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The effect of resource provisioning and sugar composition of foods on longevity of three *Gonatocerus* spp., egg parasitoids of *Homalodisca vitripennis*

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Abstract

The effect of dietary supplements on the longevity of male and female *Gonatocerus ashmeadi*, *G. triguttatus* and *G. fasciatus* (Hymenoptera: Mymaridae), was determined in the laboratory. Treatments included: water only, 3:1 honey–water solution, floral and extrafloral nectars from five different plants (excised stems from *Fagopyrum esculentum*, *Lobularia maritima*, *Phacelia tanacetifolia*, *Anethum graveolens* and *Vicia faba*), honeydew from *Coccus hesperidum* and *Homalodisca vitripennis* (formally *H. coagulata*), a commercially available food supplement (Eliminade) and citrus foliage. Additionally, the sugar composition of each food resource was determined using HPLC and whole flower extracts. Honey–water and *F. esculentum* nectar significantly increased longevity of male and female *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* up to 1860%, 1323% and 1459%, respectively, when compared with water. For both sexes and all three parasitoid species, survival on citrus foliage, *H. vitripennis* excrement, and *P. tanacetifolia* flowers was equivalent to that on water only. The longevity of *G. ashmeadi* and *G. triguttatus* was up to 539% higher on Eliminade compared with water only, however there was no significant effect of Eliminade on survival of *G. fasciatus*. *Coccus hesperidum* honeydew increased survival times up to 665% for all mymarid species compared with citrus foliage alone. HPLC analysis indicated that food resources most beneficial to *Gonatocerus* parasitoids possessed a high proportion of glucose (up to 44%) and fructose (up to 53%), suggesting that sucrose may not be as important for parasitoid survival. Citrus and *P. tanacetifolia* flowers contained favorable proportions of glucose and fructose, but the inability of *Gonatocerus* spp. to benefit from this may be related to flower morphology which could prevent access to nectar.

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Keywords: *Anethum graveolens*; Cicadellidae; Conservation biological control; Eliminade; *Fagopyrum esculentum*; *Gonatocerus*; Hemiptera; *Homalodisca coagulata*; Honeydew; Hymenoptera; *Lobularia maritima*; Mymaridae; *Phacelia tanacetifolia*; *Vicia faba*

1. Introduction

The glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), [formally *H. coagulata* (Say) (Takiya et al., 2006)] is native to the southeast United States and likely invaded California in the late 1980s possibly through movement of ornamental plants from Florida (Sorensen and Gill, 1996). It has since become a major threat to many Californian agricultural and ornamental plant industries due to its ability to vector the plant pathogenic bacterium, *Xylella fastidiosa* Wells et al. This pathogen resides exclusively in xylem tissue causing lethal scorch-like diseases in a wide range of host plants including commercial crops such as grapes, stone fruit, almonds and citrus, and many ornamental and native plants (Hopkins and Adlerz, 1988; Purcell and Saunders, 1999; Hopkins and Purcell, 2002).

Pierce’s Disease caused the rapid destruction of over 300 acres of vineyard in Temecula Valley during 1997–1999 (CDFA, 2002), and in 2000, a classical biological control...
program was initiated to reduce the density and spread of *H. vitripennis* and *Xylella*-related diseases. Three non-host feeding mymarid egg parasitoids, *Gonatocerus ashmeadi* Girault, *G. triguttatus* Girault, and *G. fasciatus* Girault (Hymenoptera: Mymaridae) have been released in California, and are being evaluated for their ability to suppress *H. vitripennis* populations. *Gonatocerus ashmeadi* is a solitary endoparasitoid that has been resident in California since 1978 (Huber, 1988). Genetic analyses indicate it is native to the southeast USA and probably invaded California with *H. vitripennis* (Vickerman et al., 2004). *Gonatocerus triguttatus* is a solitary endoparasitoid native to Texas and central Florida, and has been imported from Texas and released in California since 2001 (CDFA, 2003). *Gonatocerus fasciatus* is a gregarious parasitoid that was introduced to California from Texas in 2002 (CDFA, 2003). Widespread establishment of *G. triguttatus* and *G. fasciatus* in California has not been confirmed, but post-release recoveries of these two parasitoids have been made (Morgan, D., C DFA, personal communication).

Floral and extrafloral nectar are significant sources of nutrients for most adult Hymenoptera and can maximize parasitoid longevity, fecundity, searching activity and parasitism rates (Takasu and Lewis, 1995; Tylianakis et al., 2004; Irvin et al., 2006). Understorey management (i.e., the deliberate management of flowering plants beneath fruit-bearing plants in orchards and vineyards) is potentially one way to enhance parasitoid populations in agricultural systems thereby leading to improved pest control by natural enemies (Gurr et al., 2000; Landis et al., 2000; Gurr et al., 2004). Understorey management can decrease abundance and increase parasitism of leaffoppers in Californian vineyards (Daane and Costello, 1998; Nicholls et al., 2000) and this strategy should be evaluated for potential at controlling *H. vitripennis*. Plant candidates that have shown potential for enhancing parasitoid populations in orchards and vineyards include buckwheat (*Fagopyrum esculentum* Moench) (Irvin et al., 2000; Nicholls et al., 2000; Berndt et al., 2002; Tylianakis et al., 2004; Irvin et al., 2006), alyssum (*Lobularia maritima* L.) (Chaney, 1998; Irvin et al., 2006), *Phacelia tanacetifolia* Benth. (Baggen and Gurr, 1998), dill (*Anethum graveolens* L.) (Baggen and Gurr, 1998), and extrafloral nectaries of broad bean (*Vicia faba* L.) (Bugg et al., 1989).

