

Evaluation of floral resources for enhancement of fitness of *Gonatocerus ashmeadi*, an egg parasitoid of the glassy-winged sharpshooter, *Homalodisca vitripennis*

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Received 18 April 2006; accepted 8 September 2006

Available online 16 September 2006

Abstract

The effect of five different food resources (*Coccus hesperidum* honeydew, honey-water, *Lobularia maritima*, *Fagopyrum esculentum* and water only) on the longevity, fecundity, and progeny fitness of female *Gonatocerus ashmeadi*, an egg parasitoid of the glassy-winged sharpshooter (*Homalodisca vitripennis*) was investigated in the laboratory. Female *G. ashmeadi* survived 208–405% longer (up to 11.7 days longer) when fed *C. hesperidum* honeydew, honey-water, *L. maritima* and *F. esculentum* compared with water only. Similarly, all food treatments resulted in up to 378% more progeny (up to 80 more total progeny) than those females provisioned with water only. Mean percentage female progeny ranged from 70 to 81% and did not significantly differ between food treatments. The highest daily fecundity (22–29 progeny) occurred on the first day of oviposition for each food treatment and generally declined as female *G. ashmeadi* aged. Females deposited 61–100% of offspring within the first 5 days, after which, daily fecundity usually declined to 0–6 offspring per day. For all experimental diets except *L. maritima*, the number of male progeny oviposited remained relatively constant over time (~2 males per oviposition bout) but the proportion of males oviposited increased as female *G. ashmeadi* aged, as oviposition rates declined and fewer female eggs were subsequently laid. There was a strong positive correlation between hind tibia length (HTL) and total progeny produced by *G. ashmeadi* fed honey-water. There was no correlation between HTL and longevity of female *G. ashmeadi* fed honey-water. Mean HTL of *G. ashmeadi* progeny did not significantly differ between food treatments. Estimated progeny fecundity based on HTL, indicated that offspring produced by females provided with water only was 12–15 eggs or 9–12% lower than females that had access to food resources. This difference was not statistically significant. Female progeny that were oviposited by females 9–13 days of age with access to food resources were significantly larger and contained on average 14–17 more eggs than those female progeny resulting from eggs deposited by females 1–8 days of age.

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Keywords: Cicadellidae; *Coccus hesperidum*; Conservation biological control; *Fagopyrum esculentum*; Fecundity; Hemiptera; *Homalodisca coagulata*; Honeydew; Hymenoptera; *Lobularia maritima*; Longevity; Mymaridae

1. Introduction

In California, the egg parasitoid *Gonatocerus ashmeadi* Girault (Hymenoptera: Mymaridae) is the key natural enemy of glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), [formally named

Homalodisca coagulata (Say) (Takiya et al., 2006)] (Pilkington et al., 2005). *H. vitripennis* is native to the southeast United States, established in California around 1990 (Sorensen and Gill, 1996), and successfully invaded French Polynesia (Tahiti) in 1999, Hawaii in 2004, and Easter Island in 2005 (Pilkington et al., 2005). *H. vitripennis* is currently present in 23 of 58 Californian counties (CDFA, 2005) and is a major threat to many agricultural and ornamental plant industries due to its ability to vector the xylem dwelling plant pathogenic bacterium, *Xylella fastidiosa*

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Wells et al. Different strains of *Xylella* cause scorch-like diseases in a wide range of host plants including grapes (worth \$3.2 billion [US] to California), almonds (\$1.6 billion), stone fruit (\$905 million), and *X. fastidiosa* strains outside of the USA threaten citrus (\$1.05 billion) (CDFA, 2005). Ornamental plants and shade trees such as oleanders, oaks, elms, maples, and sycamores are also susceptible to infection by *X. fastidiosa* (Hopkins and Adlerz, 1988; Purcell and Saunders, 1999; Hopkins and Purcell, 2002).

Gonatocerus ashmeadi is a solitary endoparasitoid and has been resident in California since 1978 (Huber, 1988). Genetic studies indicate this parasitoid is native to the southeast USA and probably invaded California with *H. vitripennis* (Vickerman et al., 2004). Naturally occurring populations of *G. ashmeadi* in California have been augmented with mass reared individuals from populations found in the southeastern USA and northeastern Mexico (CDFA, 2003). However, average parasitism rates of *H. vitripennis* eggs in spring and summer are only 12–19% and may not be sufficient to provide acceptable control of this pest in California (Pilkington et al., 2005). Methods to enhance the suppression of *H. vitripennis* populations by *G. ashmeadi* require investigation, and resource provisionment in the field may be one method that could increase parasitoid efficacy.

