/25/05
9/8/05
10/4/05
10/26/05
11/15/05
5/2/06
6/6/06
7/6/06
8/8/06
9/5/06
10/2/06
Collection Date

Table 1.1. Total number of egg masses collected and number of parasitoids and *H. vitripennis* nymphs that emerged from egg masses at each site.

Site	Total egg masses	*H. vitripennis* nymphs	*G. ashmeadi*	*G. walker-jonesi*	*G. novi-fasciatus*	*G. morgani*	*G.* spp.[a]	*Ufens* spp.	Total parasitoids	Unknown (molded)[b]	Unde-veloped[c]	Totals[d]
Riverside	192	370	406	38	31	1	67	76	619	181	132	1,302
Temecula	127	468	238	91	17	0	100	0	446	64	51	1,029
Corona	52	25	140	4	4	0	7	0	155	23	7	210
Totals, 3 interior sites	371	863	784	133	52	1	156	76	1,220	268	190	2,541
Percentages 3 interior sites	--	34.0%	30.9%	5.2%	2.0%	0.04%	6.8%	3.0%	48.0%	10.5%	7.5%	100%
Mission Viejo	187	152	120	420	27	8	238	2	815	246	51	1,264
San Juan Cap.	284	228	39	1,028	44	40	288	0	1,439	340	151	2,158
Irvine	96	343	75	228	3	61	109	0	476	42	7	868
Totals, 3 coastal sites	567	723	234	1,676	74	109	635	2	2,730	628	209	4,290
Percentages 3 coastal sites	--	16.9%	5.5%	39.1%	1.7%	2.5%	14.8%	0.05%	63.6%	14.6%	4.9%	100%
Grand total	938	1,586	1,018	1,809	126	110	809	78	3,950	896	399	6,831
Percentages grand total	--	23.2%	14.9%	26.5%	1.8%	1.6%	11.8%	1.1%	57.8%	13.1%	5.8%	100%

[a] *Gonatocerus* spp. indicates the egg was parasitized by some species of *Gonatocerus* but after dissection following lack of emergence over the 25-day holding period, the species identity could not be determined.

[b] Because of mold, it could not be determined if specimens in this category were unemerged *H. vitripennis* nymphs or some species of parasitoid.

[c] Specimens in this category were undeveloped *H. vitripennis* eggs.

[d] Totals and percentages are calculated excluding data from the first column (number of egg masses collected) and the total number of parasitoids.

Chapter 2

Biology and host specificity of *Gonatocerus deleoni* (Hymenoptera: Mymaridae), a potential biocontrol agent of *Homalodisca vitripennis* (Hemiptera: Cicadellidae) in California, USA

Abstract

The host-specificity and biological traits of *Gonatocerus deleoni* Triapitsyn, Logarzo & Virla (Hymenoptera: Mymaridae), a potential candidate for biological control of the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), were determined under laboratory conditions. *Gonatocerus deleoni* is a solitary egg parasitoid native to Argentina, originally reared from sentinel eggs of *Tapajosa rubromarginata* (Signoret) (Cicadellidae). With GWSS as a fictitious host, *G. deleoni*'s average development time from oviposition to adult emergence was 18.8 ± 1.4 days, with males developing faster than females (18.0 ± 1.3 days, males; 19.0 ± 1.3 days, females). The average parasitism rate on 1-8 day old eggs was 45.7% but this was significantly affected by the age of the egg, ranging from 1.4% to 69.9% (egg ages 8 and 3, respectively). The average sex ratio was 0.34 (males: total) and sex ratio was not significantly affected by egg age. *Gonatocerus deleoni* were able to develop in eggs of GWSS and *Homalodisca liturata* Ball (both in the tribe Proconiini), but were unable to develop on eggs of *Graphocephala*

atropunctata (Signoret) (different tribe, same subfamily) or *Erythroneura elegantula* Osborn (different subfamily).

Introduction

The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar), is a leafhopper native to the southeastern U.S. It was first detected in California in 1990 (Sorensen and Gill 1996). After its arrival, GWSS became a major pest due to its ability to transmit the plant bacterium *Xylella fastidiosa* Wells et al., which causes Pierce's disease in grapevines as well as diseases in other plants, including almond and oleander. Currently, there is no cure available for the diseases caused by *X. fastidiosa*, and the only treatments in use are vector control and removal of infected plant material. Economic losses caused by *X. fastidiosa* in California include an estimated $U.S. 52 million in damage to oleanders used in highway median plantings (Costa et al. 2000) and $U.S. 37.9 million to grape growers in San Diego and Riverside counties in 1998-99 (Siebert 2001). The use of parasitoids to control GWSS is part of the current strategy to reduce Pierce's disease spread and is of particular importance in urban areas and farmland under integrated pest management or organic pest control strategies (Hix et al. 2003). To date, most GWSS biological control research has focused on egg parasitoids. Glassy-winged sharpshooters typically oviposit in masses of 4-15 eggs arranged in rows directly beneath the lower leaf epidermis of suitable leaves.

The exploration for parasitoids as potential biological control candidates in the U.S., Mexico, and South America has produced numerous species in the families Mymaridae and Trichogrammatidae (Triapitsyn et al. 1998, 2003; Triapitsyn and Phillips 1996, 2000; Hoddle and Triapitsyn 2004; Logarzo et al. 2004; de León et al. 2006; Triapitsyn and Bernal 2009). To date, the mymarids *Gonatocerus ashmeadi* Girault, *G. fasciatus* Girault, *G. morrilli* (Howard), *G. morgani* Triapitsyn, *G. triguttatus* Girault, *G. walkerjonesi* Triapitsyn, and *Anagrus epos* Girault have been reared and released in California to aid in the control of GWSS (CDFA 2003, Morse et al. 2006).

Gonatocerus deleoni Triapitsyn, Logarzo & Virla was first reared from sentinel eggs of *Tapajosa rubromarginata* (Signoret) from the Mendoza Province of Argentina. This area has been shown to be climatically similar to southern California (Jones 2003). Recently, *G. deleoni* was identified as a distinct species in the subgenus *Cosmocomoidea* Howard, apparently in the *morrilli* species group (Triapitsyn et al. 2008, 2010). The natural hosts of *G. deleoni* in Argentina are unknown (Triapitsyn et al. 2008, 2010); its biology and both field (using sentinel eggs of several leafhopper species) and laboratory host range studies have been studied by Dr. Guillermo A. Logarzo and collaborators in Argentina (unpublished data). Their field host range studies revealed that *G. deleoni* parasitizes only eggs of *T. rubromarginata* and it would not attack eggs of Cicadellini (Triapitsyn et al. 2008). Here we report the results from tests to evaluate (1) the host range of *G. deleoni* to anticipate possible non-target effects

and (2) other biological traits relevant to eventual field release if approved through the appropriate process.

We tested *G. deleoni* on three species of leafhoppers occurring in California, smoketree sharpshooter, *Homalodisca liturata* Ball; blue-green sharpshooter, *Graphocephala atropunctata* (Signoret); and western grape leafhopper, *Erythroneura elegantula* Osborn, in each case using *H. vitripennis* to compare parasitism ability. Species used in host specificity tests were chosen based on taxonomic relatedness to the target host (GWSS) because of the likelihood of shared physiological traits and similar egg morphology. *Homalodisca liturata* is the closest related species to GWSS in California, and is placed in the same genus (tribe Proconiini of the subfamily Cicadellinae). *Graphocephala atropunctata* is a less well related sharpshooter in the tribe Cicadellini of the Cicadellinae. *Erythroneura elegantula* is in the tribe Erythroneurini of the subfamily Typhlocybinae and is even more distantly related to GWSS. Among the Proconiini, only *H. vitripennis*, *H. liturata*, and several seldom collected species in the genus *Cuerna* exist in California. It is unclear how many species in the tribe Cicadellini exist in California, but many are difficult to rear in laboratory conditions. We chose to use *G. atropunctata* in our studies based on availability and ease of rearing.

Both *G. atropunctata* and *E. elegantula* are considered pests of grape, the former also being a vector of Pierce's disease (Redak et al. 2004). Both *E.*

elegantula and *H. liturata* oviposit eggs horizontally beneath leaf epidermis tissue similar to *H. vitripennis*, while *G. atropunctata* oviposits its eggs perpendicularly into leaf and stem tissue. *Homalodisca liturata* arranges its eggs in masses similar to GWSS, while *E. elegantula* and *G. atropunctata* oviposit eggs singly. Eggs of *H. liturata* are smaller, but similar in size to those of *H. vitripennis*, while the eggs of the remaining leafhoppers tested are considerably smaller. Our host-specificity tests were conducted in artificially small environments under no-choice conditions and thus, our results err on the side of over-estimation of the possible non-target host range.

Materials and Methods

Gonatocerus deleoni and *H. vitripennis* colony maintenance

Our colony of *G. deleoni* began in the University of California-Riverside (UC Riverside) quarantine facility on 7 March 2007 from a shipment of adult parasitoids from Mendoza, Argentina. This was the F2 generation, which originally emerged in Buenos Aires, Argentina, from sentinel eggs of *T. rubromarginata* prior to shipment; parasitoids were reared on GWSS eggs from their arrival at UC Riverside until our studies began in June 2009 (Triapisyn et al. 2008). The colony was maintained in cages through a process similar to that described by Krugner et al. (2008). Briefly, parasitoids were provided 4-12 euonymous (*Euonymus japonica* L.) leaves infested with 0-72 hour old egg masses laid by our lab colony of GWSS. Parasitoids emerged in approximately

18 days, at which point they were transferred to another rearing cage containing new egg masses. The cages were kept under constant temperature and light regiments (21–24℃, 40–60% RH, and 16:8 h L:D)

Our colony of GWSS was field collected from Agricultural Operations at UC Riverside during the spring and summer of 2009 and held individually in 2-liter plastic soda bottle cages similar to those described by Boyd et al. (2007). The GWSS were provided fresh cowpea, *Vigna unguiculata* (L.) Walp, and basil, *Ocimum basilicum* L., alternating every 10 days with euonymus for egg mass collection. GWSS also were provided plants for oviposition as listed below for the species evaluated in the host specificity testing. The GWSS colony was checked daily for newly laid egg masses to ensure accurate determination of the age of each egg mass used in tests. Additional GWSS adults and nymphs were kept in Bug Dorms (Bug Dorm 2, BioQuip Products, Rancho Dominguez, CA) to mature adults and provide an additional source of eggs.

GWSS egg age suitability

GWSS from our lab colony were held on euonymus plants for 24 hours to collect egg masses. Leaves on these plants with eggs were then assigned randomly to egg age treatments of 1, 2, 3, 4, 5, 6, 7, or 8 days before exposure to parasitoids in a randomized complete block design of eight replicates over time with 2-6 parasitoid females per date (total of 19-34 parasitoids per egg age; total of 221 wasps). Individual egg masses on euonymus leaves containing 4-10

eggs were introduced into a 148 mL vial containing a single 24-48-h-old adult female *G. deleoni*. These females were kept in a rearing chamber with access to males prior to use in the experiments to allow for mating and development of eggs. The parasitoids were given 24 hours to oviposit and were then removed. Leaves were held under laboratory conditions (21–24℃, 40–60% RH, and 16:8 h L:D) and monitored daily for parasitoid emergence and GWSS egg hatch. Eggs were dissected after 27 days to check for non-emerged individuals. To determine levels of unknown GWSS egg mortality for each treatment, control vials with egg masses were held without parasitoids and were processed as above. Egg masses that produced 100% male parasitoid broods were not included in the analyses (n=11), as these strongly suggested oviposition by an unmated female.

Adult parasitoid longevity

Gonatocerus deleoni developing in GWSS eggs were checked daily for emergence. Adult parasitoids were removed and randomly assigned to one of three treatments: (1) provided with honey and water, (2) with water alone, or (3) no food. Adults were kept in screen-topped bottom-capped vials similar to those described above. Parasitoids provided food were allowed access to a honey-water mixture applied to the outer surface of the screen and those assigned water obtained this via a wet cotton ball pushed through the lid of the vial. Adult survivorship was monitored daily until all parasitoids were dead.

Species used for host specificity testing

Host specificity studies were conducted on the eggs of three cicadellid species in comparison with GWSS eggs. All host species were kept under constant temperature and light regimes (28-32℃, 50–7 0% RH, and 16:8 h L:D). Smoketree sharpshooters, *H. liturata*, were provided from a laboratory colony reared by the California Department of Food and Agriculture originally collected in citrus groves in Riverside, CA in the spring and summer of 2009. These sharpshooters were reared on cowpea and sunflower, *Helianthus* spp., with euonymus used for egg mass collection and host specificity testing. Egg masses were collected 0-24 hours after oviposition and were transferred to the laboratory for immediate use.