Several studies have demonstrated that flower morphology and corolla length can prevent some parasitic hymenopterans from exploiting nectar (Patt et al., 1997) and not all nectar are beneficial to parasitoids (Wäckers, 2001). Additionally, some parasitoids benefit from feeding on honeydew excreted by homopterans (Johnson and Stafford, 1985; Miller, 1989). The benefit from floral and extrafloral resources and homopteran honeydew derived by *Gonatocerus* spp. released for *H. vitripennis* control in California is unknown. Consequently, the research undertaken here sought to determine whether food resources such as floral nectars, extrafloral nectar, arthropod waste products, honey–water solutions, and a commercially available insect food spray could enhance the longevity of male and female *G. ashmeadi*, *G. triguttatus* and *G. fasciatus* in the laboratory. Additionally, the sugar composition of each food resource was determined using high performance liquid chromatography (HPLC) analysis. The results of these studies will allow further identification of nutrition sources that may enhance mymarid parasitoid activity against *H. vitripennis* in commercial citrus orchards and vineyards.

2. Materials and methods

2.1. Insect colonies

Laboratory colonies of *H. vitripennis*, *G. ashmeadi*, *G. triguttatus* and *G. fasciatus* were maintained at the University of California, at Riverside. Parasitoid colonies were held at 26 ± 2 °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; [see Irvin and Hoddle, 2005 for plant maintenance details]).

2.2. Plant material

Plants of *A. graveolens* (cv. ‘Bouquet’), *V. faba* (cv. ‘Windsor’), *P. tanacetifolia*, *L. maritima* (cv. Easter Bonnet White) and *F. esculentum* were grown from seed in a greenhouse at 26 ± 5 °C under natural L14:10D light. Synchronous blooming was ensured by performing staggered sowings at 10–14 day intervals. *Phacelia tanacetifolia* seed was sourced from Wildseed Farms, Fredricksburg, TX and all other seed was obtained from Johnny’s Selected Seeds, Albion, ME. All plants were fertilized every two weeks with Miracle-Gro (20 ml/3.51 of water, Scotts Miracle-Gro Products Inc., Marysville, OH). Plant material for experiments was cut immediately prior to treatment set-up and flowering stems and leaflets were placed through perforated lids attached to 130 ml vials containing tap water.

2.3. Investigating parasitoid longevity

Ten to twenty replicates of each treatment [water only, honey–water (3:1 Natural Uncooked Honey, Wild Mountain Brand, Oakland, CA) with no *H. vitripennis* eggs provided, honey–water with hosts provided, *P. tanacetifolia*, *F. esculentum*, *L. maritima*, *A. graveolens*, *V. faba*, *C. limon* (L.) Burn], the commercial food spray Eliminade mixed with water (1:1), *Coccus hesperidum* L. honeydew, *H. vitripennis* excrement, and citrus foliage (‘Eureka’ lemon) as a control] were set up in the laboratory in a randomized complete block design at 26 ± 2 °C and 30–40% RH under a L14:10D photoperiod. Plant treatments consisted of an 8-cm length of flowering stem or leaflet placed through the lid of a 130 ml plastic vial (40 dram Plastic Vial, Thornton Plastics, Salt Lake City, UT) filled with tap water and sealed with a cotton wool plug. Cut flowers were used to prevent parasitoids drowning or...
getting trapped in free-water and soil, and to more economically use available space for these experiments. Previous studies investigating floral use by parasitoids have also used cut flowers (Idris and Grafius, 1997; Patt et al., 1997; Irvin et al., 1999; Damon et al., 1999; Jacob and Evans, 2000; Wäckers, 2004). Furthermore, Wade and Wratten (2006) demonstrated no significant difference in survival of Aphidius arvi Haliday (Braconidae) between intact and excised flowers of *L. maritima, F. esculentum, P. tanacetifolia* and *A. graveolens*. A second 130 ml plastic vial with ventilation (three 2 cm holes [one on the bottom, and one on each of two sides] covered with mesh netting [80 μm mesh width Jeliff Corporation, Southport, CT]) was inverted and attached to the lid of the vial holding the water and plant material. One newly emerged (≤ 24 h old) naive female and male parasitoid were placed inside the inverted vial that covered the test material. Parasitoid longevity was recorded daily and plant material was replaced every three days until parasitoids died.

Honey–water and Eliminate treatments consisted of three droplets of solution placed with an eye-dropper on the lid of a 130 ml plastic vial containing the parasitoid pair. Food was applied once to the lid at the beginning of the experiment and replaced every three days. *Homalodisca vitripennis* excrement was collected from two adult females that were caged in a plastic vial on an eight centimeter tip of young ‘Eureka’ lemon foliage. Excreta from the feeding females accumulated in the vial where the parasitoid pair was present. Similarly, an eight-centimeter tip of ‘Eureka’ lemon foliage infested with *C. hesperidum* was caged with the parasitoid pair for the *C. hesperidum* honeydew treatment. ‘Eureka’ lemon foliage without *C. hesperidum* or *H. vitripennis* was used as a treatment control for comparison to *C. hesperidum* and *H. vitripennis* excreta treatments, to assess potential bias of an increase in humidity from transpiring foliage, which could effect parasitoid survival.

The honey–water with hosts treatment consisted of honey–water and 10 *H. vitripennis* eggs (~24-48 h old) laid on ‘Eureka’ lemon leaves. *Homalodisca* eggs were replaced every two days for five consecutive replacements. Water was supplied to parasitoids in all treatments via a moist cotton ball placed on the moist top of the inverted 130 ml plastic vial. Parasitoid longevity was recorded daily until death for each sex and males were not replaced once dead.

All longevity data was square root transformed prior to analysis in SAS (1990). A three-way analysis of variance (ANOVA) was used to determine if a three-way interaction effect (parasitoid species × food treatment × parasitoid sex) and two-way interaction effects (species × treatment; sex × treatment; sex × species) were significant. Since all interactions effects were significant, one-way ANOVA’s were used to determine the effect of treatment on longevity for each sex of each parasitoid species, and to determine the effect of parasitoid species on longevity for male and female wasps fed on each food treatment. All data sets with significant F-values were analyzed using Tukey’s multiple comparison of means test at the 0.05 level of significance to determine where significant treatment effects existed.

Results comparing species are presented for treatments which produced maximum survival for each sex. The effect of sex on daily survivorship rates across parasitoid species was determined using Studentized t-test comparisons. Means presented here have been back-transformed.