Floral, extrafloral nectar, and honeydew can maximize parasitoid longevity, fecundity, searching activity, parasitism rates, and increase female sex ratios (Tylianakis et al., 2004; Berndt and Wratten, 2005; Irvin et al., 2006b). However, herbicide-use in citrus orchards and vineyards removes potential floral resources (e.g., weeds) (Dent, 1995; Gurr et al., 2003). Extreme orchard hygiene is a potential problem to *Gonatocerus* spp. egg parasitoids as citrus flowers or *H. vitripennis* excreta have no nutritive value for these parasitoids (Irvin et al., 2006a). Understorey management (i.e., the deliberate manipulation of flowering plants beneath fruit-bearing plants in orchards and vineyards) is potentially one-way to enhance parasitoid populations in agricultural systems that can improve pest control by natural enemies by providing food and shelter (Gurr et al., 2000, 2004). Food resources that have shown potential for enhancing parasitoid populations in orchards and vineyards include buckwheat (*Fagopyrum esculentum* Moench) (Tylianakis et al., 2004; Irvin et al., 2006b), alyssum (*Lobularia maritima* L.) (Chaney, 1998; Irvin et al., 2006b), and insect honeydew (Johnson and Stafford, 1985; Miller, 1989).

It has been demonstrated in the laboratory that honey-water and *F. esculentum* can significantly increase the longevity of male and female *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* up to 1760%, 1223%, and 1359%, respectively, when compared with water (Irvin et al., 2006a). Furthermore, survival of female *G. ashmeadi* provided citrus foliage infested with soft scale (*Coccus hesperidum* L. [a common citrus pest]) was 565% higher than parasitoids on citrus foliage alone, and *L. maritima* increased female survival 252% compared with water. It is unknown whether these food resources which greatly increased longevity

could also enhance *Gonatocerus* spp. fecundity or the fitness of resulting progeny. *G. ashmeadi* is the key natural enemy of *H. vitripennis* egg masses in California (Pilkington et al., 2005) and was chosen for these studies investigating food resources on parasitoid fitness. Consequently, work presented here investigated the influence of honey-water, *C. hesperidum* honeydew, and nectar from *F. esculentum* and *L. maritima* flowers on fitness of *G. ashmeadi*.

2. Materials and methods

Laboratory colonies of *H. vitripennis* and *G. ashmeadi* were maintained at the University of California, at Riverside. Parasitoid colonies were held at $26 \pm 2^\circ\text{C}$ and 30–40% RH under a L14:10D photoperiod and reared on *H. vitripennis* eggs laid on ‘Eureka’ lemon leaves, a preferred lemon variety for *H. vitripennis* oviposition and parasitoid foraging (Irvin and Hoddle, 2004). Plants of *L. maritima* (cv. Easter Bonnet White) and *F. esculentum* (obtained from Johnny’s Selected Seeds, Albion, ME) were grown from seed and synchronous blooming was ensured by performing staggered sowings at 10–14 day intervals (see Irvin and Hoddle, 2005a and Irvin et al., 2006a for details on maintaining all experimental plants).

2.1. Effect of food treatment on *G. ashmeadi* longevity and fecundity

Ten to 19 replicates of each treatment, water only, honey-water (3:1 Natural uncooked honey, Wild Mountain Brand, Oakland CA), *F. esculentum*, *L. maritima*, and *C. hesperidum* honeydew were set up in the laboratory in a randomized complete block design at $26 \pm 2^\circ\text{C}$ and 30–40% RH under a L14:10D photoperiod. Treatments were set up as described by Irvin et al. (2006a) and one newly emerged (≤ 24 -h-old) presumably mated naive female *G. ashmeadi* was placed inside inverted 130 ml plastic vials (40 dram Plastic Vial, Thornton Plastics, Salt Lake City, UT) that covered the test material. Those females that had not mated (producing only male progeny) were excluded from the male progeny totals and sex comparison analyses. Honey-water and flowering plant material were replaced every day. Approximately 35 *H. vitripennis* eggs (< 72 -h-old [preferred by *G. ashmeadi* for oviposition {Irvin and Hoddle, 2005b}]) laid on ‘Eureka’ lemon leaves were provided daily to each female *G. ashmeadi* until death. Daily egg number for presentation was selected based on previous fecundity studies for *G. ashmeadi* (Pilkington and Hoddle, 2006). Exposed leaves bearing host egg masses were placed into Petri dishes (9 × 1 cm, Becton Dickinson Labware, Becton Dickinson and Co., Franklin Lakes, NJ) lined with moist filter paper (9 cm Whatman Ltd. International, Maidstone, England) and left at 26°C for 3 weeks to allow parasitoids to emerge. Leaves sometimes decayed which often prevented successful insect emergence, therefore unemerged eggs were dissected and the numbers of unemerged parasitoids were recorded and included in progeny calculations.

Emerged female progeny were stored in 75% ethanol and hind tibia length (HTL) was determined using the protocol outlined below.