Adult blue-green sharpshooters, *G. atropunctata*, were obtained from a 2-year-old laboratory colony at UC Riverside originally collected from Temecula, CA. We maintained the blue-green sharpshooter colony in Bug Dorms provided with fresh basil plants. Blue-green sharpshooter eggs were collected by placing 10-15 adults onto individual basil plants in bottle cages as described above for our GWSS colony maintenance. Blue-green sharpshooter adults were given 24 hours to oviposit before basil plants containing eggs were used in the studies. Because blue-green sharpshooter eggs are oviposited endophytically and are exceedingly difficult to locate without damaging the plants or eggs, numbers of host eggs provided to the parasitoids were estimated using control plants which

were handled the same except no parasitoids were introduced into the bottle cages.

Adult western grape leafhoppers, *E. elegantula*, were collected from a commercial vineyard in Napa, CA on 7 October 2009. We maintained this leafhopper colony in wood frame cages described by Krugner et al. (2008) on grapevines until they were used in the host specificity studies within a month after collection. New grapevines were introduced into the western grape leafhopper colony for 24 hours for oviposition. Grapevines with 0-24 hour old eggs were then used in our experiments.

Host species suitability testing

When eggs from each host species listed above were 0-24 hours old, they were exposed to 48-72-h-old naïve (no prior oviposition experience) female *G. deleoni*. Parasitoids were kept in rearing chambers with access to males prior to use in the experiments to allow for mating and the development of eggs. Host specificity testing was conducted in individual 148 mL plastic vials described by Krugner et al. (2009). Initial tests were conducted with individual female *G. deleoni* given 24 hours to oviposit in order to assess the ability of each female to find and parasitize hosts. If no parasitoids emerged from these hosts, then secondary trials were conducted using 10 females, given their lifespan (up to 18 days) to parasitize hosts. Submitting multiple females at once to search for hosts simulates competition conditions, which can increase parasitoid readiness for

host acceptance (Carbone and Rivera 2003). Furthermore, allowing parasitoids to oviposit for an extended time without access to target hosts simulates host deficiency and can also increase parasitoid readiness for host acceptance (Withers and Browne 2004).

Each non-target host species was tested separately but concurrently with egg masses of the target species, GWSS. To control for possible brood effects, one female wasp from a brood was randomly assigned to the test host species, with a second female from the same brood introduced to GWSS eggs. In the multiple female trials, equal numbers of females from each of two broods were introduced into the paired cages, to achieve the total of 10 females per test cage. These studies were replicated 4-10 times per day on 3 dates for each non-target host species, achieving 24 total replicates with single females on *H. liturata* and 15 replicates each with 10 females with *G. atropunctata* and *E. elegantula*. To control for plant volatile association behavior (Godfray 1994; Krugner et al. 2008), both target and non-target eggs were provided using the same plant species substrate. To evaluate host viability and estimate numbers of host eggs present, in addition to the cages with introduced parasitoids, half of the cages containing host material were randomly assigned to a control treatment without parasitoids. For both the 24-h and 18-d trials, parasitoid behavior was monitored for 1 h to observe initial host discrimination and parasitoid behavior. All cages were held for emergence and host eggs that did not produce emergence after 27 days were dissected to check for non-emerged individuals.

Statistical analysis

In the studies on GWSS egg age suitability, differences in treatment effects were analyzed using Analysis of Variance (Proc ANOVA). When results indicated significant differences among treatments, multiple comparisons were made using Tukey's HSD test with $P = 0.05$. Differences in treatment effects in longevity studies were analyzed by comparing survival curves using a log rank test. All tests were performed with Statistical Analysis Software (SAS) 9.2 for Windows (SAS Institute Inc., Cary, NC).

Results

GWSS egg age suitability

The average rate of GWSS parasitism (= total number of parasitoids emerged + dissected, divided by the number of host eggs) for all egg ages tested was 45.7 %. There were significant differences in parasitism rate between egg ages ($df = 7, 206$, $F = 32.8$, $P < 0.0001$), ranging from a low of 1.4% to a high of 69.9% (for egg ages 8 and 3, respectively) *Gonatocerus deleoni* showed a significantly higher rate of parasitism when parasitizing young GWSS eggs (1-3-d-old) than when parasitizing older eggs (5-8-d-old) (Fig. 1).

GWSS nymphs typically began emerging at day 9 after oviposition and *G. deleoni* adults as early as day 14 in 4-day old eggs**.** The overall average developmental time of *G. deleoni* was 18.8 days. Host age had a significant

effect on the developmental time of *G. deleoni* (*df* = 7, 688, *F* = 9.0, *P* = <0.0001), with 3 and 4-d-old eggs resulting in the shortest developmental times (Fig. 2). However, host age had no apparent effect on the sex ratio of *G. deleoni* offspring (*df* = 7, 143, *F* = 1.6, *P* = 0.1325).

No pre-mating or pre-ovipositional period was observed. Parasitoids typically began mating shortly after emergence, often with several males attempting to gain access to an emerging female. Host searching behavior was observed shortly after emergence. Host searching behavior consisted of rapid drumming with the antennae while walking and frequently changing direction on leaf surfaces. Oviposition behavior often was observed within 15 minutes of the female being introduced into a cage containing a GWSS egg mass. Oviposition behavior consisted of extension of the ovipositor to the surface of the host egg, probing with the ovipositor inside the egg, visible pumping of the abdomen (this is possibly the act of oviposition), and finally, retraction of the ovipositor from the egg. These behaviors in aggregate typically lasted 20-60 minutes. Parasitized eggs could be detected after 3-7 days due to a slight darkening of the host egg. After 7-14 days, the parasitized egg became dark brown, indicating the parasitoid's pupal stage, based on dissection of eggs in this age range (data not shown).

Adult parasitoid longevity

There was no significant difference ($\chi^2 = 2.2$, $df = 1$, $P = 0.14$) between the longevity of males receiving water (5.46 ± 0.30 days) and males receiving no food or water (4.77 ± 0.37 days) (Fig. 3). Females provided water lived significantly longer than those receiving no food or water ($\chi^2 = 4.3$, $df = 1$, $P = 0.04$; 6.05 ± 0.36 vs. 4.86 ± 0.35 days, respectively). Both sexes lived for significantly longer periods of time when provided honey and water (males: $\chi^2 = 61.6$, $df = 1$, $P < 0.001$; females: $\chi^2 = 61.3$, $df = 1$, $P < 0.001$), with males living 13.57 ± 0.94 days and females living 16.08 ± 1.11 days. Longevity of males versus females did not differ significantly for any of the three food treatments ($\chi^2 = 2.4$, $df = 1$, $P = 0.12$).

Host species suitability testing

When *G. deleoni* were tested on *H. liturata* eggs on euonymus leaves, an average of 2.13 ± 0.29 parasitoids emerged per cage (Table 1). However, when *G. deleoni* were tested on *G. atropunctata* and *E. elegantula*, no parasitoids emerged. *Gonatocerus deleoni* oviposition behavior was observed on *H. vitripennis*, *H. liturata*, and *E. elegantula*, but not with *G. atropunctata*.

Discussion

GWSS egg age suitability

Gonatocerus deleoni showed an average rate of parasitism of 45.7 % for all ages tested, with higher rates of parasitism on 1-3-d-old eggs and lower rates on 5-8-d-old eggs. This trend is typical of parasitoids of *H. vitripennis*, as shown in Figure 1. The lower rates of parasitism in host eggs older than four days may indicate either an inability to develop in developing nymphal tissue or some inhibition of *G. deleoni* development by the developing embryo's physiology. One possible mechanism that can explain the successful development of some *G. deleoni* in eggs of advanced age is oviposition directly into the developing embryo. This would terminate the developing nymph, possibly allowing the parasitoid to develop (Eidmann 1934). The fact that we observed low levels of parasitism in host eggs approaching the normal time for nymphal emergence may not actually indicate the ability to develop in advanced host nymphs, but rather the ability to develop in sterile host eggs, which occurs in GWSS populations at low, but regular levels (Al-Wahaibi 2004).

The parasitism rates produced by *G. deleoni* are similar to those found for *G. ashmeadi* with 1, 2, and 3-d-old GWSS eggs, but *G. ashmeadi* exhibited lower parasitism in 4-d-old and older eggs (Irvin and Hoddle 2005a, Fig. 1). *Gonatocerus deleoni* parasitism rates are similar to those seen with *G. triguttatus* for 4-6-d-old GWSS eggs, lower for 7 and 8-d-old eggs, and higher for 1-3-d-old

eggs (Irvin and Hoddle 2005a). Parasitism rates are higher than those found with *G. fasciatus* across all host ages (Irvin and Hoddle 2005a). *Gonatocerus deleoni* parasitism rates are similar to those seen with *A. epos* for 4, 6, and 7-d-old GWSS eggs, higher for 1-3 and 5-d-old eggs, and lower for 8-d-old eggs (Krugner et al. 2009).

Gonatocerus deleoni developed fastest on 4-d-old GWSS eggs and slower on 1-3 and 5-6 d-old eggs (Fig. 2). We hypothesize this is due to the necessity of developing parasitoid larvae to attain a certain level of maturity in host eggs in order to compete successfully with relatively mature host embryos. Parasitoids may only be able to develop in 4-d-old and older non-sterile host eggs when oviposited directly into the developing embryo. Parasitoids developing in young host eggs may be relatively free from nutrient competition from developing host embryos and thus, those developing slowly were able to survive. With increasing host egg age, only fast-developing parasitoid larvae may be able to survive. With still greater host egg age, only parasitoid eggs deposited in host embryos or in sterile eggs may have been able to survive.

The overall average developmental time of 18.8 days for *G. deleoni* was similar to that seen for other species of *Gonatocerus* at similar temperatures. *Gonatocerus ashmeadi* developed from egg to adult in 18.6 days at 20℃ and 13.4 days at 24℃ (Chen et al. 2006). *Gonatocerus triguttatus* developed in 22.3 days at 20℃ and in 13.0 days at 25℃ (Pilkington and Hoddle 2007). *Anagrus*

epos development took longer, developing from egg to adult in 31.9 days at 23.8℃ (Krugner et al. 2009). Host egg age had no a pparent effect on the sex ratio of *G. deleoni* offspring. This result is similar to the sex ratios found across all GWSS host ages provided to *G. triguttatus* (Irvin and Hoddle 2005a).

Adult parasitoid longevity

Provisioning both sexes with honey and water produced life spans approximately threefold longer than when provided no food or water. Water is regularly available in agricultural and urban settings in the form of irrigation, rainfall, or dew, however nectar resources can be scarce in many settings, particularly when weed control is implemented. Creation of nectar sources by adding flowering cover crops or reducing weed control might improve *G. deleoni* persistence in the field by increasing longevity.

Gonatocerus deleoni appears to have a moderately short to average life span compared with other species of *Gonatocerus* parasitizing *H. vitripennis*. Female *G. deleoni* have a much shorter life span than female *G. ashmeadi*, which lived for an average of 46.5 days, and a more similar, but shorter life span than female *G. fasciatus* and *G. triguttatus*, which lived for an average of 24.8 and 17.1 days, respectively (Irvin and Hoddle 2005b).

The similar adult life spans of male versus female *G. deleoni* contrasts with the shorter life spans of male versus female *G. ashmeadi* and *G. fasciatus*. Male *G. ashmeadi* and *G. fasciatus* lived only 13.5 and 4.0 days, respectively

whereas females lived 46.5 and 24.8 days (Irvin and Hoddle 2005b). However, the similar adult life spans of male and female *G. deleoni* is comparable to the longevity of *G. triguttatus* sexes, with adult male and female life spans of 17.0 and 17.1 days, respectively (Irvin and Hoddle 2005b).

Host species suitability testing

While well documented serious non-target effects of introduced parasitoids are rare over the past 45 years, a high level of host discrimination for introduced biological control agents is generally considered of paramount importance (Hoddle and Syrett 2002). Understanding the host specificity of an introduced parasitoid can reduce the likelihood of it attacking and developing on unexpected non-target hosts. Standardized and thorough test protocols are not yet in place for determining the host specificity of arthropod control agents like those described for weed biological control agents (Wapshere 1974). In addition, testing arthropod biological control agents is often more difficult than testing those used for weed control due to less well understood phylogenies and the difficulty in rearing multiple insect host species. Despite these difficulties, we chose our non-target species based on a modified centrifugal-phylogenetic method described by Boyd and Hoddle (2007).