### 2.4. Extraction and analyses of soluble carbohydrates

Floral and extrafloral nectar typically consists of phloem, or phloem and xylem secreted by specialized stomata (Davis et al., 1988; Gaffal et al., 1998; Razem and Davis, 1999; Fahn and Shimony, 2001; Wist and Davis, 2006). Xylem is generally greater than 95% water with some amino acids (Anderson et al., 1989), therefore simple soluble sugars present in flowers would mostly consist of phloem present in vascular tissues and nectar. Consequently, whole flowers were used for HPLC in the current study to estimate the amount of soluble carbohydrates in nectar. For each flowering treatment (*L. maritima, F. esculentum, C. limon, P. tanacetifolia, A. graveolens*), 10 newly opened (~24–36 h of age) flowers from 12 individual plants were excised, wrapped in aluminium foil, immediately placed into liquid nitrogen, and stored in a ~ −80 °C freezer. Seven broad bean stipples were removed from each of 12 individual plants and treated as described. Frozen plant material was freeze-dried (LABCONCO Freeze Dry System Lyph-Lock 4.5) for three days and ground to a fine powder to assay for soluble carbohydrates.

Carbohydrates were extracted from powdered plant material following methodology of Bi et al. (2001). Ten milligrams of plant material were extracted three times, each for 8 min, in 1.2 ml of 80% ethanol in an 80 °C water bath. Microcentrifuge tubes (Fisherbrand 1.5 ml polypropylene microcentrifuge tubes, Fisher Scientific, Pittsburgh, PA) containing the combined extracts were stoppered and vortexed (Vortex Genie-2, Scientific Industries Inc, Bohemia, NY 11716) for 1 min before centrifuging (Eppendorf Minispin Plus, Brinkmann Instruments Inc, Westbury, NY 11590-0207) for 5 min (at 5000g) to obtain a clear alcohol extract.

Honey–water and Eliminate were diluted 1000-fold and 750-fold, respectively, with ultrapure water and then aliquoted into 0.5 ml samples in 1.5 ml microcentrifuge tubes prior to freezing at ~ −80 °C. *Coccus hesperi dam* honeydew was collected by placing a clean sheet of aluminium foil beneath *Yucca recurviflora* Salisb. plants infested with *C. hesperidum* for 24 h. Excreta was rinsed from the foil with 2 ml of ultrapure water which was collected. Dissolved *C. hesperidum* honeydew was filtered using 0.45 μm low protein binding, sterile Acrodisc filters (Gelman Sciences, Ann Arbor, MI) and aliquoted into 0.5 ml samples in 1.5 ml microcentrifuge tubes prior to freezing at ~ −80 °C. This procedure was repeated using clean aluminium foil and a number of equally infested plants to obtain 12 replicated samples.

*Homalodisca vitripennis* excreta was collected by placing five adult females into a 130 ml plastic vial with a plastic lid cut from the center to the edge. The vial with *H. vitripennis* was positioned over a growing tip of a potted ‘Eureka’
lemon tree by sliding the stem through the cut lid. After 24h the accumulated excreta from feeding *H. vitripennis* was filtered using 0.45 μm low protein binding, sterile Acrodisc filters, and aliquoted into 0.5 ml samples in microcentrifuge tubes prior to freezing at −80°C. This procedure was repeated using new ‘Eureka’ lemon trees and female *H. vitripennis* to obtain 12 replicated samples.

The ethanol-extracted samples were prepared for analysis by transferring 50 μl aliquots into 1.5 microcentrifuge tubes for rapid evaporation of the 80% ethanol. A gentle stream of nitrogen gas was applied to each tube through fine stainless steel tubes using a Pierce ReactiTherm III evaporation module. *Vicia faba* and *F. esculentum* ethanol-extracted samples were diluted 2-fold, while *A. graveolens*, *C. limon*, *P. tanacetifolia* and *L. maritima* were diluted 4-fold to adjust carbohydrate concentrations prior to analysis. Sugars and other non-structural carbohydrates such as alcohols in the reconstituted samples were quantified by HPLC using equipment and procedures described in Hendrix et al. (1992) and Hendrix and Wei (1994).

Total sugar concentration and the mean concentration of fructose, glucose, sucrose, sorbitol, inositol and maltose was calculated for each food sample, and compared between and within samples using Wilcoxon non-parametric analysis of variance at the 0.05 level of significance on non-transformed data. Critchlow–Fligner multiple comparisons (Critchlow and Fligner, 1991) was used to determine significant differences between means.

### 3. Results

There was a significant parasitoid sex × parasitoid species × food treatment interaction (*F* = 3.04; *df* = 24, 940; *p < 0.005). Two-way ANOVA interactions were also significant (sex × species: *F* = 8.68; *df* = 2, 66; *p < 0.005; sex × treatment: *F* = 6.56; *df* = 12, 335; *p < 0.005; species × treatment: *F* = 2.44; *df* = 24, 485; *p < 0.005). Results for one-way ANOVA’s are presented below.

#### 3.1. Treatment effects on *G. ashmeadi* survival

There was a significant effect of treatment on the longevity of female (*F* = 35.7, *df* = 12, 155, *p < 0.001) and male (*F* = 12.5, *df* = 12, 156, *p < 0.001) *G. ashmeadi* (Table 1). For females, honey–water, honey–water with hosts, *F. esculentum*, *A. graveolens*, Eliminade and *L. maritima* resulted in up to 1860% longer survival times than water alone (Table 1). Providing females with citrus foliage infested with *C. hesperidum* significantly enhanced longevity by 665% compared with citrus foliage alone. Female *G. ashmeadi* survival times on honey–water were 269% longer than female *G. ashmeadi* provisioned with honey–water and hosts (Table 1).

For male *G. ashmeadi*, honey–water, Eliminade, honey–water with *H. vitripennis* eggs, and *F. esculentum* resulted in up to 550% greater longevity in comparison to the water only treatment (Table 1). *Coccus hesperidum* honeydew produce the highest male *G. ashmeadi* longevity of all resource treatments. Male longevity was not significantly greater when provided *H. vitripennis* excrement compared with citrus foliage only.