2.2. Using HTL to predict *G. ashmeadi* longevity and realized fecundity

HTL as a measure of parasitoid size has been positively correlated with parasitoid longevity and fecundity (Bernal et al., 1999; Jervis et al., 2003). HTL is easily measured and is less sensitive to distortions that result from preparation to measure when compared to other insect body parts that could be assessed (Bai, 1986). The relationship between HTL and realized fecundity for *G. ashmeadi* was determined in the following way. Female parasitoids ($n = 12$) were maintained on honey-water and were provided excess *H. vitripennis* eggs until death. At time of death, females had the right metathoracic tibia removed and mounted on a microscope slide in Canada balsam and covered with a glass cover slip. The length of each tibia was measured to within 0.003 mm using an optical scale lens positioned in the eye piece of a compound microscope at 160 \times magnification. Tibiae were measured from the point of attachment to the femur to the top of the first tarsal segment at the base of the spur. The relationship between female HTL and longevity, and between female HTL and total number of progeny produced (realized fecundity) was determined using linear regression in SAS (1990). The latter relationship was used to predict potential fecundity of progeny reared under experimental conditions by measuring the HTL of female progeny and then estimating their fecundity from the regression analysis. For each food treatment that was being assessed, a maximum of 10 progeny were measured for each experimental female for each day that oviposition resulted in successful progeny production. A total of 2224 female *G. ashmeadi* progeny were slide mounted and HTL determined for each day and food treatment that produced female progeny.

2.3. Statistical analysis

Cox's Proportional Hazard Regression Model (Cox, 1972) was used to analyze longevity data and survival functions were separated using likelihood ratio tests (Casella and Berger, 2002). A sequential Bonferroni multiple comparison procedure was conducted to offer family wise error protection (Holm, 1979). Male and female progeny and total progeny data for each experimental treatment were square root transformed to normalize distributions. A BOXCOX analysis indicated a squared transformation was necessary for percentage female data prior to analysis. Mean male progeny, mean female progeny, mean total progeny, and mean percentage female progeny were compared between food treatments using one-way analysis of variance (ANOVA) on transformed data in SAS (1990). Tukey's Studentized Range test at the 0.05 level of significance was used to separate means. For each food treatment,

daily fecundity and proportion of male progeny were calculated for each day and graphed to determine trends over time. Additionally, a one-way ANOVA was conducted to determine differences in daily fecundity between the five food treatments for days 1–4, after which all control parasitoids had died. Tukey's Studentized Range test at the 0.05 level of significance was used to separate means.

To further explore the influence of parasitoid age on sex allocation, the mean number of male progeny produced per ovipositing female was calculated for each day within each treatment. Regression ANOVA's were performed for each treatment to determine whether slopes were significant. Parasitoid age was divided into three categories 1–4, 5–8 and 9–13 days, and mean progeny HTL and estimated progeny fecundity was calculated for each female/day combination to remove pseudo-replication (a maximum of 10 progeny tibiae were measured per female on each day). A two-way ANOVA was performed in SAS (1990) on untransformed HTL and estimated progeny fecundity data to determine interaction effects. Tukey's Studentized Range test at the 0.05 level of significance was used to separate means. Means and SEM's presented in figures were calculated from non-transformed data.

3. Results

3.1. Effect of food treatment on *G. ashmeadi* longevity and fecundity

Mean survival times of female *G. ashmeadi* on diets were significantly different and ranged from 2.6 ± 0.2 days in the water only treatment to 14.3 ± 1.9 days in the *C. hesperidum* treatment (Likelihood ratio test = 68.30, $df = 4$, $p < 0.005$; Fig. 1). Female *G. ashmeadi* survived 208–405% longer (up to 11.7 days longer) when fed *C. hesperidum* honeydew, honey-water, *L. maritima*, and *F. esculentum* compared with water only (Fig. 1). Realized fecundity ranged from 29.6 ± 4.5 progeny in the water only treatment to 110.4 ± 15.0 progeny in the *C. hesperidum* treatment (Fig. 1). *C. hesperidum* honeydew, honey-water, *L. maritima*, and *F. esculentum* resulted in up to 378%, 198%, and 273% more male progeny ($F = 9.61$, $df = 4$, 52, $p < 0.005$), female progeny ($F = 8.43$, $df = 4$, 52, $p < 0.005$), and total progeny ($F = 11.78$, $df = 4$, 63, $p < 0.005$), respectively, than those females provisioned with water only (Fig. 1). However, results were not significant when comparing female progeny between *F. esculentum* and water (Fig. 1). There was no significant difference in progeny production or survival between plant candidates *L. maritima* and *F. esculentum*, although females provisioned with honey-water and *C. hesperidum* honeydew survived up to 79% longer compared with *L. maritima*. Mean percentage female progeny ranged from 70 to 81% and did not significantly differ between food treatments ($F = 2.21$, $df = 4$, 51, $p = 0.08$; Fig. 1).