Gonatocerus deleoni was able to successfully parasitize *H. vitripennis* and *H. liturata*, but not *G. atropunctata* or *E. elegantula,* even when 10 females at a time were confined together in a cage. Although *H. liturata* is native to California,

it also is a vector of *X. fastidiosa* and thus, decreasing its levels may be considered a positive result of releasing *G. deleoni* into the environment. The ability of *G. deleoni* to successfully parasitize *H. liturata* is shared by all tested species of *Gonatocerus* introduced to date for the control of GWSS (Boyd and Hoddle 2007, Krugner et al. 2008). Oviposition behavior was seen on *H. vitripennis*, *H. liturata*, and *E. elegantula*. Oviposition into eggs of these species may be explained by their similar egg placement beneath leaf tissue compared with *T. rubromarginata* (Virla et al. 2005). Oviposition behavior was not observed on *G. atropunctata* eggs, which may be due to their more concealed placement deeper into plant tissues. *Gonatocerus deleoni* is a solitary parasitoid (i.e. one parasitoid at most can develop per host egg), so one possible reason for the lack of successful development in *E. elegantula* could be due to the insufficient size of the host egg to allow for complete parasitoid development. Eggs of *E. elegantula* are less than half the size of those of *H. vitripennis*. Precise egg measurements are not known for the green sharpshooter, *Draeculacephala minerva* Ball, another California sharpshooter, but Boyd and Hoddle (2007) speculated that its eggs are likely similar in size to those of a congener species, *D. mollipes* (Say), which produces eggs 1.35 mm long by 0.25 mm wide (Gibson 1915). If this is correct, while *D. minerva* oviposits eggs beneath leaf epidermis tissue similar in fashion to the GWSS (Freitag 1951), its small egg size, roughly half that of GWSS eggs, would likely prevent successful development of *G. deleoni*.

Previous research showed that *G. deleoni* was outcompeted under laboratory conditions by *G. ashmeadi* (Hoddle et al. 2008). Competition is likely to occur in areas where potentially competing species of parasitoids are already present and these findings should be taken into account when deciding whether or not to apply for a *G. deleoni* release permit. It appears the poor performance of *G. deleoni* in the Hoddle et al. (2008) study might have been due to the small amount of time parasitoids were given to parasitize GWSS eggs (15 or 60 minutes for two separate tests). Based on behavioral observations, it appears that *G. deleoni* females take much more time to find and parasitize GWSS egg masses than do *G. ashmeadi* females (JL, personal observation). It is unclear how competition between these two species would take place in field conditions, particularly on various host plants and in different ecological microniches. Due to similar findings of longevity, developmental time, and parasitism rates compared with other species of *Gonatocerus* released, a good climatic match to its potential area of release, as well as a host range apparently limited to cicadellid eggs of similar size to GWSS, we conclude that *G. deleoni* is a suitable candidate for the biological control of GWSS in California.

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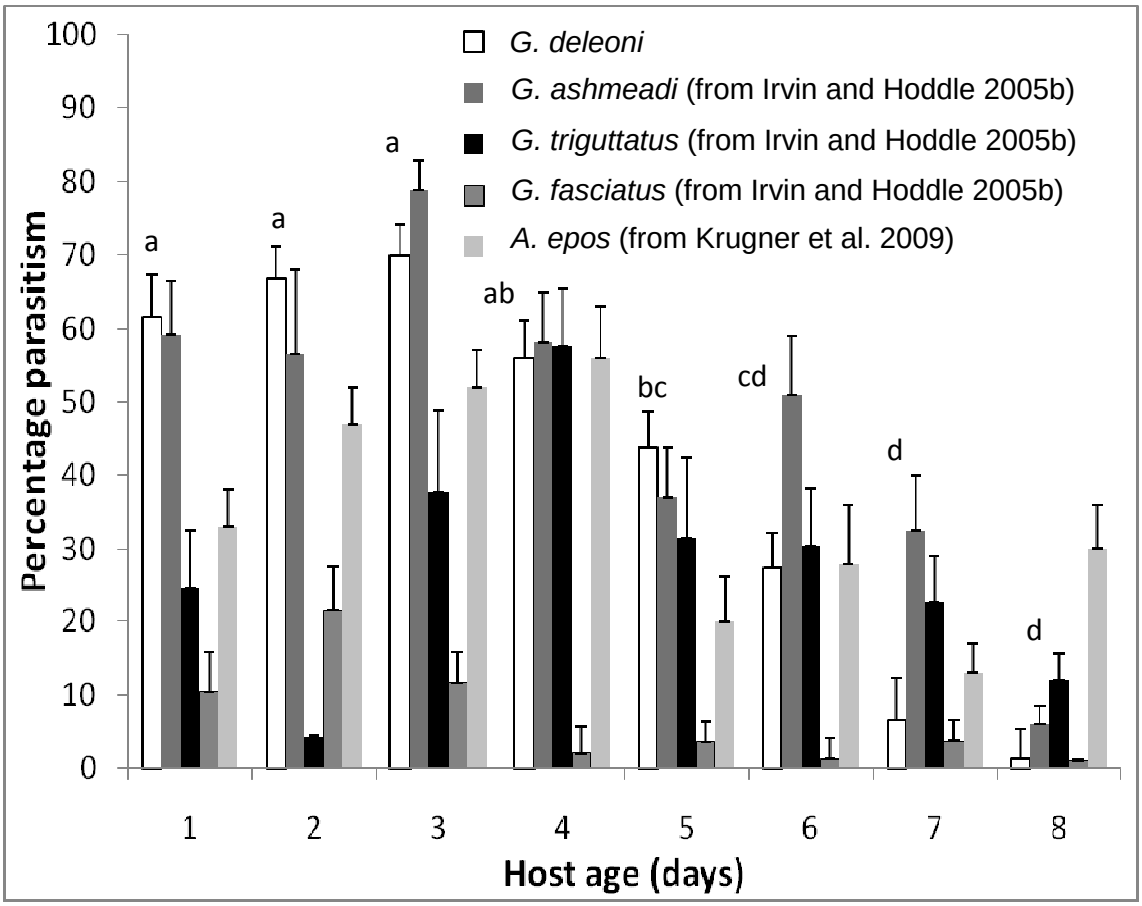

Figure 2.1. Effects of host egg age on percentage parasitism, comparing data from this *G. deleoni* study to other published studies with *Gonatocerus* spp. Mean (+ SE) *G. deleoni* percent parasitism at various host egg ages followed by the same letter are not significantly different (Tukey's HSD test, $P = 0.05$)

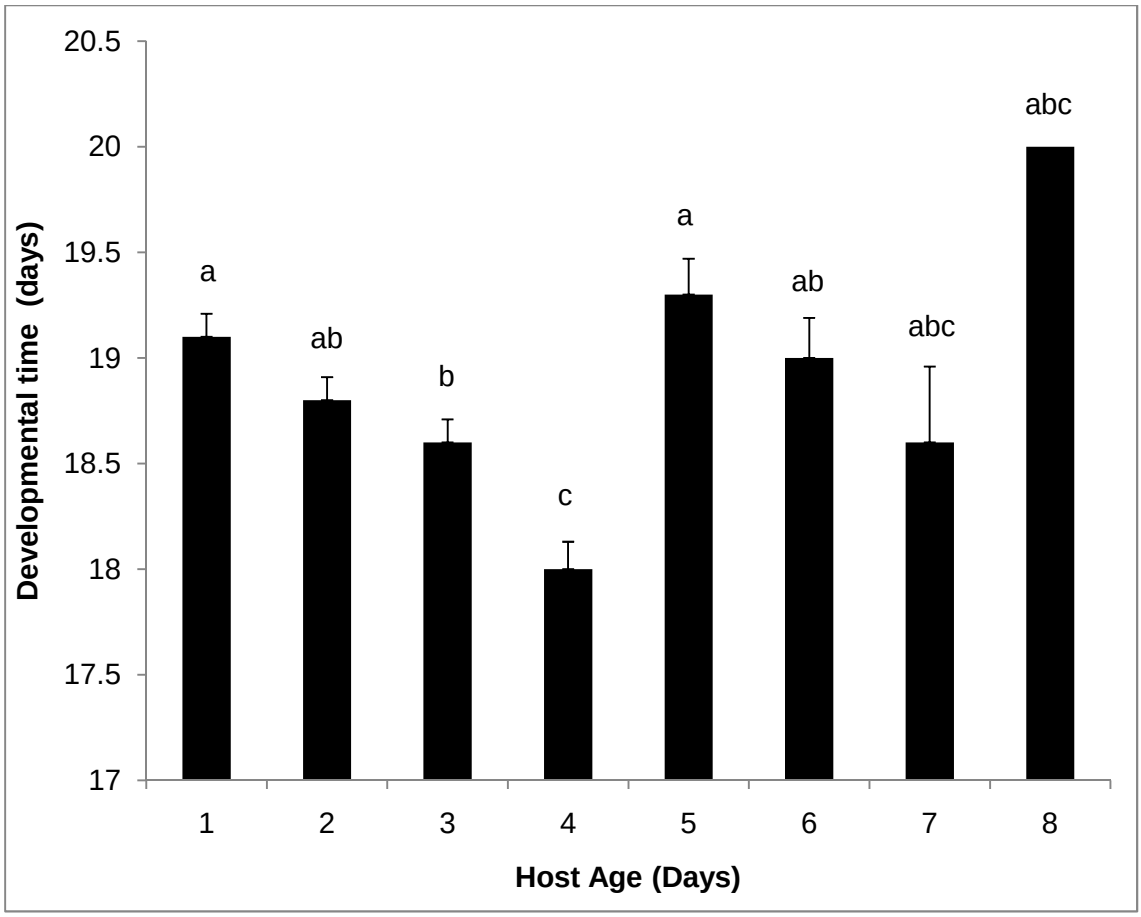

Figure 2.2. Effects of host age on developmental time of *G. deleoni.* Average developmental times (+ SE) followed by the same letter are not significantly different (Tukey's HSD test, $P = 0.05$)

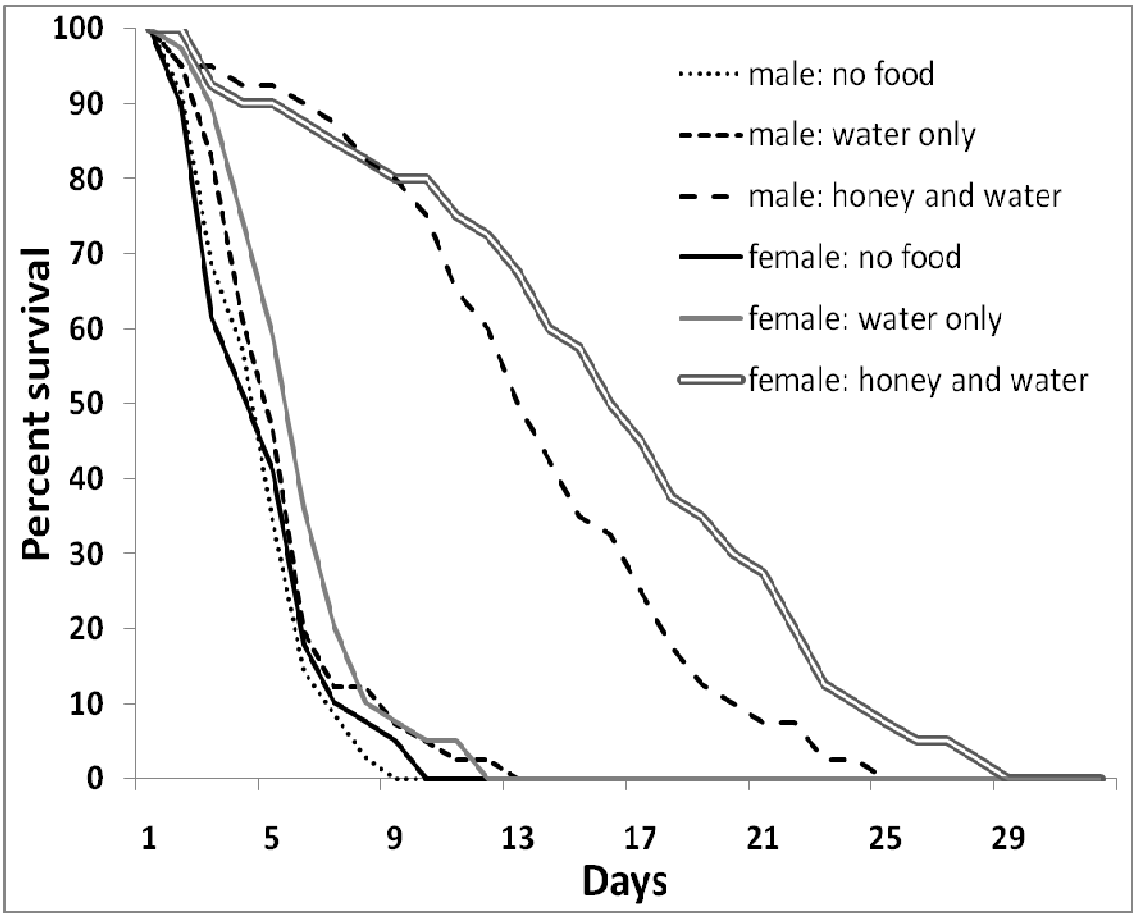

Figure 2.3. Longevity of male versus female *G. deleoni* provided access to honey and water, water, or no food