#### 3.2. Treatment effects on *G. triguttatus* survival

There was a significant effect of treatment on the longevity of female (*F* = 27.13, *df* = 12, 153, *p < 0.001) and male (*F* = 20.2, *df* = 12,152, *p < 0.001) *G. triguttatus* (Table 2). For females, honey–water, *F. esculentum*, honey–water with *H. vitripennis* eggs, *A. graveolens*, *L. maritima* and Eliminade resulted in up to 1323% longer survival times in comparison to the water only treatment (Table 2). Honey–water resulted in the highest survival time for female *G. trigutta-

### Table 1

Significant differences for female and male *Gonatocerus ashmeadi* [different letters (A, B, C…) indicate significant (*p < 0.05*) differences in survival between food treatments; asterisks indicate significant (i = *p < 0.05*; ii = *p < 0.01*; iii = *p < 0.001*) differences in survival between sexes within food treatments; different Roman Numerals (i, ii, iii…) indicate significant (*p < 0.05*) differences in survival between species within food treatments]

<table>
<thead>
<tr>
<th>Food resource</th>
<th>Female <em>G. ashmeadi</em></th>
<th>Male <em>G. ashmeadi</em></th>
<th>Differences between sexes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean longevity (days) ± SEM</td>
<td>Differences between foods</td>
<td>Differences between spp.</td>
</tr>
<tr>
<td>Honey–water</td>
<td>46.5 ± 6.8 A</td>
<td>i</td>
<td>13.5 ± 3.7 A</td>
</tr>
<tr>
<td><em>Coccus hesperidum</em></td>
<td>17.3 ± 2.6 B</td>
<td>i</td>
<td>14.3 ± 2.4 A</td>
</tr>
<tr>
<td>Honey–water with hosts</td>
<td>139 ± 18 BC</td>
<td>i</td>
<td>11.0 ± 2.1 ABC</td>
</tr>
<tr>
<td><em>Fagopyrum esculentum</em></td>
<td>13.8 ± 1.8 BC</td>
<td>i</td>
<td>8.8 ± 0.9 ABCD</td>
</tr>
<tr>
<td><em>Anethum graveolens</em></td>
<td>10.2 ± 1.8 BCD</td>
<td>i</td>
<td>4.1 ± 0.5 DE</td>
</tr>
<tr>
<td>Eliminade</td>
<td>9.8 ± 2.3 BCD</td>
<td>i</td>
<td>11.2 ± 2.3 AB</td>
</tr>
<tr>
<td><em>Lobaria maritima</em></td>
<td>8.8 ± 1.4 CDE</td>
<td>i</td>
<td>4.9 ± 1.5 CDE</td>
</tr>
<tr>
<td><em>Vicia faba</em></td>
<td>4.5 ± 0.4 DEF</td>
<td>i, ii</td>
<td>4.8 ± 0.5 BCDE</td>
</tr>
<tr>
<td><em>Hymenosiga vitripennis</em></td>
<td>3.0 ± 0.4 F</td>
<td>i</td>
<td>2.0 ± 0.2 E</td>
</tr>
<tr>
<td>Citrus foliage control</td>
<td>2.6 ± 0.3 F</td>
<td>i</td>
<td>2.3 ± 0.3 E</td>
</tr>
<tr>
<td>Water</td>
<td>2.5 ± 0.3 F</td>
<td>i</td>
<td>2.6 ± 0.3 E</td>
</tr>
<tr>
<td><em>Phacelia tanacetifolia</em></td>
<td>2.2 ± 0.2 F</td>
<td>i</td>
<td>2.7 ± 0.2 E</td>
</tr>
<tr>
<td><em>Citrus limon</em></td>
<td>1.8 ± 0.2 F</td>
<td>i</td>
<td>1.8 ± 0.2 E</td>
</tr>
</tbody>
</table>
Food resource | Female *G. triguttatus* | Male *G. triguttatus* | Differences between sexes
--- | --- | --- | ---
Honey–water | 17.2 ± 2.9 | 17.1 ± 2.0 | A
Aegopodium podagraria | 13.4 ± 2.3 | 11.8 ± 1.2 | AB
Homalodisca vitripennis | 12.9 ± 1.6 | 12.3 ± 1.8 | AB
Anethum graveolens | 9.6 ± 0.9 | 5.6 ± 0.7 | CDEF
Coccus hesperidum | 8.9 ± 1.5 | 7.2 ± 0.8 | BCDE
Lobularia maritima | 7.7 ± 1.9 | 8.5 ± 1.3 | BCD
Eliminade | 5.8 ± 1.0 | 9.7 ± 1.1 | AC
Vicia faba | 4.8 ± 1.1 | 4.9 ± 0.8 | DEFG
Citrus limon | 2.3 ± 0.2 | 2.6 ± 0.3 | FG
Phacelia tanacetifolia | 2.2 ± 0.3 | 2.3 ± 0.3 | FG
Homalodisca vitripennis | 2.1 ± 0.3 | 2.0 ± 0.4 | G
Citrus foliage control | 1.8 ± 0.2 | 2.4 ± 0.1 | FG
Water | 1.3 ± 0.2 | 1.8 ± 0.2 | G

**Table 2**
Significant differences for female and male *Gonatocerus triguttatus* (see Table 1)

For male *G. triguttatus*, honey–water, honey–water with *H. vitripennis* eggs, *F. esculentum*, Eliminade, *L. maritima* and *A. graveolens* resulted in up to 950% longer survival times in comparison to the water only treatment (Table 2). Honey–water resulted in the highest survival time for male *G. triguttatus*. The provisioning of *F. esculentum* significantly enhanced male longevity by 211% and 241% when compared with *A. graveolens* and *V. faba*, respectively.

Providing *G. triguttatus* with citrus foliage infested with *C. hesperidum* significantly enhanced survival of females and males by 494% and 300%, respectively, when compared with the citrus foliage control (Table 2).