The highest daily fecundity (22–29 progeny) occurred on the first day of oviposition for each food treatment and gen-

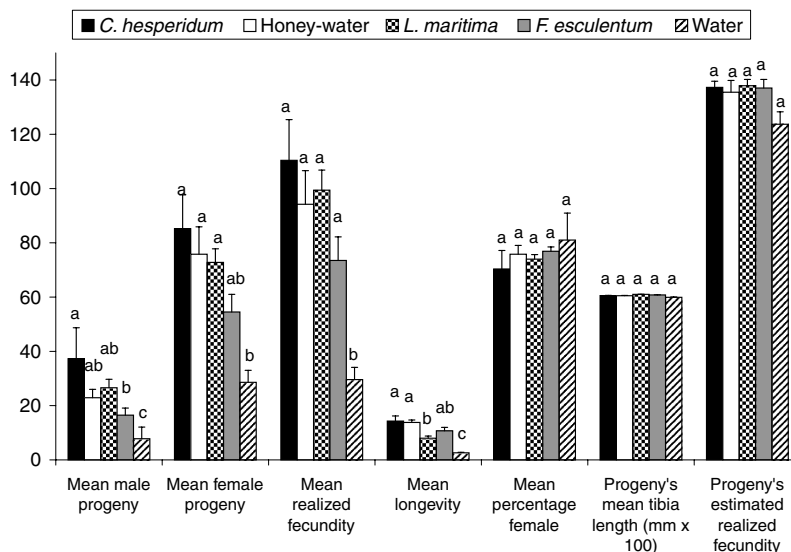


Fig. 1. Mean progeny, longevity, percentage of females, progeny size and estimated progeny fecundity when female *Gonatocerus ashmeadi* were provided five different food sources in the laboratory (different letters indicate significant [$p < 0.05$] differences between food treatments; error bars indicate +SEM).

erally declined as female *G. ashmeadi* aged (Fig. 2). Females laid 61–100% of offspring in the first 5 days of life, after which, daily fecundity usually declined to 0–6 offspring per day (Fig. 2). There was no significant difference in daily fecundity between the five food treatments for day 1, whereas, treatment had a significant effect on daily fecundity for days 2–4 (Table 1). For days 2 and 3, daily fecundity was 2–8 times greater in the food treatments compared with water, however, this comparison was only significant for *L. maritima* and honey-water (Table 1). *L. maritima* also resulted in significantly higher daily fecundity than *C. hesperidum* honeydew and *F. esculentum* (Table 1). For day 4, daily fecundity was 4–6 times greater in all food treatments compared with water, while no other significant differences between treatments occurred on this day (Table 1).

Female *G. ashmeadi* without continuous access to males to mate with generally oviposited a higher proportion of eggs that developed into male offspring with increasing age (Fig. 2). Regression analyses showed that over time there was no significant change in numbers of male progeny oviposited daily by female *G. ashmeadi* fed *C. hesperidum* honeydew ($F = 0.14$, $df = 1, 12$, $p = 0.71$), honey-water ($F = 3.42$, $df = 1, 11$, $p = 0.09$), *F. esculentum* ($F = 2.51$, $df = 1, 11$, $p = 0.14$), and water ($F = 1.19$, $df = 1, 2$, $p = 0.39$). Those females provided with *L. maritima* oviposited a significantly lower number of male progeny with increasing age (regression equation: $y = -0.3521x + 5.308$; $R^2 = 0.59$, $F = 14.62$, $df = 1, 10$, $p < 0.01$; Fig. 3).

3.2. Using HTL to predict *G. ashmeadi* longevity and realized fecundity

There was a strong positive correlation between realized fecundity and HTL ($F = 35.10$, $df = 1, 11$, $p < 0.005$; Fig. 4). There was no correlation between HTL and longevity of female *G. ashmeadi* ($F = 1.09$, $df = 1, 11$, $p = 0.32$).

3.3. Effect of food treatment on *G. ashmeadi* progeny

There was no significant interaction between age and food treatment for mean progeny HTL ($F = 0.95$, $df = 6, 111$, $p = 0.53$) and estimated progeny fecundity ($F = 0.85$, $df = 6, 111$, $p = 0.53$). However, age had a significant effect on progeny HTL ($F = 11.11$, $df = 2, 11$, $p < 0.005$) and progeny fecundity ($F = 11.05$, $df = 2, 11$, $p < 0.005$). Progeny that were oviposited 9–13 days after female emergence were significantly larger and contained on average 14–17 more eggs than those deposited 1–8 days after emergence (Fig. 5). Overall mean HTL of progeny ranged from 0.559 to 0.608 mm and did not significantly differ between food treatments ($F = 1.43$, $df = 4, 111$, $p = 0.23$; Fig. 1). Estimated progeny fecundity of offspring produced by females provided with water only was 12–15 eggs lower than those females provided with *C. hesperidum* honeydew, honey-water, *L. maritima* or *F. esculentum*. However, this result was not statistically significant ($F = 1.52$, $df = 4, 111$, $p = 0.20$; Fig. 1).