Table 2.1. Summary of *G. deleoni* host specificity studies

Insect/plant species tested	Emerged wasps per cage ± SE	Emerged hosts per cage ± SE	Oviposition behavior
H. liturata on euonymus	2.13 ± 0.29	1.96 ± 0.20	yes
GWSS on euonymus	3.08 ± 0.41	1.88 ± 0.37	yes
G. atropunctata on basil	0	4.13 ±1.10	no
GWSS on basil	3.13 ± 0.64	1.53 ± 0.51	yes
E. elegantuta on grape	0	6.86 ± 1.35	yes
GWSS on grape	3.42 ± 0.52	1.50 ± 0.43	yes

Chapter 3

Biology of *Pseudoligosita plebeia* (Hymenoptera: Trichogrammatidae), an Egg Parasitoid of *Homalodisca* spp. (Hemiptera: Cicadellidae) Collected From Northwestern Mexico as a Potential Biocontrol Agent of *H. vitripennis* in California

Abstract

Pseudoligosita plebeia (Perkins) (Hymenoptera: Trichogrammatidae) is a potential candidate for the biological control program targeting the glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), in California. Little is known about the biology of *P. plebeia*. Here we report the results of laboratory studies describing the longevity of *P. plebeia* adults provided alternative food resources, their ability to parasitize different age *H. vitripennis* eggs, lifetime offspring production when provided steady access to excess host eggs, and levels of mature ovarian eggs present when wasps were held without access to hosts. *Pseudoligosita plebeia* is a gregarious parasitoid, with up to 6 adults emerging from a single *H. vitripennis* egg. When provided with honey and water, water alone, or no food or water, *P. plebeia* adult females lived an average of 64.1, 2.3, and 2.0 days, respectively. *Pseudoligosita plebeia* were able to successfully parasitize all ages of *H. vitripennis* eggs (1-8 days old), with higher parasitism in young host eggs (1-3 days old) than in old host eggs (5-7 days old).

An increasing trend in offspring production was seen for *P. plebeia* from adult age 2 to 26 days followed by a decreasing trend, with offspring produced up to age 75 days. *Pseudoligosita plebeia* are at least partially synovigenic, as females contained fewer mature eggs at younger ages (1 and 3 days old) than at older ages (5, 11, 15, and 31 days old). Our results provide foundational information regarding the biology of *P. plebeia* useful it its further evaluation as a potential biological control agent in California.

Introduction

The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar), a leafhopper native to the southeastern U.S. and northeastern Mexico, first was detected in southern California in 1990 (Sorensen and Gill 1996). After its arrival, GWSS became a major pest due to its ability to transmit the plant bacterium *Xylella fastidiosa* Wells et al., which causes diseases in multiple crops and ornamentals, including Pierce's disease of grapevines, almond leaf scorch, phony peach disease, and oleander leaf scorch (Blua et al. 1999). Economic losses caused by *X. fastidiosa* in California include an estimated $U.S. 52 million in damage to oleanders used in highway median plantings (Costa et al. 2000) and $U.S. 37.9 million to grape growers in San Diego and Riverside counties in 1998-99 (Siebert 2001). The use of parasitoids to control GWSS is part of the current strategy to reduce Pierce's disease spread and is of particular importance in urban areas and farmland under integrated pest management or organic pest

control strategies (Hix et al. 2003). To date, most GWSS biological control research has focused on egg parasitoids. Glassy-winged sharpshooters typically oviposit in masses of 4-15 eggs arranged in rows directly beneath the lower epidermis of leaves.

The exploration for parasitoids as potential biological control candidates in the U.S., Mexico, and South America has produced numerous species in the families Mymaridae and Trichogrammatidae (Triapitsyn et al. 1998, 2003, Triapitsyn and Phillips 1996, 2000, Hoddle and Triapitsyn 2004, Logarzo et al. 2004, de León et al. 2006, Triapitsyn and Bernal 2009). To date, all species of parasitoids released for GWSS control have been in the family Mymaridae and consist of the following: *Anagrus epos* Girault, *Gonatocerus ashmeadi* Girault, *Gonatocerus fasciatus* Girault, *Gonatocerus morgani* Triapitsyn, *Gonatocerus morrilli* (Howard), *Gonatocerus triguttatus* Girault, and *Gonatocerus walkerjonesi* Triapitsyn (Morse et al. 2006). Two species in the family Trichogrammatidae, *Ufens principalis* Owen and *Ufens ceratus* Owen, parasitize *H. vitripennis* in southern California, however, these species are difficult to raise under laboratory conditions due to the fact that they will parasitize *Homalodisca* spp. eggs only for several hours after host oviposition (Al-Wahaibi et al. 2005).

Another species of Trichogrammatidae, *Pseudoligosita plebeia* (Perkins), first was reared from eggs of *Homalodisca liturata* Ball from Sonora, Mexico in July of 2007 (Triapitsyn and Bernal 2009). This culture was lost in 2009 due to

contamination of another parasitoid species combined with seasonal low availability of *H. vitripennis* eggs. In 2010, we re-collected egg masses (see details below) and re-established a colony inside UC Riverside's Quarantine Facility. Here we report the results from laboratory studies on *P. plebeia* and provide information on its basic biology, longevity, and ability to parasitize varying age *H. vitripennis* eggs.

Materials and Methods

Collection and Maintenance of *P. plebeia*

We collected *Homalodisca* spp. egg masses on horseweed, *Conyza canadensis* (L.), in Miguel Alemán, Sonora, Mexico from 4-7-VII-2010. These leaves with egg masses were transported to the University of California-Riverside quarantine facility on July 9th 2010 under USDA-APHIS permit P526P-10-02056. From these egg masses, a single male and female *P. plebeia* emerged, which were used to initiate a colony. Our colony was maintained by exposing emerged parasitoids to 3-10 detached euonymus leaves (*Euonymous japonica* Thunb., cv. 'Silver King') containing *H. vitripennis* egg masses every two weeks. All parasitoids and leaves containing egg masses were housed in acrylic containers (10 x 10 x 15 cm) described by Krugner et al. (2008). The detached leaves were kept fresh by inserting their stems into foam sheets (9.7 x 9.7 cm), which were floated on 3 cm of water within the bottom of the cage; two sides of each cage was covered with fine mesh screening to allow air flow. These cages were kept

under constant temperature and light regiments (21–24°C, 40–60% RH, and 16:8 h L:D).

Maintenance of *H. vitripennis*

H. vitripennis adults were collected from citrus trees in Field 7H at Agricultural Operations at UC Riverside from Fall 2010 through Fall 2011. These adults were housed in Bug Dorms (Bug Dorm 2, BioQuip Products, Rancho Dominguez, CA) and provided with potted Silver King euonymus; cowpea, *Vigna unguiculata* (L.) Walp. cv. California Blackeye #5; and basil, *Ocimum basilicum* L. Fresh plants were provided every seven days or earlier to replace any plants appearing unhealthy. *H. vitripennis* were kept at constant temperature and light regiments (28-32℃, 50–70% RH, and 16:8 h L:D).

Longevity of *P. plebeia* Adults

Adult males and females that had eclosed over the previous 24 hours were randomly assigned to one of three treatments: (1) with honey and water, (2) with water alone, or (3) no food or water. Each parasitoid was kept in a separate 147 mL vial with a screen top. Honey was provided via a honey-water mixture applied to the screen and water was provided via a moist cotton ball inserted through the lid. Survivorship was monitored daily until all parasitoids expired. Each treatment was replicated 30 times for females. Males were scarce at the time of the experiment, and thus, treatments of no food or water and honey and

water were replicated 25 times and the water alone treatment was replicated 24 times.

Effect of Host Egg Mass Age on Parasitism

To determine which ages of eggs were suitable for *P. plebeia* oviposition and development, individual mated females were exposed to a single 0-7 day old *H. vitripennis* egg mass in a no-choice setting. *H. vitripennis* adults in our lab colony were held on euonymus, basil, and cowpea for 7 days. Euonymus were inspected daily and leaves that contained egg masses were labeled and left on the plant to avoid egg degradation or developmental changes that might be caused by leaf removal. Each euonymus plant was removed from the *H. vitripennis* colony after one day of exposure and replaced with a new euonymus plant, to preserve the quality of leaves that contained egg masses. Basil and cowpeas were replaced every three days or earlier if plants appeared unhealthy. After 7 days, all labeled leaves with egg masses with 5-10 eggs to be used in the experiment were removed. Individual 10-15 day old adult female *P. plebeia* were randomly assigned to one of seven treatments, i.e. egg ages 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, or 6-7 days following oviposition. Each female was placed in a 160 mL vial with a mesh screen lid containing an egg mass inserted through a foam disk and floated in a metal tray of water (after Krugner et al. 2009). Females were given 24 hours to oviposit and then were removed. Egg masses were monitored for emergence and dissected after 40 days. The number of unemerged

parasitoids, unidentifiable egg contents, and unemerged *H. vitripennis* nymphs were recorded. Most egg ages had 9 replicates on each date and the entire age assembly was replicated on four dates. Due to limitations in egg production, egg ages 5-6 and 6-7 had 8 replicates on the third date (total n=36 for 5 treatments, 35 for the other two).

Lifetime Fecundity of *P. plebeia*

Nine mated, 2-3 day old female *P. plebeia* emerging from separate egg masses were placed into 160 mL vials containing a 0-1 day old egg mass. For each surviving female, this egg mass was replaced every three days over her adult life in order to provide a constant supply of excess host eggs of a suitable age for oviposition. After removal, each egg mass was monitored for emergence with dissection after 40 days. Egg masses were provided until each female had expired. A second group of nine females was treated similarly on a later date, except our lab colony of *H. vitripennis* did not produce enough egg masses to allow for continued egg mass replacement until all parasitoids had died and this study was terminated on day 51 following emergence.

Egg Load Measurements

To estimate the compliment of eggs *P. plebeia* emerges with and the number of eggs generated throughout her life, adult females were dissected and their mature eggs counted. Adult females were provided with honey and water and held with access to males and without access to host material. They were

randomly assigned to be placed in alcohol after 1, 3, 5, 11, 15, or 31 days, with 20 replicates for each of these six ages. They were then placed in a droplet of water, dissected, stained with toluidine blue, pressed with a cover slip to extrude their eggs into the surrounding water, and mature eggs were counted.

Statistical Analysis

For the studies of GWSS egg age suitability, differences in treatment effects were analyzed using Analysis of Covariance (Proc ANCOVA). Differences in treatment effects for studies of egg load measurements were analyzed using Analysis of Variance (Proc ANOVA). When results indicated significant differences among treatments, multiple comparisons were made using Tukey's HSD test with $P = 0.05$. Differences in treatment effects in longevity studies were analyzed by comparing survival curves using a log rank test. All tests were performed with Statistical Analysis Software (SAS) 9.2 for Windows (SAS Institute Inc., Cary, NC).

Results

Longevity of *P. plebeia* Adults

There was no significant effect of provisioning water on the longevity of both sexes when compared with individuals not receiving water (males: $\chi^2 = 1.3$, df = 1, $P = 0.25$; females: $\chi^2 = 0.4$, df = 1, $P = 0.53$). Males receiving no water lived 1.88 ± 0.15 days, males receiving water lived 1.86 ± 0.17 days, females

receiving no water lived 2.03 ± 0.20 days, and females receiving water lived 2.27 ± 0.28 days (Fig. 1). Females provided honey and water lived significantly longer than those receiving only water ($\chi^2 = 66.9$, df = 1, $P < 0.0001$), with those provided honey and water living 64.13 ± 5.08 days. Similarly, males provided honey and water lived significantly longer than those receiving only water ($\chi^2 = 53.1$, df = 1, $P < 0.0001$), with those provided honey and water living 36.88 ± 5.19 days. When provisioned with honey and water, females lived significantly longer than males of the same food regimen ($\chi^2 = 12.4$, df = 1, $P < 0.0005$), however, no significant differences in longevity were found between sexes on the other two food regimens (water alone: $\chi^2 = 0.1$, df = 1, $P = 0.88$; no food or water: $\chi^2 = 0.5$, df = 1, $P = 0.49$).

Effect of Host Egg Mass Age on Parasitism

Host egg age had a significant effect on the number of *P. plebeia* offspring that emerged from or developed fully inside host eggs (F = 13.34, df = 6, 239, $P < 0.0001$) (Fig. 2), while the effect of trial date was insignificant (F = 6, 239, $P = 0.908$). An average of 0.4 to 5.6 offspring developed (for egg ages 7 and 1 days old, respectively) from each egg mass held for 24 h with a single female parasitoid. *Pseudoligosita plebeia* showed significantly higher production of offspring when parasitizing young host eggs (1-3 days old) than when parastizing older host eggs (5-7 days old) (Fig. 2).