### 3.3. Treatment effects on *G. fasciatus* survival

A significant effect of treatment on the daily longevity of female (F = 23.34, df = 12, 147, p < 0.001) and male (F = 6.57, df = 12, 147, p < 0.001) *G. fasciatus* was detected (Table 3). For females, honey–water, honey–water with hosts, *F. esculentum* and *V. faba* resulted in up to 1459% greater longevity in comparison to the water only treatment. The honey–water treatment produced the longest average survival time of all food treatments, and resulted in 198% greater survival than females provisioned with honey–water and hosts (Table 3).

For male *G. fasciatus*, *F. esculentum* and honey–water with hosts resulted in up to 513% longer survival times compared with water only. *Fagopyrum esculentum* produced the highest survival time for male *G. fasciatus*, and led to significantly greater longevity (357% increase) when compared with *L. maritima* (Table 3). Providing *G. fasciatus* with citrus foliage infested with *C. hesperidum* significantly enhanced survival of females and males by 325–642% when compared with the citrus foliage control.

### 3.4. Interspecific longevity comparisons across treatments

There was a significant interaction between species and treatment (female: F = 6.07, df = 27, 454, p < 0.0001; male: F = 3.71, df = 27, 454, p < 0.0001). The maximum mean survival times for females of all three parasitoid species were produced on the honey–water treatment where mean
survival of female G. ashmeadi, G. triguttatus and G. fasciatus was 46.5 ± 6.8, 17.2 ± 2.9, and 24.8 ± 2.0 days, respectively (F = 11.12, df = 2, 33, p < 0.001; Tables 1–3). When provisioned with honey–water, female G. ashmeadi survived 270% and 188% longer than G. triguttatus and G. fasciatus, respectively. There was no significant difference in survivorship rates between G. triguttatus and G. fasciatus supplied with honey–water (Tables 1–3).

The maximum mean survival times for male G. ashmeadi, G. triguttatus and G. fasciatus were produced on the C. hesperidum, honey–water and F. esculentum treatments, respectively (means = 14.3 ± 2.4, 17.1 ± 2.0, and 8.2 ± 2.1 days) (Tables 1–3). When male parasitoids were provisioned with honey–water (F = 11.10, df = 2, 33, p < 0.001) male G. ashmeadi and G. triguttatus survived 338–428% longer than male G. fasciatus. Survival of male G. ashmeadi fed C. hesperidum honeydew (F = 3.61, df = 2, 31, p < 0.05) was 183–199% greater than G. triguttatus and G. fasciatus. Survival of male G. triguttatus was equivalent to G. fasciatus on this treatment (Tables 1–3).

3.5. Comparing male and female longevity

Parasitoid sex had a significant effect on overall longevity with females significantly outliving males for G. ashmeadi (t = 4.39, df = 201, p < 0.0001) and G. fasciatus (t = 5.11, df = 171, p < 0.0001). There was no significant effect of sex on overall longevity of G. triguttatus (t = 0.22, df = 188, p > 0.05) (Tables 1–3) shows longevity difference between sexes for individual treatments.

3.6. Extraction of soluble carbohydrates

There were significant differences in sugar concentrations across food resources (Table 4). Honey–water largely consisted of fructose and glucose, while Eliminade consisted of sucrose, glucose and fructose (Table 4). Glucose, fructose and sucrose were the constituents of H. vitripennis excreta. In addition to these three sugars, C. hesperidum honeydew contained maltoose; citrus, F. esculentum, P. tanacetifolia and V. faba had inositol; while L. maritima and A. graveolens contained inositol and sorbitol (Table 4).

There was a significant difference in the total sugar concentration and the concentration of each individual sugar type between food resources (Table 4). The total sugar concentration in honey–water was 742% higher than Eliminade, and both of these foods were up to 234–1739 mM higher compared with all remaining food resources. Similarly, honey–water was 2199–2891% higher in fructose and glucose compared with Eliminade, and these food resources resulted in up to 913.6 mM higher concentrations of fructose and glucose compared with all other foods (Table 4). Honey–water and C. hesperidum honeydew contained 55.8 mM and 0.2 mM of maltose, while no further food resources contained this sugar. The concentration of sucrose was 13983% higher in Eliminade compared with P. tanacetifolia flowers, and these food resources contained a significantly higher proportion of sucrose than all other foods (Table 4).

4. Discussion

4.1. Importance of floral resources for parasitoids

Honey–water and F. esculentum significantly increased longevity of male and female G. ashmeadi, G. triguttatus, and G. fasciatus compared with water indicating that resource procurement may extremely important for enhancing the survival of these parasitoid species in agroecosystems. Irvin and Hoddle (2006) demonstrated that supplying G. ashmeadi with an adequate food supply increased realized fecundity by up to 378% compared with females fed water. Increased longevity of female parasitoids resulting from resource procurement, reduced searching times for food because of deliberate provisioning, and possible increased fitness resulting from resource acquisition may enhance biological control of H. vitripennis by myrmcid parasitoids because longer lived parasitoids may encounter and parasitize more hosts (Jervis et al., 1996; Sirot and Bernstein, 1997).

Food resources that were most beneficial to Gonatocerus parasitoids (honey–water, F. esculentum and C. hesperidum honeydew) had a high proportion of glucose and fructose. Sucrose may not be important for parasitoid survival since honey–water resulted in maximum parasitoid longevity, yet did not contain measurable sucrose levels (honey typically contains ~1% sucrose [White et al., 1962]). Additionally, Rogers (1985) reported that sucrose rich nectars are less attractive to common Hymenoptera pollinators than those containing fructose and glucose.

Honey–water and C. hesperidum honeydew were very beneficial for parasitoid survival and were the only food resources that contained maltose, suggesting maltose may be beneficial for parasitoid survival. Citrus flowers contained similar proportions of glucose and fructose to honey–water, and possessed a significantly higher total sugar concentration compared with C. hesperidum honeydew and F. esculentum. Therefore, the inability of citrus flowers to enhance parasitoid survival may be attributable to flower morphology inhibiting parasitoid access, or poor nectar secretion once flowering stems are severed from the plant and used in laboratory experiments which affected longevity estimates.