4. Discussion

Honey-water, *F. esculentum*, *C. hesperidum* honeydew, and *L. maritima* significantly increased longevity of female *G. ashmeadi* and enhanced realized fecundity up to 273% when compared with the water only treatment. These results indicate that resource procurement is not only essential for enhancing *G. ashmeadi* survival, but is also important for increasing *G. ashmeadi* realized fecundity. Results from comparing daily fecundity between food treatments demonstrated that *L. maritima* and honey-water resulted in significantly 3–8 times more offspring than water on days 2 and 3, while numbers of offspring oviposited on the fourth day was significant and 4–6 times greater in all food treatments compared with water (all control parasitoids died

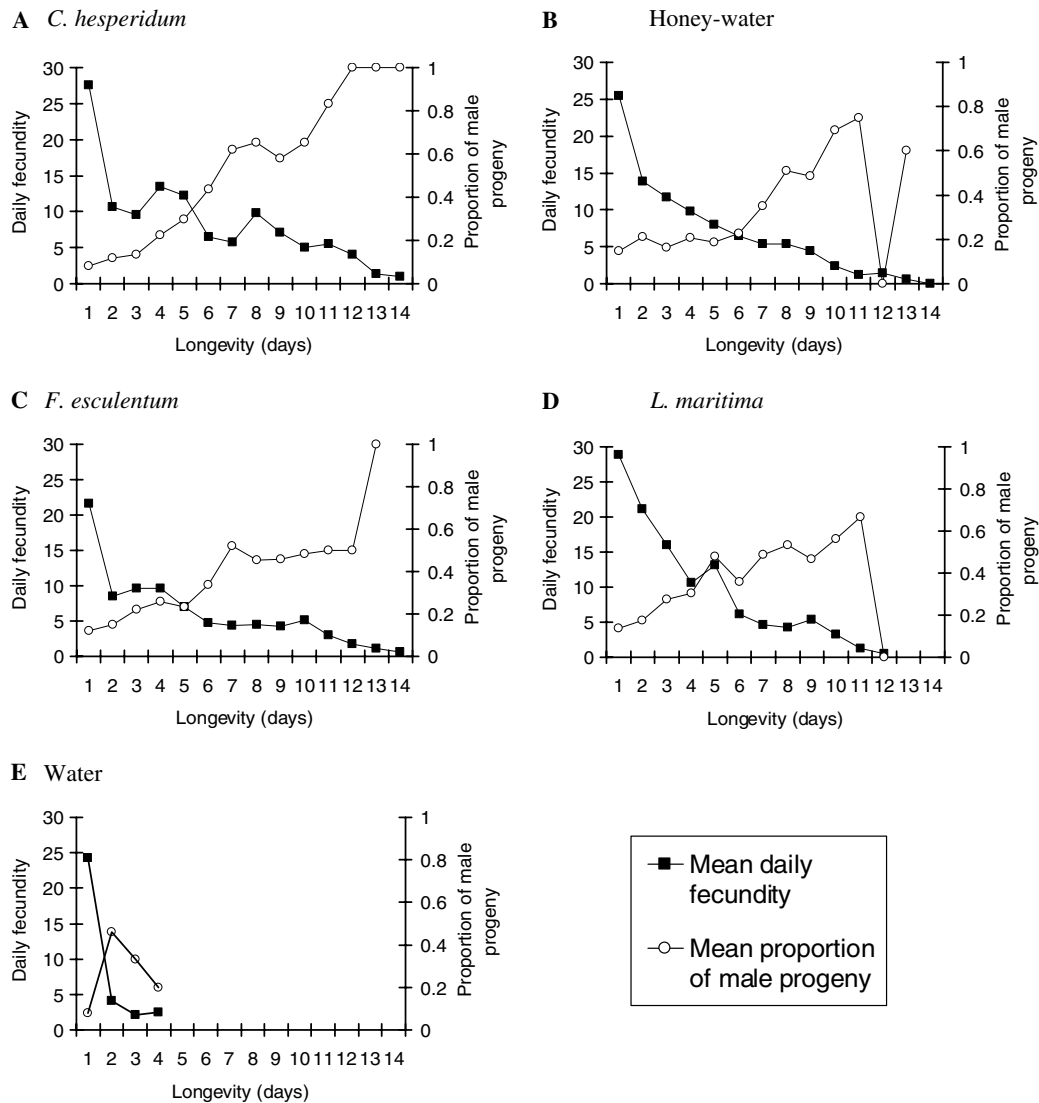


Fig. 2. Mean daily fecundity and proportion of male progeny produced per day for female *Gonatocerus ashmeadi* provided with five different food resources in the laboratory.