Lifetime Fecundity of *P. plebeia*

Data from ovipositing *P. plebeia* showed an apparent positive trend in offspring production, from a minimum of 2.0 ± 0.97 offspring produced by 2-3 day old females over 3 days, to a maximum of 12.1 ± 1.17 offspring produced by 24-26 day old females (Fig. 3). Older wasps produced a declining number of offspring but one 77-78 day old wasp produced two offspring among a total of 123 offspring over her life. One female from the nine wasps set up during the first trial died on day 15 and did not produce any offspring. She was possibly sterile and was not included in the analysis. Total fecundity for the first nine wasps (day of death) was 0 (15), 52 (48), 57 (36), 85 (48), 106 (60), 114 (39), 122 (69), 123 (75), and 135 (69) offspring per female whereas the second group of nine produced 34 (21), 51 (27), 64 (51), 74 (45), 102 (this and all later females were terminated on day 54), 102, 128, 138, and 147 offspring.

Egg Load Measurements

Adult female *P. plebeia* age had a significant effect on the number of mature eggs contained in her ovaries after she had been held with honey and water but without access to host eggs ($F = 27.92$, $df = 5, 114$, $P < 0.0001$) (Fig. 4). Young *P. plebeia* (1 and 3 days old) contained significantly fewer mature eggs than older *P. plebeia* (5, 11, 15, and 31 days old) (Fig. 4). The number of mature eggs did not significantly differ among parasitoids of 5, 11, 15, and 31 days old. On average, 1-day-old females possessed the fewest mature eggs,

with 6.15 ± 1.12 eggs, and 11-day-old females the most eggs, with 32.9 ± 2.82 eggs. Young females appeared to possess a greater number of immature eggs, however, these were not recorded for our analyses. Our dissections failed to detect presence of resorbed eggs.

Discussion

Pseudoligosita plebeia produced up to four offspring per host egg when ovipositing singly. When multiple females were allowed to oviposit on host eggs (as was the case in our colony), up to 13 developing larvae were found per egg. These high numbers of developing *P. plebeia* found in colony conditions are likely not typical, as they were only seldom discovered and did not successfully emerge from the host eggs. A maximum of six adults were observed emerging per egg. *Pseudoligosita plebeia* showed higher production of offspring on 1-3 day old host eggs than on 5-7 day old eggs (Fig. 2). This trend is consistent with other parasitoids of *H. vitripennis* (Irvin and Hoddle 2005, Krugner et al. 2009, Lytle et al. 2011). The reduced parasitism of older host eggs is likely explained by an inability of *P. plebeia* to develop in developing nymphal host tissue or by an inhibition of *P. plebeia* development by the host embryo's physiology. The low numbers of *P. plebeia* developing in old host eggs approaching eclosion of nymphs may not necessarily indicate an ability to develop in advanced host nymphs, but likely the ability to develop in sterile host eggs, which are known to

be present in *H. vitripennis* egg masses in low, but consistent numbers (Al-Wahaibi 2004, Al-Wahaibi and Morse 2009).

When provided host eggs throughout their lifetime, *P. plebeia* displayed an apparent ability to replenish eggs, as they were able to produce offspring in greater numbers than were found in eggs from our dissection studies. Also, *P. plebeia* apparently produced fewer offspring when young (0-2 days old) than when old (21-23 days old) (Fig 3). The delayed production of eggs was also supported by the results of the dissections, as young females (1 and 3 days old) contained significantly fewer mature eggs than did mature females (5, 11, 15, and 31 days old) (Fig. 4). Delayed production of eggs indicates synovigeny and the continual production of eggs throughout a portion of the parasitoid's lifetime is a life history trait common among synovigenic species (Godfray 1994). We did not detect egg resorption in our egg load measurement studies. However, this result may not indicate the inability to resorb eggs, as our experimental design included the provisioning of honey, which may provide enough nutrients to eliminate the need for egg resorption. Similar egg load measurement studies have shown that parasitoids resorb eggs when experiencing starvation, but may not show signs of resorption when honey-fed (Heimpel et al. 1997).

Pseudoligosita plebeia has a longer adult lifespan when provided honey than *G. ashmeadi*, the dominant egg parasitoid species of *H. vitripennis* egg parasitoid in most areas of California, which lives for an average of 46.5 days

(Irvin and Hoddle 2005). This potentially long lifespan could allow *P. plebeia* to locate and parasitize a large number of host eggs. However, *P. plebeia* has a significantly reduced lifespan when not provided access to honey. Access to nectar sources could provide *P. plebeia* a great advantage for finding and parasitizing *H. vitripennis* eggs in a field setting. This is of particular importance due to the largely synovigenic nature of its egg production (based on dissection data). As *P. plebeia* showed a delayed production of mature eggs (Fig. 4), with only an average of 13.2 eggs present in the ovaries by day three after eclosion, the average lifespan of 2.26 days for females when only given access to water indicates access to water alone in the field would be insufficient to allow for *P. plebeia* to produce very many offspring. Water is typically available in agricultural and urban settings as dew, irrigation, or rainfall, but nectar sources may be scarce in many settings, particularly when weed control is practiced. Creation of nectar sources by planting flowering cover crops or borders or by allowing some weeds to persist may improve *P. plebeia* persistence and its ability to establish by increasing longevity and egg production. It is unclear how *P. plebeia* would perform under field conditions in the presence of competing parasitoids, but due to its unique life history and potentially long life span, we conclude that *P. plebeia* warrants further research regarding its potential as a biological control agent of *H. vitripennis*.

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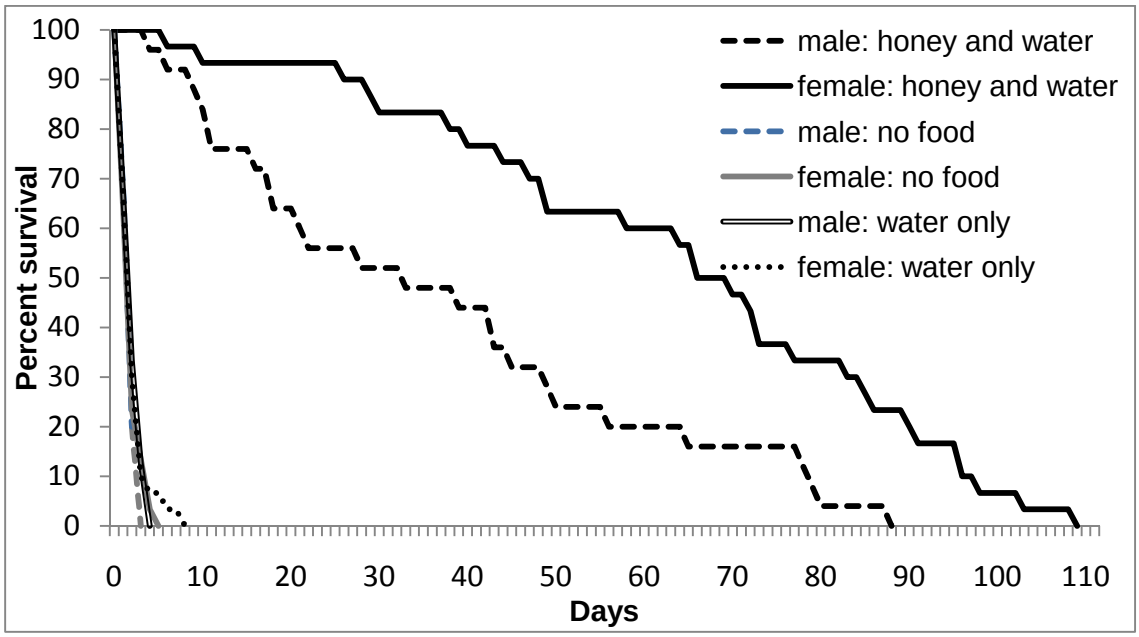

Figure 3.1. Longevity of male vs. female adult *P. plebeia* provided access to honey and water, water, or no food.

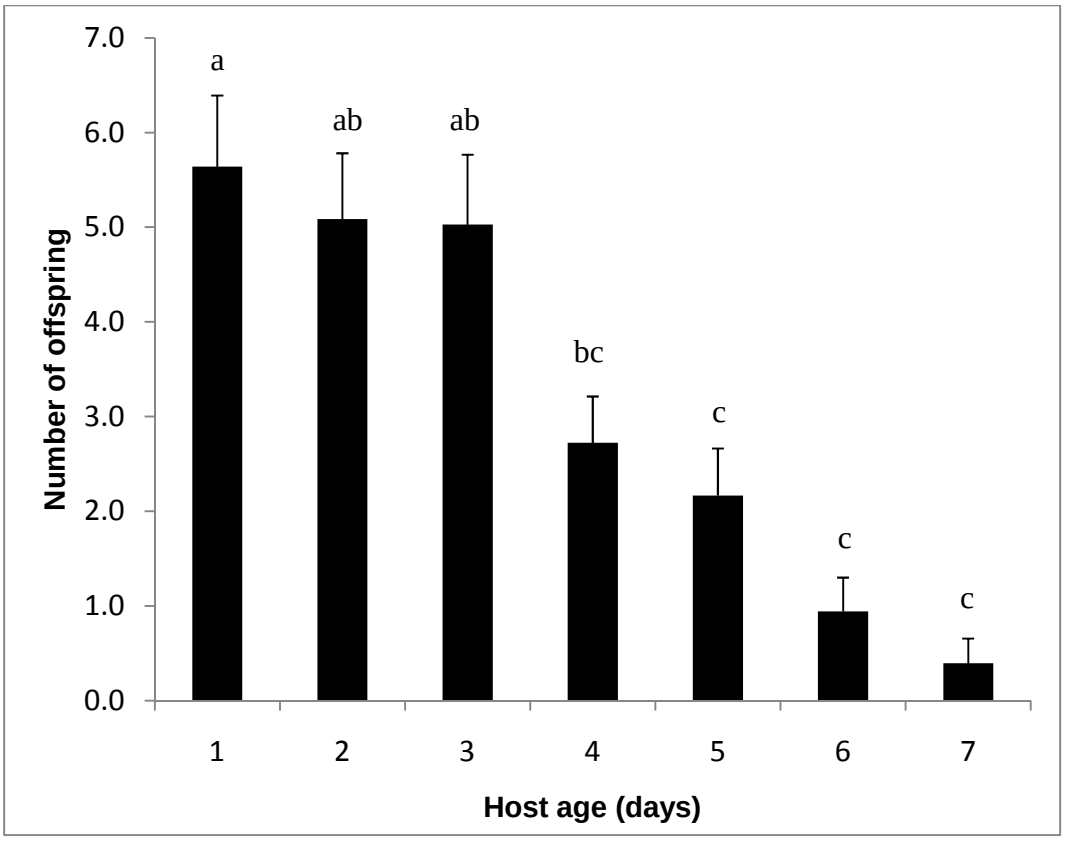

Figure 3.2. Effects of host egg age on *P. plebeia* parasitism. Mean number (± SE) of parasitoid offspring that developed from a single *H. vitripennis* egg mass as a function of egg mass age post oviposition. A single 10-15 day old *P. plebeia* was left on each egg mass for 24 h. Means listed with the same letter are not significantly different (Tukey's HSD test, $P = 0.05$).