The results of this work suggest that herbicide-treated citrus orchards may lack essential food resources for Gonatocerus spp. survival since longevity of parasitoids on citrus flowers and H. vitripennis excreta was equivalent to that on water. Sowing flowering plants, in particular, F. esculentum as an understorey in citrus orchards could potentially provide nectar to Gonatocerus spp. and this management practice needs to be evaluated in the field as a strategy for enhancing parasitoid efficacy against H. vitripennis.
Table 4
Sugar content (mM/10 mg dry weight of flower) of potential food sources [different letters (A, B, C...) indicate significant differences in sugar concentration between food resources within sugar categories; different Roman Numerals (i, ii, iii...) indicate significant differences in sugar concentrations within food resources].

<table>
<thead>
<tr>
<th>Food resource</th>
<th>Total sugar</th>
<th>Fructose</th>
<th>Glucose</th>
<th>Maltose</th>
<th>Inositol</th>
<th>Sorbitol</th>
<th>Sucrose</th>
<th>Comparisons between sugars $\chi^2$, df, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Honey–water</td>
<td>1739.2 ± 218 A</td>
<td>913.6 ± 112 A i</td>
<td>769.7 ± 99 A ii</td>
<td>55.8 ± 0.8 A iii</td>
<td>0 ± 0 A iv</td>
<td>0 ± 0 A iv</td>
<td>0 ± 0 A iv</td>
<td>$\chi^2 = 147.98$, df = 10, $p &lt; 0.005$</td>
</tr>
<tr>
<td>Eliminade</td>
<td>254.3 ± 63 B</td>
<td>31.6 ± 27 B i</td>
<td>35.0 ± 2.8 B i</td>
<td>0 ± 0 B ii</td>
<td>0 ± 0 A ii</td>
<td>0 ± 0 A ii</td>
<td>167.8 ± 2.1 B iii</td>
<td>$\chi^2 = 146.32$, df = 10, $p &lt; 0.005$</td>
</tr>
<tr>
<td>Citrus limon</td>
<td>8.9 ± 0.2 B</td>
<td>3.4 ± 0.1 C i</td>
<td>4.3 ± 0.1 C ii</td>
<td>0 ± 0 B iii</td>
<td>0.7 ± 0.5 B iv</td>
<td>0 ± 0 A iii</td>
<td>0.5 ± 0.1 C v</td>
<td>$\chi^2 = 140.85$, df = 5, $p &lt; 0.005$</td>
</tr>
<tr>
<td>Anethum graveolens</td>
<td>5.5 ± 0.1 D</td>
<td>1.4 ± 0.1 D i</td>
<td>1.7 ± 0.1 D ii</td>
<td>0 ± 0 B iii</td>
<td>0.4 ± 0.0 C iv</td>
<td>1.4 ± 0.1 B i</td>
<td>0.5 ± 0.1 C v</td>
<td>$\chi^2 = 142.77$, df = 10, $p &lt; 0.005$</td>
</tr>
<tr>
<td>Phaeacites tanacetifolia</td>
<td>5.2 ± 0.2 D</td>
<td>1.9 ± 0.1 E i</td>
<td>1.8 ± 0.1 D i</td>
<td>0 ± 0 B ii</td>
<td>0.3 ± 0.0 D iii</td>
<td>0 ± 0 A ii</td>
<td>1.2 ± 0.1 D iv</td>
<td>$\chi^2 = 149.82$, df = 10, $p &lt; 0.005$</td>
</tr>
<tr>
<td>Coccus hesperidum</td>
<td>3.9 ± 0.2 E</td>
<td>1.5 ± 0.2 D i</td>
<td>1.7 ± 0.1 D i</td>
<td>0.2 ± 0.0 C iii</td>
<td>0 ± 0 A iii</td>
<td>0 ± 0 A iii</td>
<td>0.6 ± 0.1 C iv</td>
<td>$\chi^2 = 133.74$, df = 10, $p &lt; 0.005$</td>
</tr>
<tr>
<td>Vicia faba</td>
<td>2.9 ± 0.3 F</td>
<td>0.2 ± 0.0 F i</td>
<td>1.5 ± 0.2 DE ii</td>
<td>0 ± 0 B iii</td>
<td>0.3 ± 0.1 CD iv</td>
<td>0 ± 0 A iii</td>
<td>0.9 ± 0.1 E v</td>
<td>$\chi^2 = 145.23$, df = 10, $p &lt; 0.005$</td>
</tr>
<tr>
<td>Lobularia maritima</td>
<td>2.7 ± 0.1 F</td>
<td>1.1 ± 0.1 G i</td>
<td>1.2 ± 0.1 E ii</td>
<td>0 ± 0 B iii</td>
<td>0.2 ± 0.0 D iv</td>
<td>0.1 ± 0.0 C iii</td>
<td>0 ± 0 G v</td>
<td>$\chi^2 = 149.82$, df = 10, $p &lt; 0.005$</td>
</tr>
<tr>
<td>Fagopyrum esculentum</td>
<td>2.5 ± 0.1 F</td>
<td>1.3 ± 0.0 D i</td>
<td>0.9 ± 0.0 F ii</td>
<td>0 ± 0 B iii</td>
<td>0.1 ± 0.0 E iv</td>
<td>0 ± 0 A iii</td>
<td>0.2 ± 0.0 G v</td>
<td>$\chi^2 = 133.74$, df = 10, $p &lt; 0.005$</td>
</tr>
<tr>
<td>Homalodisca vitripennis</td>
<td>0.8 ± 0.1 G</td>
<td>0.3 ± 0.0 H i</td>
<td>0.4 ± 0.1 G ii</td>
<td>0 ± 0 B iii</td>
<td>0 ± 0 A iii</td>
<td>0 ± 0 A iii</td>
<td>0 ± 0 H iv</td>
<td>$\chi^2 = 145.23$, df = 10, $p &lt; 0.005$</td>
</tr>
<tr>
<td>Water</td>
<td>0 ± 0 H</td>
<td>0 ± 0 I</td>
<td>0 ± 0 I</td>
<td>0 ± 0 I</td>
<td>0 ± 0 I</td>
<td>0 ± 0 I</td>
<td>0 ± 0 I</td>
<td>—</td>
</tr>
</tbody>
</table>