Table 1
 Mean daily fecundity (\pm SEM) for days 1–4 when female *Gonatocerus ashmeadi* were provided with one of five different food resources in the laboratory (different letters indicate significant [$p < 0.05$] differences between food treatment; test statistics are indicated)

Food treatment	Day 1	Day 2	Day 3	Day 4
<i>Lobularia maritima</i>	28.9 \pm 1.8 A	21.1 \pm 2.8 A	16.0 \pm 0.8 A	10.6 \pm 1.8 A
Honey-water	25.4 \pm 2.6 A	13.9 \pm 2.8 AB	11.8 \pm 1.4 AB	9.8 \pm 1.7 A
<i>Coccus hesperidum</i>	27.5 \pm 3.1 A	10.7 \pm 3.4 BC	9.5 \pm 1.2 BC	13.5 \pm 2.0 A
<i>Fagopyrum esculentum</i>	21.6 \pm 2.1 A	8.5 \pm 0.8 BC	9.7 \pm 1.1 BC	9.6 \pm 1.6 A
Water	24.2 \pm 3.2 A	4.2 \pm 1.7 C	2.1 \pm 1.8 C	2.5 \pm 2.5 B
<i>F</i>	1.39	8.72	22.64	8.38
df	4, 63	4, 62	4, 57	4, 51
<i>p</i>	0.25	<0.005	<0.005	<0.005

after day 4). This result indicates that enhanced realized fecundity from resource procurement was not only due to increased longevity, allowing females more time to parasitize hosts, but also to a direct increase in fecundity due to improved nutrition. Increased longevity and fecundity of female parasitoids resulting from resource procurement may enhance biological control of *H. vitripennis* because

increased longevity may increase host and mate encounter rates, and enhanced fecundity could result in a higher proportion of *H. vitripennis* eggs being parasitized leading to greater pest suppression. Additional studies have demonstrated that resource acquisition can positively affect parasitoid searching efficiency (Kazmer and Luck, 1995; West et al., 1996), generation time, primary sex ratio

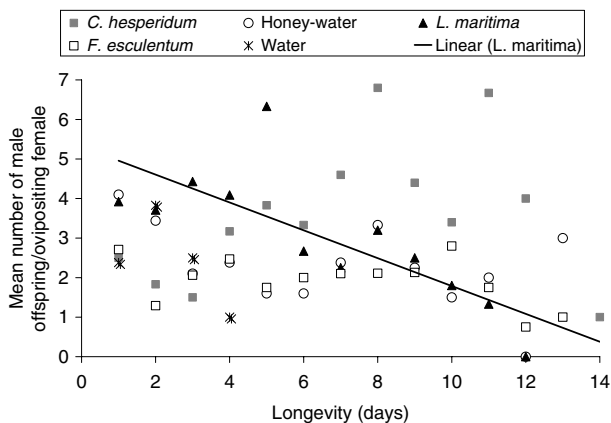


Fig. 3. The mean number of male offspring produced per ovipositing female with increasing parasitoid age when female *Gonatocerus ashmeadi* were provided five different food sources in the laboratory (regression line shows significant [$p < 0.01$] correlation for the *L. maritima* diet. There were no significant relationships for the other diets).

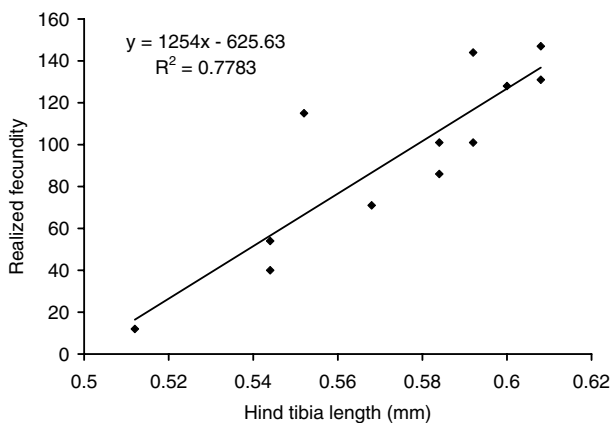


Fig. 4. The relationship between hind tibia length and realized fecundity when female *Gonatocerus ashmeadi* were fed honey-water in the laboratory.

(Berndt and Wratten, 2005), and egg viability (Jervis et al., 1996), which collectively could enhance biological control of target pest species.

Progeny size did not differ significantly between food treatments, suggesting that host size and quality may be more important than female nutrition for determining progeny size. Estimated progeny fecundity ranged between 124 and 138 eggs, and was statistically equivalent between treatments. This suggests that in the field, the realized fecundity of these offspring would be largely governed by longevity, which could not be predicted from HTL in the current study. This iterates the importance of resource subsidies for enhancing *G. ashmeadi* longevity in the field environment.

