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Spatial and temporal variation in sugar availability for insect parasitoids in agricultural fields and consequences for reproductive success



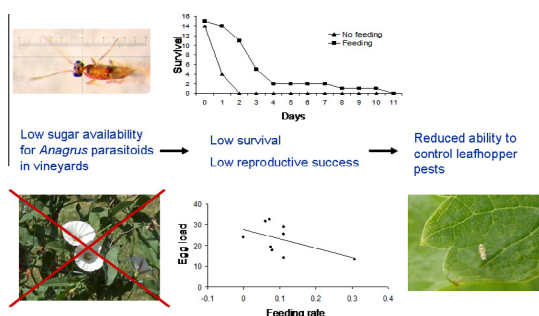
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HIGHLIGHTS

- Feeding on honey enhanced the longevity of *Anagrus* parasitoids in the laboratory.
- Feeding rates and survival of *Anagrus* parasitoids in the field were low, suggesting many die of starvation.
- Feeding rates differed among field sites and decreased as the season progressed.
- Feeding rates had a weak positive effect on the reproductive success of *Anagrus* females in the field.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 29 April 2013

Accepted 20 July 2013

Available online 27 July 2013

Keywords:

Anagrus

Erythroneura

Vitis

Vineyard

Nectar

Cold anthrone test

Egg load

ABSTRACT

Our ability to improve biological control of insect pests depends on our understanding of factors that limit the reproductive success of their natural enemies in the field. Pest control by parasitoids in agricultural fields can be compromised by the availability of sugar resources. We studied sugar availability for *Anagrus* parasitoids that parasitize the eggs of leafhopper pests (genus *Erythroneura*) in vineyards in northern California. Laboratory assays indicated that parasitoids survive less than a day on average without feeding, and ~3 days when supplied with honey. We captured foraging parasitoids in five unsprayed vineyards in northern California and applied a simple chemical test to assay the presence of sugar in their abdomens. Parasitoid abundance decreased throughout the day (08:00–16:00 h), suggesting that many parasitoids survive less than a day in the field. The proportion of feeding (sugar-positive) individuals was generally low (~10% on average); it varied among field sites and decreased as the season progressed (from July to October). Parasitoids captured in the afternoon were more often found with sugar in their guts compared to those captured in the morning hours. This may be explained by a direct positive effect of sugar on parasitoid survival, but may also result from longer-lived parasitoids simply having more time to locate scarce sugar resources. The egg loads of female parasitoids were negatively related to sugar availability, suggesting that sugar increases oviposition success, although we could not rule out a causal role for other site-date specific factors. Overall, our results suggest that *Anagrus* parasitoids in vineyards may be limited by sugar availability, and that adding sugar resources such as flowering cover crops, especially towards the end of the season, could potentially improve their efficacy as biological control agents. However, this is only likely to be successful in combination with the elimination of other factors that compromise parasitoid success.

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1. Introduction

Modern agriculture has simplified the agricultural landscape, increased the field size of crops, and reduced the amount of

non-crop habitat. It has been suggested that, collectively, these changes often contribute to an increase in herbivore densities (Bianchi et al., 2006; Matson et al., 1997). One of the basic explanations for this pattern is that natural enemies such as predators and parasitoids may be more abundant and/or efficient in reducing herbivore densities in more diverse plant habitats (Root, 1973). Diverse vegetation can provide habitat essential to perpetuate

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natural enemies by providing food resources, alternative hosts, overwintering grounds and shelter habitats (Altieri and Letourneau, 1982; Coll, 1998). In particular, establishment of floral vegetation can provide adult parasitoids with sugar resources that are required for energy and physiological maintenance, thereby potentially enhancing their efficiency as biological control agents (Heimpel and Jervis, 2005).