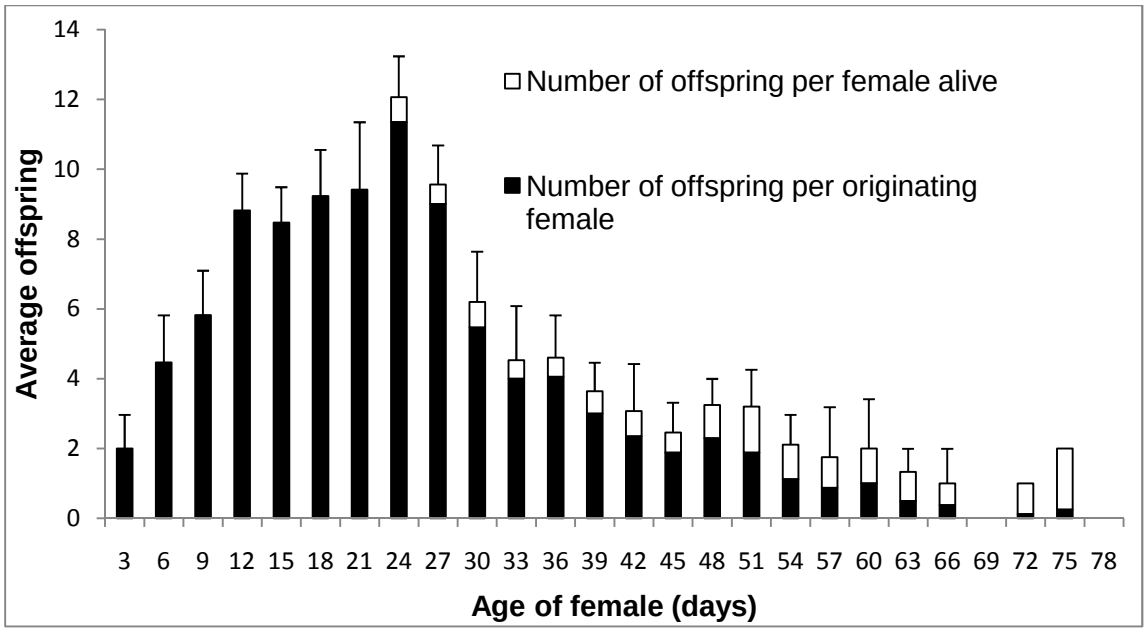

Figure 3.3. Lifetime *P. plebeia* offspring production (± SE) when provided a single 0-1 day old *H. vitripennis* egg mass every third day (i.e. data for day 6 are the number of offspring per female when a single 2-3 day old mated female was held on an egg mass for 3 days). Data for days 6-54 are for 17 wasps (one wasps from the first trial with 9 wasps did not produce any offspring and was excluded from the analysis) whereas later data are for 8 wasps (the second trial with 9 wasps was terminated on day 54 due to insufficient availability of host egg masses). To avoid over-estimation of offspring produced when few females remained alive, we show both offspring produced per female alive at that time (stacked bar and errors) and per female initiating the experiment (solid portion of the bar).

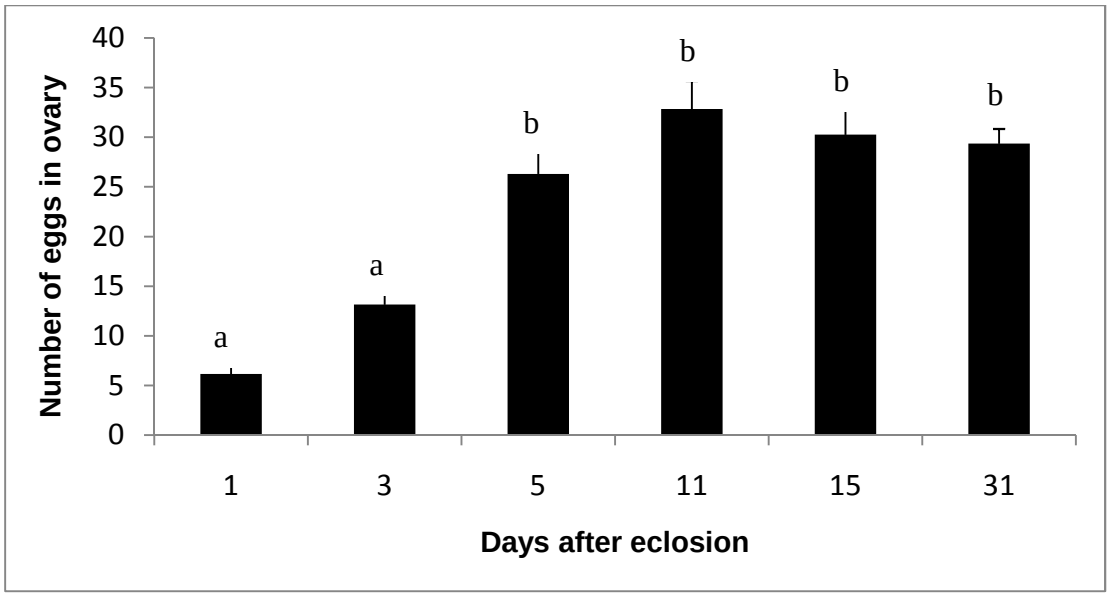

Figure 3.4. Effects of *P. plebeia* age on the number of mature eggs found in their ovaries. Mean number (± SE) of mature eggs dissected from the ovaries of *P. plebeia* females of various ages held with honey and water but without access to host egg masses. Means depicted with the same letter are not significantly different (Tukey's HSD test, $P = 0.05$; n=20 per wasp age).

Chapter 4

Sex Ratio Dynamics of *Gonatocerus ashmeadi*, a Parasitoid of the Glassy-winged Sharpshooter in California

Abstract

We examined whether *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae), a quasi-gregarious egg parasitoid of *Homalodisca vitripennis* (Germar), produces precise sex ratios under a field setting. Other studies have shown *G. ashmeadi* to exhibit strongly female biased sex ratios with low variance in the number of males produced per host under laboratory conditions. Field collected *G. ashmeadi* tend to produce much less female biased sex ratios with high variance in male numbers. We found significant positive effects of proportion parasitism and host density on sex ratio. Proportion parasitism also had a positive effect on sex ratio variance.

Introduction

In parasitic Hymenoptera that develop gregariously in hosts or quasi-gregariously in host patches (e.g., egg parasitoids) and when mating is mostly confined among relatives, progeny sex ratios are frequently female biased (Godfray, 1994). The mechanism responsible for these biased sex ratios is Local Mate Competition (LMC): when a few foundresses exploit a patch of hosts, males are competing for access to mates and thus it pays for parental females to

allocate more female offspring to better utilize host resources; whereas as the number of foundresses increases, progeny sex ratios approach equality as males then have mating opportunities with unrelated females (Hamilton, 1967; West, 2009). In extreme situations, where only a single foundress exploits a host or patch of hosts, the optimal strategy would be allocation of the minimum number of sons necessary to inseminate their sisters. Broods without male offspring would result in female offspring dispersing unmated and being destined to lay only male offspring, which will incur severe fitness costs for parental females. On the other hand, the production of superfluous sons would waste host resources better allocated for the development of female offspring. This strategy would result in low variance, i.e. precise sex ratios (Green et al, 1982; Hardy et al., 1992).

The sex ratio variance of broods laid by single females and how it is affected by various factors has been the subject of intense theoretical and empirical investigations (e.g., Nagelkerke, 1996; Nagelkerke & Hardy, 1994; Hardy et al. 1998). However, the variance of sex ratios in situations where multiple females exploit a host either simultaneously or sequentially, including superparasitism, has received less attention. Avoiding the production of unmated daughters may still hold an advantage for superparasitzing females exhibiting precise sex ratios. Also, successive foundresses without precise sex ratios may experience reduced fitness if they deviate from local sex ratio optima and thus sex ratio precision may still provide fitness gains when multiple foundresses are present (Nagelkerke,

1996). Luck et al. (2001) demonstrated that although the overall sex ratios produced by successive *Trichogramma pretiosum* Riley foundresses were found to become more male biased than those produced by previously ovipositing foundresses, the variance of sons allocated remained low, i.e. precise sex ratios were obtained. Also, *Telenomus fariai* Lima produced similar numbers of sons and fewer daughters when superparasitizing compared to ovipositing alone, thereby increasing sex ratios produced while maintaining sex ratio precision (Rabinovich et al., 2000). The fig wasp, *Pegoscapus silvestrii* (Grandi), apparently uses a similar sex allocation strategy, with successive foundresses each allocating similar numbers of sons, but later foundresses allocating fewer total offspring (Ramirez-Benavides et al., 2009). A counter example comes from *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae), which exhibited increased sex ratio variance with superparasitizing females compared to those initially parasitizing hosts (Orzack, 1986). Orzack concluded that the increase in sex ratio variance may be due to an inability of superparastizing females to produce precise sex ratios or that precision has low selective pressure for superparasitizing females. The later hypothesis is supported in part by sex ratio theory, as the superparasitizing female's offspring may have increased outbreeding opportunities (Kolman, 1960; Hamilton, 1967); however, Nagelkerke's models (1996) predicted an advantage to precise sex ratios even when multiple foundresses were present.

In this study, we investigated the field-based sex allocation of *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) developing on eggs of *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae). *Gonatocerus ashmeadi* has been found in California since 1978 (Huber, 1988) and likely was an accidental introduction, subsisting on eggs of the native smoketree sharpshooter, *Homalodisca liturata* (Ball) (Vickerman et al., 2004). Since its detection in 1990, the glassy-winged sharpshooter, *H. vitripennis*, an important vector of Pierce's disease, has served as an egg host for *G. ashmeadi* in California. *Homalodisca vitripennis* oviposits its eggs as conspicuous egg masses typically consisting of 2-15 eggs, laid on the underside of leaves. *Homalodisca vitripennis* typically has two broods per year in California, one in late spring and the second in late summer, with an occasional third brood in early winter. *Gonatocerus ashmeadi* is a solitary parasitoid that produces highly female biased sex ratios when ovipositing singly in laboratory studies, typically in the range of 71-88% female (Irvin & Hoddle, 2005, 2006; Chen et al., 2006; Pilkington & Hoddle, 2006; J.L., personal observation). In field settings, parasitism rates of *H. vitripennis* can be extremely high, at times reaching 100% parasitism (Lytle & Morse, in press).

Here we document the proportion males produced by *Gonatocerus* species on *H. vitripennis* under field settings. Our data were collected initially to observe the temporal and spatial distribution of egg parasitism of egg parasitoids of *H. vitripennis* in southern California. From this larger data set, we sought to analyse

field sex ratios of *G. ashmeadi*, given that parasitoid densities under field conditions can be substantially higher than those used in previously reported laboratory experiments. We then investigated how sex ratio variance was affected by the population density of *G. ashmeadi* as extrapolated from parasitism rates. Lastly, we discuss how observed sex ratio variance is explained in the context of the population biology of this species coupled with theoretical expectations.

Materials and Methods

H. vitripennis egg mass collection

The California Department of Food and Agriculture (CDFA) conducted biweekly surveys for egg masses in southern California beginning in 2000 to track the rates of *H. vitripennis* egg parasitism by various species in California. A total of 142 sites were surveyed, with a total of 20,000 egg masses collected to date. From this data set, we selected data from 2006 to 2009, because methods used to process field collected egg masses were inconsistent prior to this point in time; these variable methods may have affected reported parasitoid emergence rates. From available data, we eliminated any egg masses from which more than one parasitoid species emerged, or the sex of any parasitoid could not be confirmed, or in which egg contents were unidentifiable. The resulting edited data set contained data from 36 sites located in Fresno County (GPS location: 36.86655, -119.77467), Imperial County (33.37453, -116.02066), Kern County

(35.233818, -118.57279; 35.42918, -119.04550; 35.304, -119.07983; 35.37731, -119.02975; 35.41024, -118.97379; 35.397, -118.94809), Los Angeles County (34.05974, -117.80969), Orange County (33.520932, -117.633405; 33.522099, -117.571784), Riverside County (33.70671, -116.86388; 33.90738, -117.42057; 33.97137, -117.33831; 33.53749, -117.08687; 33.73428, -116.38773; 33.89163, -117.42737; 33.82088, -117.56654; 33.75743, -117.49611; 33.970129, 117.339327), San Bernardino County (34.14487, -117.60211; 34.11035, -117.64745; 34.06765, -117.22319; 34.05783, -117.11220; 34.08821, -117.38348), San Diego County (33.18147, -117.17122; 33.30881, -116.98352; 33.31811, -116.98157), Tulare County (36.07683, -119.03999), and Ventura County (34.37173, -118.91936; 34.29028, -118.95844; 34.39235, -118.84258; 34.31487, -118.89416; 34.38396, -118.91911; 34.38366, -118.97534; 34.24712, -119.10550).

Density estimates of *H. vitripennis* egg masses were gathered during each collection at each field site by conducting two-minute searches for sharpshooter egg masses, replicated five times, each time on a different tree. These timed searches consisted of visually inspecting the underside of leaves around the entire perimeter of each tree, and counting each intact sharpshooter egg mass (i.e. only those from which nymphs or parasitoids had not yet emerged). If fewer than 10 egg masses were collected during the density estimates, nearby trees were searched for up to 60 additional minutes until ten egg masses had been collected or that time expired. Each leaf that contained an egg mass was

removed from the tree and placed in a plastic bag with moist paper towels for transport to the lab and further processing.

Rearing parasitoids and determining brood sex ratios

Each leaf was washed gently with a cotton ball soaked in a 2% bleach solution: this was done to prevent mould and fungi from growing on the leaves during incubation. Leaves were then rinsed with water and placed individually in Petri dishes lined with moistened filter paper to keep the leaves hydrated. Petri dishes were sealed with Parafilm® (Pechiney Plastic Packaging, Menasha, WI) and kept under constant temperature and light regiments (21–24℃, 40–60% RH, and 16:8 h L:D) for 25 days. Egg masses were checked regularly for parasitoid and sharpshooter emergence, at which point emerged individuals were identified, recorded, and stored in 95% alcohol. Decayed egg contents were sometimes unidentifiable by this methodology and thus they were not used in our analyses.