Comparisons between treatments: $\chi^2 = 144.32$, df = 10, $p < 0.005$, $\chi^2 = 140.85$, df = 10, $p < 0.005$, $\chi^2 = 142.77$, df = 10, $p < 0.005$, $\chi^2 = 149.82$, df = 10, $p < 0.005$, $\chi^2 = 133.74$, df = 10, $p < 0.005$, $\chi^2 = 145.23$, df = 10, $p < 0.005$. 
The current study may have underestimated the benefit of floral resources because parasitoids were presented with cut stems which may produce less nectar after harvest compared to uncut flowers (Jacob and Evans, 2000). However, Wade and Wratten (2006) demonstrated no significant difference in survival of Aphidius arvi Halidy (Braconidae) between intact and excised flowers of L. maritima, F. esculetum, P. tanacetifolia and A. graveolens. The current study was an exploratory investigation of the relationship between nectar composition and parasitoid longevity. HPLC analysis was conducted on samples containing whole flowers, therefore the sugar composition values for nectar are conservative estimates as phloem, xylem and other non-sugar compounds from the flowers would have been included in the analyses. Further research may be required to investigate sugar composition of the nectar only, and the presence of amino acids, lipids and antioxidants that may also be beneficial for parasitoid survival.

4.2. Identifying a suitable plant candidate for understorey planting

Phacelia tanacetifolia has increased parasitoid longevity in the laboratory (Baggen et al., 1999) and shown potential as an understorey plant for increasing the abundance of parasitoids in apple orchards (van der Bosch and Telford, 1964). However, the current study showed that for both sexes and all three Gonatocerus species tested, longevity on P. tanacetifolia was equivalent to that on water only. Jacob and Evans (2000) similarly found no effect of P. tanacetifolia on survival of the ichneumonid parasitoid Bathypelectes curculionis (Thomson). HPLC results presented here showed that P. tanacetifolia contained high proportions of glucose and fructose, which are probably beneficial for Gonatocerus spp. survival, and these flowers contained a significantly higher total sugar concentration compared with C. hesperidum, F. esculetum and L. maritima, which all promoted greater longevity of these parasitoids. Therefore, the inability of Gonatocerus spp. to benefit from nectar produced by P. tanacetifolia may be related to flower morphology. The long corolla of P. tanacetifolia may prevent exploitation by parasitoids (Jervis et al., 1993; Baggen et al., 1999) and outward pointing hairs, present on the style and ovary, and scale-like appendages within corollae, may limit nectar access by the Gonatocerus species studied here (Baggen et al., 1999; Jacob and Evans, 2000).

Vicia faba extranatural nectar increased survival of both male and female G. fasciatus but did not significantly increase the survival of male and female G. ashmeadi and G. triguttatus compared with water only. Extrafloral nectars tend to be sucrose rich and less attractive to many Hymenoptera compared with hexose rich floral nectars (Baker et al., 1978), and results from the current study showed that V. faba contained a significantly higher proportion of sucrose compared with all flowering treatments. Irvin et al. (1999) found that providing V. faba to Dolichogenidea tasmanica (Cameron) (Braconidae), in the laboratory increased longevity by 74% compared with water and this translated to an increase in parasitoid abundance and parasitism of a lepidopteran pest in apple orchards (Irvin et al., 2000). Extrafloral nectars are easily accessible for parasitoids and large amounts of nectar are available for extended periods throughout the growing season (Stapel et al., 1997; Yokoyama, 1978). However, our results suggest that V. faba may not be the most suitable understorey management option if all three Gonatocerus spp. are to benefit from floral resource provisioning for enhanced activity against H. vitripennis.

Chaney (1998) ranked 22 flowering plant species, including P. tanacetifolia, F. esculetum and L. maritima, for their potential as in-field ‘insectary plants’ for California grown lettuce crops. Lobularia maritima showed the greatest potential because no other plant tested flowered as quickly when sown from seed or attracted as many beneficial insects. In work presented here, L. maritima and A. graveolens significantly enhanced survival of female G. ashmeadi and G. triguttatus, but not G. fasciatus. Fagopyrum esculentum significantly enhanced survival of both sexes of all three parasitoid species, and parasitoids provisioned with F. esculetum lived longer than those provided with either L. maritima or A. graveolens. This result was most pronounced for G. triguttatus and G. fasciatus. Sowing F. esculetum in orchards can increase parasitism of key pests (Irvin et al., 2000; Tylianakis et al., 2004) and has led to lower abundance and higher parasitism of leafhoppers in vineyards (Nicholls et al., 2000; English-Loeb et al., 2003). Fagopyrum esculentum has good agronomic performance as it germinates easily, has a short sowing-flowering time, its seed is inexpensive and readily available. These agronomic characteristics combined with the beneficial effects F. esculentum has for parasitoids makes it a good candidate understorey plant for use in integrated pest management programs (Bowie et al., 1995) and our results suggest it may be the most beneficial plant for use with Gonatocerus spp. parasitoids.

4.3. The importance of sternorrhynchan excreta as a food source for parasitoids in orchards

Coccus hesperidum honeydew increased survival times by up to 565% for both sexes of all three Gonatocerus spp. compared with citrus foliage alone. Lopez et al. (2004) similarly found an 8-fold increase in survival of G. ashmeadi fed Dialeurodes citri (Ashmead) (Homoptera: Aleyrodidae) honeydew compared with water. In the current study, there was no significant difference in survival of parasitoids between the citrus foliage alone and water only treatments. This demonstrates that C. hesperidum honeydew enhanced parasitoid survival and not shelter, alternative food sources (such as aphid honeydew) or micro-climate, which the citrus foliage control treatment may have potentially provided. These results suggest that in citrus orchards, low non-damaging C. hesperidum populations may be beneficial for enhancing parasitoid survival and could enhance
biological control of *H. vitripennis*. However, soft scale honeydew may not be a reliable food source for parasitoids because honeydew producers are often aggressively tended by ants (*Johnson and Stafford, 1985*), and it is unknown if *Gonatocerus* spp. forage in areas of infested citrus trees where they would encounter soft scale honeydew.