Gonatocerus parasitoids are generally classified as pro-ovigenic (Jervis and Copland, 1996), consequently we would expect daily fecundity not to significantly differ between food and water treatments since pro-ovigenic species emerge with their full complement of mature eggs (Jervis and Copland, 1996). Not surprisingly, this occurred on

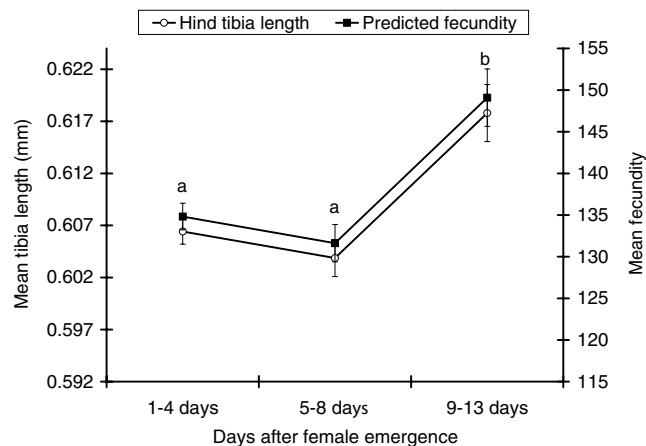


Fig. 5. Mean hind tibia length and predicted realized fecundity (based on hind tibia length) of female progeny oviposited 1–13 days after female *Gonatocerus ashmeadi* emergence (different letters indicate significant [$p < 0.05$] differences between ages; error bars indicate \pm SEM).

day 1 (i.e., the day of female emergence), however, daily fecundity was not equivalent between alyssum and honey-water, and water treatments for days 2–4, suggesting that *G. ashmeadi* may be partially syn-ovigenic. Irvin et al. (2006a) reported that female *G. ashmeadi* given access to honey-water survived 269% longer than those provided with honey-water and hosts. This may indicate that in the absence of hosts, females reabsorb mature eggs and redirect energy into host seeking and survival, a characteristic of syn-ovigenic species (Jervis et al., 1996). Further research investigating potential fecundity, egg maturation and oosorption is required to determine the ovigeny mechanism of *G. ashmeadi*.

Irvin et al. (2006a) demonstrated that citrus flowers and *H. vitripennis* excrement did not significantly enhance *G. ashmeadi* longevity and had nutritional value equivalent water. Results from work presented here suggest that low non-damaging *C. hesperidum* populations in citrus orchards may be beneficial for enhancing *G. ashmeadi* survival and fecundity. However, soft scale honeydew may not be a reliable food source for parasitoids because honeydew producers are often tended aggressively by ants (Johnson and Stafford, 1985), and it is unknown if *G. ashmeadi* forages in areas of infested citrus plants where they would encounter *C. hesperidum* honeydew.

Sowing flowering plants, such as *F. esculentum* and *L. maritima*, as an understorey in citrus orchards could potentially provide nectar to *G. ashmeadi*. Increased parasitoid longevity from resource subsidization may be most advantageous in early spring when parasitoid populations are low and hosts are scarce (Triapitsyn et al., 2003; Pilkington et al., 2005). Deliberate sowing of useful flowering plants that would provide food for *G. ashmeadi* may further enhance parasitism rates by reducing foraging time and energy expenditure associated with searching for nutrition, and by increasing the time available for host location and exploitation (Sirot and Bernstein, 1997). Energy requirements could be greater in the field since parasitoids may

encounter predators (e.g., ants), hyperparasitoids, difficult environmental conditions, and need to travel long distances to find hosts. All of these impediments to host location that would increase energy expenditure were eliminated in these laboratory studies.

Floral feeding is not always beneficial for the outcomes of a biological control program. Hyperparasitism or predation of parasitoids may be higher at close proximity to floral patches and may lead to significantly higher mortality of foraging adult parasitoids (Rosenheim, 1998; Stephens et al., 1998). Provisioning of floral resources may also enhance pest fitness (Irvin et al., 2006b; Baggen et al., 1999), in turn masking any effect of floral resources on the natural-enemy population and herbivore population (Lavandero et al., 2006). Baggen et al. (1999) demonstrated that 'selective resource subsidies' can benefit parasitoids and not the target pest. Further research is required to evaluate whether potential plant candidates for *G. ashmeadi* enhancement may act as potential reservoirs for *H. vitripennis* or *X. fastidiosus*.

In this and a previous study (Irvin et al., 2006a), *F. esculentum* and *L. maritima* have shown high potential as understory plant candidates for enhancing *G. ashmeadi* populations, and both plants have good agronomic characteristics. *L. maritima* is a semi-perennial plant that flowers quickly when sown from inexpensive and readily available seed (Chaney, 1998). *F. esculentum* is an annual that germinates easily, has a short sowing-flowering time. Seed of *F. esculentum* is also inexpensive and readily available (Bowie et al., 1995). *F. esculentum* may be more beneficial for *G. ashmeadi* since nectar enhances survival of both sexes of *G. ashmeadi* (Irvin et al., 2006a). Further investigation is required to determine the ability of *F. esculentum* and *L. maritima* to increase parasitism of *H. vitripennis* by *G. ashmeadi* in the field, and whether a combination of both plant species than either alone is more beneficial for parasitoids. A combination of *F. esculentum* and coriander (*Coriandrum sativum* L.) significantly increased the longevity of male *Dolichogenidea tasmanica* (Cameron) (Hymenoptera: Braconidae) than either plant species on their own (Irvin et al., 1999), possibly by providing a wider range of amino acids, sugars, and proteins required by the parasitoid (Hagen, 1986).