Statistical analysis

The variance ratio, R, was used to describe the variance of sex ratios across brood sizes and also across different densities of parasitism: R = 1 indicates a binomial distribution, whereas R >1 and R <1 indicate over- and under- dispersion, respectively (Krackow et al., 2002). The Meelis test statistic, *U*, was used to assess the significance of sex ratio deviations from binomality: negative *U* values indicate underdispersed (precise) sex ratios, while positive *U* values indicate overdispersed sex ratios (Krackow et al., 2002). To investigate

the effect of parasitism rate and egg mass (host) density on brood sex ratios, we used logistic regression, which is appropriate for proportional data. The percentage of deviance explained is an approximate analogue of r^2 for logistic models. The advantage of using Generalized Linear Models is that data need not be transformed to fit Gaussian assumptions (e.g., Crawley, 1993; Wilson & Hardy, 2002). To explore the effect of parasitism rates on sex ratio variance we used Spearman rank correlation. We sorted the data by increasing levels of parasitism at each site and date. The data were then split in ten groups (approximately 72 broods per group). For each group, we calculated the variance ratio R and regressed it on the mean parasitism rate of the group. Although the analysis of relationships of R with other variables may suffer from concerns regarding statistical validity (Krackow et al., 2002), in the past, the investigation of the influence of developmental mortality on sex ratio variance (R) was performed successfully (Kapranas et al., 2011). All analyses were performed using the GENSTAT statistical package (Version 13; VSN International Ltd., Hertfordshire, UK).

Results

The subset of glassy-winged sharpshooter egg masses collected from 2006-2009 used in our analysis yielded 750 broods of *G. ashmeadi* consisting of 6,624 individuals in total. Twenty-one broods of *G. ashmeadi* consisted of only one individual and were not included in our analyses as they were not informative.

The remaining *G. ashmeadi* broods were divided into three classes of densities based on the average number of egg masses found per minute (an estimate of parasitoid density) for that brood; with low, medium, and high densities defined as 0 – 0.4, >0.4 – 0.9, and >0.9 egg masses found per minute, respectively. This grouping produced class sizes of 151, 204, and 374 broods, respectively. There were insufficient numbers of broods of the other species of parasitoids recovered to conduct proper analyses; thus, we focused only on *G. ashmeadi*.

Field sex ratios of *G. ashmeadi* were female biased overall, with mean sex ratios of 0.35, 0.35, and 0.41 for low, medium, and high host densities, respectively. Both parasitism rate and egg mass density significantly influenced sex ratios (parasitism rate: $F_{1,746} = 13.73$, $P < 0.001$, %Dev = 1.76; egg mass density: $F_{1, 746} = 16.37$, $P < 0.001$, %Dev = 2.1), whereas the interaction of parasitism rate and egg mass density was not significant ($F_{1,746} = 4.604$, $P = 0.152$; Fig. 1).

However, sex ratios were significantly overdispersed (i.e. we failed to detect sex ratio precision) across different parasitism densities, with increasing overdispersion at higher densities (low density: R = 1.577, $U = 2.28$, $P < 0.05$; medium density: R = 1.869, $U = 6.05$ $P < 0.001$; high density: R = 2.255, $U = 12.09$, $P < 0.001$). Brood sex ratio variance increased significantly in those areas wherein higher parasitism density was observed (Spearman rank test: $r_s = 0.739$, $P = 0.004$; Fig. 2).

Discussion

In this paper, we show that field sex ratios of *G. ashmeadi* broods respond to changes in parasitism rates and host density, i.e. more males are produced in egg masses that are likely exploited by multiple females. Increased densities of hosts and parasitism rates indicate that field populations of these wasps are dense, leading to multiple foundresses simultaneously allocating offspring in patches of hosts. Under this scenario, sex ratios are predicted to be less female biased (Hamilton, 1996) and this is what, indeed, was observed in our study, i.e. field sex ratios were less female biased than those observed for the same species when parasitizing the same host singly in the laboratory (Irvin et al., 1996). The sex ratios shifted towards more males with increased parasitism rates, showing the ability of this species to respond to external cues in order to adjust their sex ratio optima. Nunney & Luck (1988) noted that when different mothers sequentially allocate offspring, sex ratios should not significantly change due to emergence asynchrony, which would not provide outbreeding opportunities; thus, each foundress would produce roughly equal primary sex ratios. However, Shuker et al. (2006) explained that sequentially ovipositing females will experience asymmetric LMC, with later emerging males experiencing increased competition due to the persistence of males that had emerged previously. This asymmetric LMC would explain the less female biased sex ratios produced by superparasitizing females. Our data show many broods that approach and even exceed 50% male emergence. From these data, 36.5%

of field-collected broods produced by *G. ashmeadi* contained 50% males or more. These somewhat less female biased sex ratios could result from two processes, both leading to panmictic conditions: i) excess superparasitism and wasps being able to perceive parasitized hosts (i.e. “host cues”; Werren, 1980), and ii) females responding to cues from encountering other females while foraging on host patches (i.e. “social cues”; Shuker & West, 2004). Shuker & West (2004) showed that *N. vitripennis* sex ratios changed significantly in response to both host cues and social cues. However, the effects of social cues were much smaller than those explained by host cues and only produced a significant effect in previously unparasitized hosts. They concluded that *N. vitripennis* ovipositing simultaneously in a host patch mostly relies on the same cues as when ovipositing sequentially. This could account for the results from laboratory studies with *G. ashmeadi*, which show female encounter prior to oviposition does not significantly influence sex ratios (Irvin & Hoddle, 2006). Alternately, *G. ashmeadi* may detect some cues that indicate an increased opportunity for off patch mating for their offspring and adjust their sex allocation accordingly, even under single foundress scenarios. A local abundance of host patches may serve as such a cue, as these would indicate an increased chance of a foundress’ offspring finding off patch mating opportunities.

Previous studies have found that species experiencing LMC also manifest precise sex ratios under both field and laboratory conditions (Kapranas et al., 2008; 2009; 2011). Our data show that *G. ashmeadi* produces non-precise sex

ratios on *H. vitripennis* eggs under field settings. This overdispersion was consistent across most host densities as well as parasitism rates, however, some precision was achieved at low parasitism densities that presumably correspond to low number, if not single, foundresses sex ratios (Fig. 2). This is in agreement with lab studies observing that when individual *G. ashmeadi* were offered egg masses, they produced brood sex ratios that tended to be precise, typically with only one or two males per egg mass, (Irvin & Hoddle, 2006; JL, unpublished data).

The variance of sex ratios in cases where multiple foundresses are present, including instances of superparasitism, has received some theoretical and empirical attention. Both Luck et al. (2001) and Rabinovich et al. (2000) observed precise sex ratios with increasing numbers of foundresses. However, Orzack (1986) found an increase in sex ratio variance as more foundresses allocated offspring on the same patch. One possible explanation for the absence of sex ratio precision is the increasing probability of off-patch mating with increasing foundress number, leading to situations approaching panmixis. On the other hand, Nagelkerke (1996) showed an overall benefit to precise sex ratios, even in cases where multiple foundresses were present. Increased variance of sex ratios could, however, be anticipated when the distribution of foundresses in the field is fairly dispersed and thus, the intensity of selection for producing such precise sex ratios is weak (West & Her, 1998). Another point regarding the interpretation of our field data is that we used secondary sex ratios

(sex ratio at emergence) and not primary sex ratios (sex ratios at birth). Developmental mortality could result in increased variance of secondary sex ratios, but not to completely overdispersed sex ratios, as this has been demonstrated both in laboratory and field studies (Hardy et al., 1998; Kapranas et al., 2011). In our case, the ubiquity of such highly overdispersed sex ratios likely rejects a mortality driven causal effect.

In conclusion, our study shows that mean parasitoid sex ratios and variance in agroecosystems can depart significantly from those observed under laboratory conditions. We found that increasing host densities and parasitism rates both significantly increased the proportion males that were produced as well as the variance of sex ratios. These findings are in direct contrast to laboratory studies that show *G. ashmeadi* produces precise, female-biased sex ratios when ovipositing singly. The implications of such sex ratio shifts and variances in terms of the potential for effective biological control of this species are not straightforward but they might suggest several factors that can significantly affect the population biology of parasitoid species.

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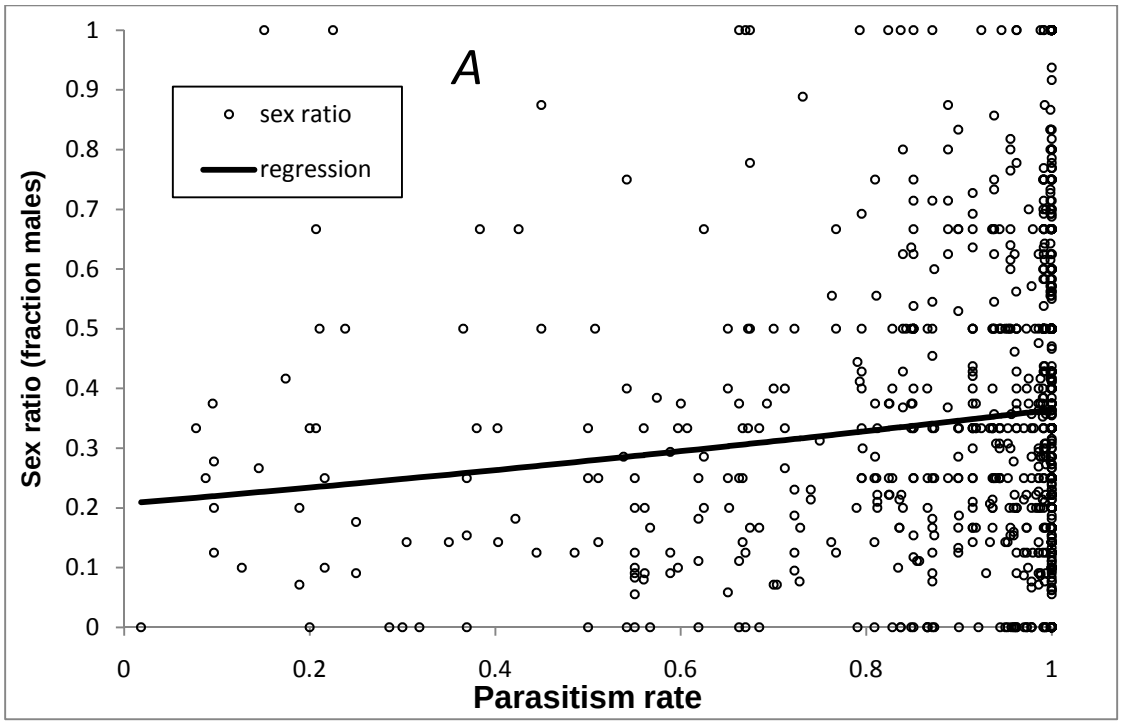
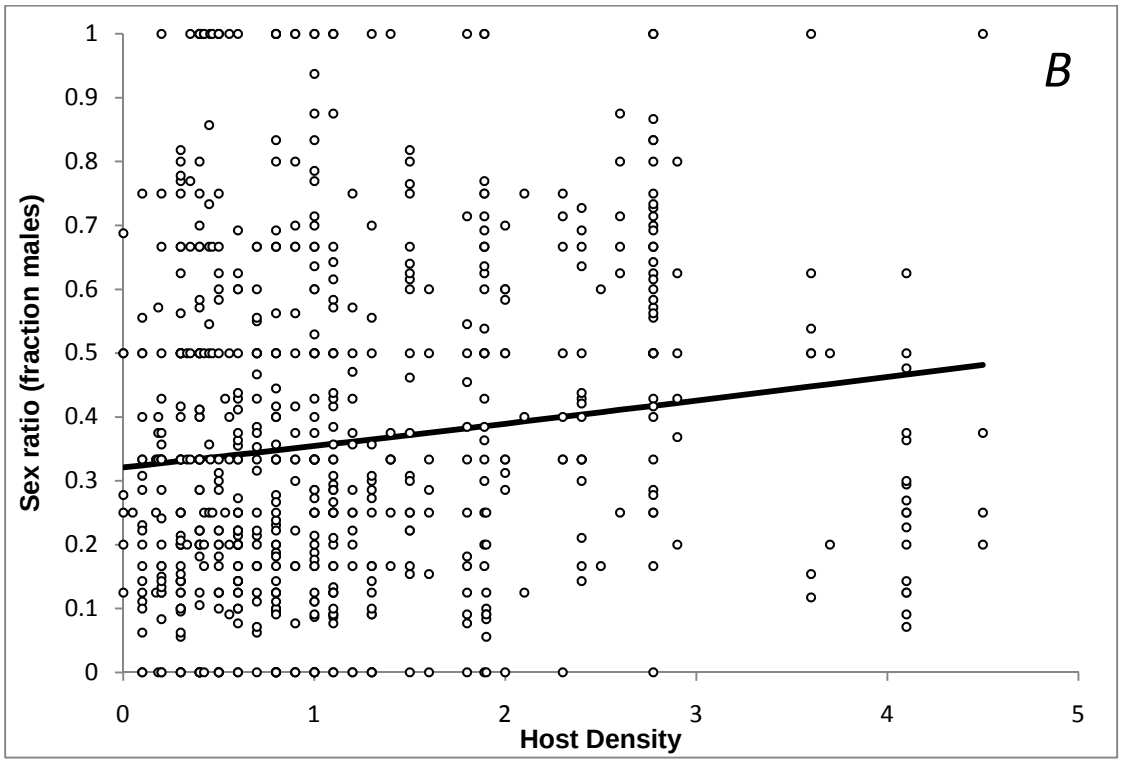
Figure 4.1. (A) Effect of parasitism rate on sex ratios of *G. ashmeadi;* (B) Effect of *H. vitripennis* egg mass density on sex ratios of *G. ashmeadi.*