In contrast to *C. hesperidum* honeydew, *H. vitripennis* excrement did not increase survival of any parasitoid species. *Homalodisca vitripennis* feed exclusively on xylem and metabolizes sugars with 99% efficiency resulting in excreta that is almost pure water (*Anderson et al., 1989*). In comparison, *C. hesperidum* feeds on phloem and produces a sugar rich honeydew that is nutritionally beneficial to a variety of parasitoid species (*Bogo and Mantle, 2000*). HPLC results in the current study demonstrated that *C. hesperidum* honeydew contained 488% more total sugar concentration compared with *H. vitripennis* excrement.

### 4.4. The potential of nutritional food sprays

Nutritional food sprays have been suggested as one way to provide resources to natural enemies in agricultural areas that either lack resources to maintain understorey plantings or such plantings would interfere with cropping practices (*Hagen et al., 1971*). Results presented here showed that the commercial food spray, Eliminade, specifically formulated as a nutritionally complete food for parasitoids (*Stephens et al., 1997*), enhanced survival of male and female *G. ashmeadi* and *G. triguttatus* up to 539% compared with water only. This result was not achieved with *G. fasciatus* where survivorship did not differ significantly from water. *Mathews and Stephens (1997)* investigated the influence of Eliminade on seven species of Hymenoptera in three families (i.e., Braconidae, Pteromalidae and Eurytomidae) and showed that all species survived longer when provisioned with Eliminade than parasitoids fed water only. *Mathews and Stephens (1997)* failed to document the concentration of Eliminade:water used in their methodology. In the current study, the 1:1 ratio used was recommended by the manufacturer, Entopath. Results showed that honey–water outperformed Eliminade in terms of enhancing parasitoid survivorship times. Eliminade may not have performed as well as honey–water because droplets of Eliminade at the concentration used in this study started to solidify after one day, and this may have prevented parasitoids being fully able to utilize this resource. Alternatively, Eliminade nutrition may not be optimal for *Gonatocerus* spp. Results showed that sucrose, the main constituent of Eliminade, may not be as beneficial for mymarid longevity since honey–water contained no sucrose, yet resulted in maximum parasitoid survival. The low concentrations of fructose and glucose, in conjunction with high viscosity of Eliminade may have attributed to its poor performance in the current study.

### 4.5. Differences in longevity between parasitoid species

Results presented here demonstrated differences in nutritional requirements between the three *Gonatocerus* species and indicate that it may be necessary to include mixed plantings of flowers in orchards and vineyards to allow all *Gonatocerus* spp. parasitoids to perform optimally. Furthermore, providing more than one flowering plant species may extend the period of nectar availability due to different flowering schedules, or supply a wider range of nutrients and amino acids to parasitoids, thereby further enhancing parasitoid longevity, fecundity and efficacy. *Irvin et al. (1999)* demonstrated in the laboratory that a combination of *F. eucalypturn* and *Coriandrum sativum* L. resulted in greater longevity of *D. tasmanica* parasitoids compared with either plant species alone.

### 4.6. Differences in longevity between parasitoid sexes and between host treatments

Female *G. ashmeadi* and *G. fasciatus* significantly out lived males suggesting that these species may exhibit ‘sib-mating’ where females live longer than males thereby allowing host seeking and oviposition, and males live long enough to mate with sisters emerging from the same egg masses (*Hardy and Cook, 1995*; *Quicke, 1997*; *Loch and Walter, 2002*). Male *G. ashmeadi*, *G. triguttatus* and *G. fasciatus* all emerge one day prior to females, remain close to the parasitized egg mass from which they emerged, and immediately begin mating with newly emerged females (*Triapitsyn et al., 1998; Irvin, personal observation*).

Female *G. ashmeadi* and *G. fasciatus* given access to honey–water survived 269% and 198% longer, respectively, than those provided with honey–water and hosts for oviposition. This may indicate that because hosts were absent, females reabsorbed eggs allowing additional energy reserves for survival (*Flanders, 1950*; *Meyerdink and Mortario, 1987*; *Heimpel et al., 1997*). Such a trait is characteristic of syn-oviginic females that have the capacity to develop eggs during adult life (*Jervis et al., 1996*). *Gonatocerus* parasitoids have generally been viewed as pro-ovigenic (*Jervis and Copland, 1996*) where females emerge with their lifetime complement of mature eggs and do not develop more eggs as they age. Conversely, there was no significant difference in *G. ashmeadi* longevity between sexes when parasitoids were provided with water only. *Hohmann et al. (1988)* similarly showed that the lifespan of female *Trichogramma platneri* (*Trichogrammatidae*) fed water only was unaffected by the presence of hosts eggs. When females were provided honey, survival was longer when hosts were absent. Further research investigating potential fecundity, egg maturation and oosorption is required to determine the ovigency mechanism of the *Gonatocerus* species used in these studies.

### 4.7. Conclusions

Resource provisioning can significantly enhance parasitoid longevity which may be very important for increasing parasitoid activity against *H. vitripennis* in commercial cropping situations. The fitness effects of resource subsi-
zation for these myrmid parasitoids has been quantified in the laboratory and positive effects demonstrated (see Irvin and Hoddle, 2006). Consequently, field trials with candidate understorey plants are required to determine if Gonatocerus spp. activity against *H. vitripennis* increases, and that understorey plants do not promote increased survivorship of immature and adult *H. vitripennis* or act as reservoirs for *X. fastidiosa* from which this pest can acquire bacteria.

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