Gonatocerus ashmeadi reproduces via arrhenotoky (Triapitsyn et al., 2003), where female offspring arise from fertilized eggs that are diploid, and haploid males arise from unfertilized eggs (Flanders, 1965). Results presented here indicate that the percentage male progeny produced by female *G. ashmeadi* generally increased from a minimum of 8% to a maximum of 100% as females aged. Similarly, an increase in male progeny in response to female age was reported by Berndt and Wratten (2005) for *D. tasmanica* fed *L. maritima* flowers. The increase in male progeny production over time in the current study may be attributable to two factors: (1) in haplodiploid parasitoids, unfertilized eggs producing males are typically laid first followed by fertilized eggs that result in females (Waage, 1982). In this

study, daily fecundity of female *G. ashmeadi* was at its highest (22–29 progeny) on the first day of oviposition and declined to <4 per day as females reached the end of their lifespan. Therefore, a reduction in the total number of oviposited eggs could result in the deposition of proportionately more male progeny to female offspring because fewer eggs are laid even though the total number of male eggs laid does not change significantly (Antolin, 1992). This scenario is most likely to have occurred as opposed to (2) a lack of sperm to fertilize eggs because regression analysis indicated that numbers of males produced over time were relatively constant for the *C. hesperidum*, honey-water, *F. esculentum*, and water treatments. Interestingly, *L. maritima* produced significantly fewer male progeny over time suggesting a positive influence of this diet on daily female production as ovipositing females aged. A similar result was found for *D. tasmanica*, in which *L. maritima* flowers increased the proportion of female offspring compared with water in the laboratory (Berndt and Wratten, 2005), and a higher proportion of female offspring were reared from sentinel hosts in *F. esculentum* field plots compared with control plots (Berndt et al., 2002). This illustrates the possibility of enhancing natural enemy efficiency through resource subsidies increasing female sex ratio (Kean et al., 2003).

The strong positive correlation between female *G. ashmeadi* size as determined by HTL and realized fecundity indicated that HTL is a useful index estimating parasitoid fitness. However, there was no significant correlation between longevity and HTL in the current study. Previous work has demonstrated a positive relationship between HTL and parasitoid longevity (Harvey et al., 1994; Bernal et al., 1999). Conversely, Pavlík (1993) found that longevity was not correlated with size for several *Trichogramma* spp. and suggested that, although size can play an important role in influencing parasitoid longevity, the influence of size can be overruled by other internal factors such as genetics, or external factors such as temperature and humidity that can influence parasitoid survival rates.

Gonatocerus ashmeadi, *G. triguttatus*, and *G. fasciatus* are currently undergoing mass rearing and release in California as part of a classical biological control program for *H. vitripennis*. Lack of suitable resources, including alternative non-host food sources has been reported as an important factor causing the failure of natural enemies to establish or realize their full potential in classical biological control programs (Stiling, 1993; Gurr and Wratten, 2000). Results presented here and by Irvin et al. (2006a) demonstrate that resource provisioning can significantly enhance the survival of male and female *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* and more specifically, resource provisioning can lead to significantly increased fecundity for *G. ashmeadi*. Further research is necessary to investigate the influence of understory plants on parasitoid abundance and parasitism of *H. vitripennis* in the field, and to determine which of the best plant candidate(s) identified from these laboratory studies prove to be of most benefit to *Gonatocerus* spp. parasitoids in the field. Field trials should

determine attractiveness of plants to *Gonatocerus* spp., synchronicity of diurnal patterns of nectar secretion with parasitoid activity periods, flower phenology, resource competition with foraging bees and syrphids, and economic and cultural compatibility with the agricultural agronomic system. Additionally, research will be required to determine whether incorporating flowering plants in an orchard understorey will act as potential reservoirs for *H. vitripennis*, *X. fastidiosa*, and predators of *Gonatocerus* species.

Acknowledgments

This work was supported in part by the California Department of Food and Agriculture (CDFA) Pierce's Disease-Glass-Winged Sharpshooter Management Research Program. David Morgan, CDFA, Mt Rubidoux Field Station, Riverside, California kindly supplied parasitoids to initiate our colonies, and Justin Nay assisted with statistical analysis. We thank Mike Lewis, Lisa Gonzalez, and Ruth Vega for assistance in the field and laboratory. Finally, we thank the anonymous reviewers who provided many helpful comments on an earlier draft of this manuscript.

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