A
sex ratio
regression
Sex ratio (fraction males)
Parasitism rate
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
0.2
0.4
0.6
0.8
1


B
Sex ratio (fraction males)
Host Density
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
1
2
3
4
5

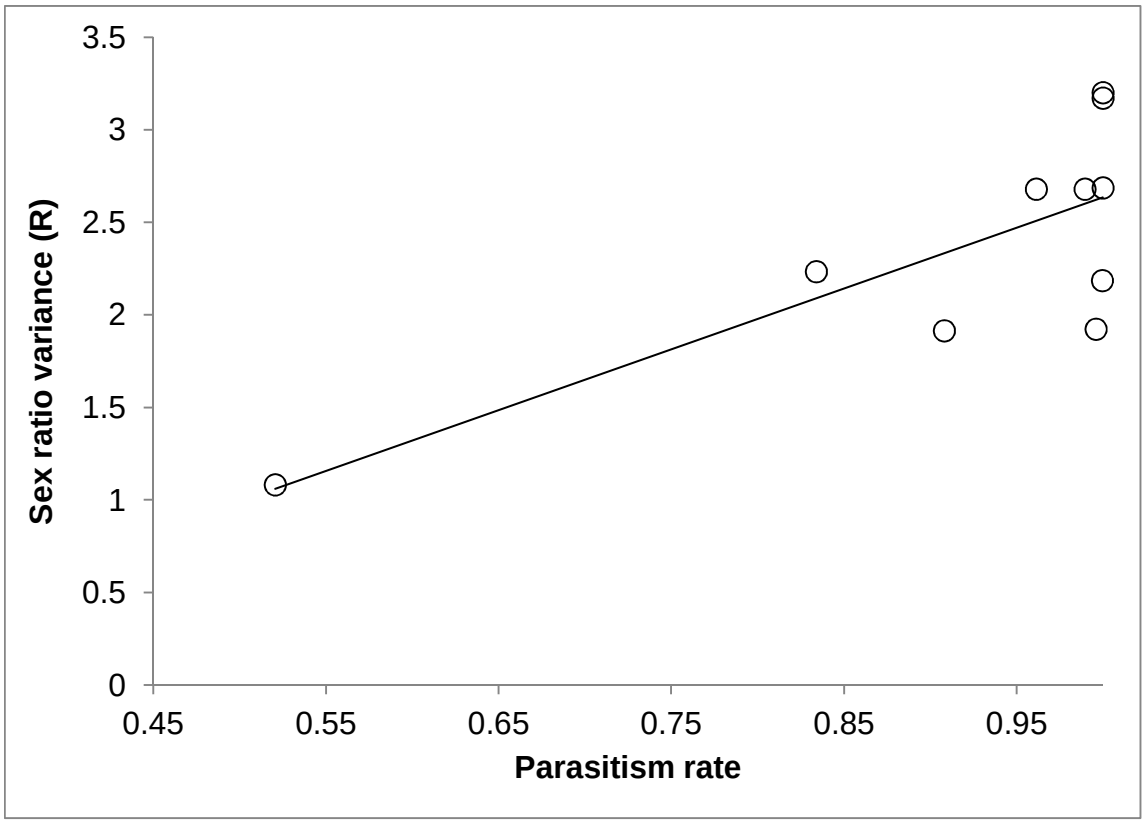


Figure 4.2. Effect of parasitism rate on *G. ashmeadi* sex ratio variance.

CONCLUSION

Findings from the studies presented herein have generated significant new information regarding the field distribution of parasitoids of the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar), biological traits of two potential candidates for the biological control of GWSS, *Gonatocerus deleoni* Triapitsyn, Logarzo & Virla and *Pseudoligosita plebeia* (Perkins), and sex ratio allocation patterns of *Gonatocerus ashmeadi* Girault in the field. Results from the two-year field study showed that lower proportions of GWSS eggs were parasitized during its first brood in spring and higher proportions were parasitized during its second brood in summer, when percentage parasitism often would reach 100%. *Gonatocerus ashmeadi* was the most prevalent parasitoid from the sites located in the interior area of southern California. However, in the coastal regions, *Gonatocerus walkerjonesi* Triapitsyn was the most commonly observed parasitoid. While my results suggest that *G. ashmeadi* is a highly effective biological control agent at interior sites, the data from coastal sites indicate *G. walkerjonesi* may be more effective at parasitizing sharpshooter eggs at coastal locations. It is not clear what factors favor the abundance of *G. walkerjonesi* in these areas, but they may include cooler temperatures found near the coast. Due to our findings of dominance in coastal sites, we believe *G. walkerjonesi* may be an effective biological control agent of GWSS at some locations within California and warrants further attention and study. *Gonatocerus walkerjonesi*

may be of particular interest if GWSS populations spread into the cooler climatic regions of central California around or in Napa County.

Gonatocerus deleoni is an egg parasitoid native to Argentina and has not yet been released in California. In order to assess its potential host range and examine its biology, experiments were conducted in the laboratory. *Gonatocerus deleoni* showed an average parasitism rate of 45.7% for all ages tested, with higher rates of parasitism on 1-3-d-old eggs and lower rates on 5-8-day-old eggs. This trend is typical of parasitoids of GWSS and likely indicates an inability to develop in the embryos of advanced nymphs. The fact that we observed low levels of parasitism in host eggs approaching the normal time for GWSS nymphal emergence does not necessarily indicate their ability to develop in advanced host embryos, but, more likely, the ability to develop in sterile host eggs, which are known to occur at a low and regular rate in GWSS populations. The parasitism rates produced by *G. deleoni* were similar to those seen by *G. ashmeadi* for most ages of GWSS eggs, however *G. deleoni* produced higher rates of parasitism on GWSS eggs that were four days and older. *Gonatocerus deleoni*'s average developmental time from oviposition to adult emergence was 18.8 d, with males developing faster than females (means of 18.0 d, males; 19.0 d, females). This developmental time is consistent with several other species of *Gonatocerus* that parasitize GWSS eggs, notably *G. ashmeadi.* The average sex ratio was 0.34 (fraction males) and sex ratio was not significantly affected by egg age. Provisioning both sexes with honey and water produced life spans approximately

threefold longer than when wasps were provided no food or water. Water is regularly available in agricultural and urban settings in the form of irrigation, rainfall, or dew, however nectar resources can be scarce in many settings, particularly when weed control is implemented. Creation of nectar sources by adding flowering cover crops or reducing weed control might improve *G. deleoni* persistence in the field by increasing longevity.

Gonatocerus deleoni was able to develop in eggs of GWSS and *Homalodisca liturata* Ball, but was unable to develop on eggs of *Graphocephala atropunctata* (Signoret) or *Erythroneura elegantula* Osborn. Although *H. liturata* is native to California, it also is a vector of *X. fastidiosa* and thus, decreasing its levels may be considered a positive result of releasing *G. deleoni* into the environment. The ability of *G. deleoni* to successfully parasitize *H. liturata* is shared by all tested species of *Gonatocerus* introduced to date for the control of GWSS. Oviposition behavior was seen on *H. vitripennis*, *H. liturata*, and *E. elegantula*. Oviposition into eggs of these species may be explained by their similar egg placement beneath leaf tissue compared with the native host of *G. deleoni*, *Tapajosa rubromarginata* (Signoret). Oviposition behavior was not observed on *G. atropunctata* eggs, which may be due to their more concealed placement deeper into plant tissues. *Gonatocerus deleoni* is a solitary parasitoid (i.e. one parasitoid at most can develop per host egg), so one possible reason for the lack of successful development in *E. elegantula* could be due to the insufficient size of the host egg to allow for complete parasitoid development;

eggs of *E. elegantula* are less than half the size of those of GWSS. Due to similar findings of longevity, developmental time, and parasitism rates compared with other species of *Gonatocerus* released, a good climatic match to its potential area of release, as well as a host range apparently limited to cicadellid eggs of similar size to GWSS, we conclude that *G. deleoni* is a suitable candidate for the biological control of GWSS in California.

Pseudoligosita plebeia is an egg parasitoid, native to Mexico, that has not yet been released in California. Experiments were conducted in the laboratory to examine its biological traits that may aid in assessing its potential as a candidate for biological control of *H. vitripennis*. *Pseudoligosita plebeia* is gregarious, with up to 6 adults emerging from a single GWSS egg. *Pseudoligosita plebeia* were able to successfully parasitize all ages of *H. vitripennis* eggs (1-8 d old), with higher parasitism in younger host eggs (1-3 d old) than in older host eggs (5-7 d old). Similar to our findings with *G. deleoni*, the ability of *P. plebeia* to develop in older GWSS eggs may be attributed to the presence of sterile host eggs. *Pseudoligosita plebeia* females are at least partially synovigenic, as they contained fewer mature eggs at younger ages (1 and 3 d old) than at older ages (5, 11, 15, and 31 d old). From the lifetime fecundity experiments, an increasing trend in daily offspring production was seen for *P. plebeia* from adult age 2 to 26 d followed by a decreasing trend with offspring produced up to age 75 d. When provided host eggs throughout their adult life, *P. plebeia* displayed an apparent ability to replenish eggs, as they were

able to produce offspring in greater numbers than were found in their ovaries through our dissections.

When provided with honey and water *P. plebeia* females lived significantly longer than those provided water alone, or no food or water (64.1, 2.3, and 2.0 d, respectively). This potentially long lifespan could allow *P. plebeia* to locate and parasitize a large number of host eggs. However, *P. plebeia* has a significantly reduced lifespan when not provided access to honey. Access to nectar sources could provide *P. plebeia* a great advantage for finding and parasitizing *H. vitripennis* eggs in a field setting. This is of particular importance due to the apparent synovigeny in this parasitoid (based on dissection data). As *P. plebeia* showed a delayed production of mature eggs, with only an average of 13.2 eggs present in the ovaries by day three after eclosion, the average lifespan of 2.26 d for females when given access only to water indicates that access to water alone in the field would be insufficient to allow for *P. plebeia* to produce very many offspring. Provision of nectar sources by planting flowering cover crops or borders, or by allowing some weeds to persist may improve *P. plebeia* persistence and its ability to establish by increasing longevity and egg production. Alternatively, studies might be done to determine how well *P. plebeia* persists on various sources of honeydew produced by a number of Hemiptera that are common on citrus and other plants harboring GWSS populations. It is unclear how *P. plebeia* would perform under field conditions in the presence of competing parasitoids, but due to its unique life history and potentially long life

span, we conclude that *P. plebeia* warrants further research regarding its potential as a biological control agent of *H. vitripennis*.

In our analyses of sex ratios of *G. ashmeadi* in field conditions, we showed that significantly greater proportions of males and higher variance of sex ratios were found associated with higher parasitism rates as well as with higher host densities. These findings largely deviate from laboratory work that shows *G. ashmeadi* produces female-biased sex ratios with low variance. The increased densities of hosts and parasitism rates found in the field indicate that populations of these wasps are dense, leading to multiple foundresses simultaneously allocating offspring in patches of hosts. Under this scenario, females may adaptively shift their sex allocation to produce a less female-biased sex ratio than would be predicted under a scenario typical of local mate competition. *Gonatocerus ashmeadi* may perceive opportunities for external mating of their offspring either from detection of host cues indicating prior parasitism or detection of other *G. ashmeadi*. Previous laboratory studies show that exposure to other *G. ashmeadi* prior to exposure to hosts did not produce a significant change to their sex allocation behaviour, indicating the detection of host cues is a more likely explanation.