Feeding on sugar may benefit parasitoid reproductive success directly, via increasing egg maturation rate (Burger et al., 2004; England and Evans, 1997; Heimpel et al., 1997a; Olson and Andow, 1998; Tylianakis et al., 2004; Zhang et al., 2011) and/or indirectly, via increasing longevity and hence the total amount of time available for foraging and oviposition (Berndt and Wratten, 2005; Geneau et al., 2012; Giron et al., 2002; Lee and Heimpel, 2008; McDougall and Mills, 1997; Schmale et al., 2001). In the field, parasitoids obtain sugar from nectar or from homopteran honeydew, although the former is generally superior in nutritional value (Wackers et al., 2008). Indeed, there is accumulating evidence for increased parasitoid abundance and parasitism levels in the field in the presence of flowering plants (Berndt et al., 2006; Ellis et al., 2005; Lavandero et al., 2005; Masetti et al., 2010; Simpson et al., 2011; Stephens et al., 1998; Tylianakis et al., 2004). Nevertheless, a number of studies manipulating floral resources have not shown a consistent increase in parasitism rates (Berndt et al., 2002; Cappuccino et al., 1999; Lee et al., 2006; Lee and Heimpel, 2005; Nicholls et al., 2000; Pfiffner et al., 2003; Winkler et al., 2010). One possible explanation for a lack of effect is that parasitoids may not be limited by sugar resources in the field (Heimpel and Jervis, 2005). For example, several studies found that a large proportion of parasitoids feed naturally on sugar resources in unsupplemented fields or plots (Casas et al., 2003; Desouhant et al., 2010; Heimpel and Jervis, 2005; Lee et al., 2006). Even if parasitoids rarely feed in the field, their lifespan may be compromised more strongly by other factors. For example, high mortality from predators, high temperatures or other adverse weather conditions may reduce the likelihood that adult parasitoids will live long enough to reach starvation (Heimpel et al., 1997b; McDougall and Mills, 1997; Roitberg et al., 1992; Rosenheim, 1998). Similarly, a positive effect of feeding on potential fecundity may be of little importance if parasitoids rarely deplete their eggs in the field (Heimpel and Jervis, 2005). Finally, several studies have found possible counter-productive effects of increased sugar consumption, e.g., reduced longevity (Ellers et al., 2011) and reduced host searching behavior (Lightle et al., 2010). The availability of sugar resources, the relative importance of different mortality risks, and the occurrence of egg limitation may all fluctuate in space and time, and hence, the contribution of sugar feeding to parasitoid reproductive success in the field is likely to be highly dynamic.

Here we studied sugar availability for two parasitoid species of the genus *Anagrus* in the field. *Anagrus* spp. parasitoids are considered the most important natural enemies of leafhoppers in the genus *Erythroneura*, major herbivores in California vineyards (Bentley, 2009). Despite evidence for positive effects of sugar feeding on longevity in the laboratory (English-Loeb et al., 2003), attempts to improve biological control of leafhoppers by *Anagrus* spp. via supplementing flowering cover crops have had limited success. In one study, the use of flowering cover crops in vineyards was shown to reduce leafhopper populations, but this was more likely caused by reduced vine vigor than by enhanced biological control by parasitoids (Costello and Daane, 2003). In two additional studies, reduced leafhopper abundance in vineyards with cover crops was not accompanied by increased parasitism rates, and was instead explained mainly by increased predator abundance (Hanna et al., 2003; Nicholls et al., 2000). In a fourth study, establishment of flowering plants between vine rows had some positive

effects on parasitoid abundance and on parasitism. However, this did not translate into reduced leafhopper abundance (English-Loeb et al., 2003). The weak effects of cover crops on parasitism by *Anagrus* in these studies may suggest that parasitoids were not limited by the availability of sugar resources, but rather that stronger limitations on their reproductive success were imposed by factors other than sugar availability. For example, insecticides and fungicides such as sulfur are commonly used in vineyards and have been shown to have strong negative effects on *Anagrus* spp. survival (Jepsen et al., 2007a; Martinson et al., 2001). Hence, supplementation of floral resources in pesticide-treated vineyards may not be effective in enhancing biological control by *Anagrus*.

We took one step back to address the basic assumptions underlying the feasibility of floral supplementation to enhance biological control by *Anagrus* parasitoids in vineyards. To rule out adverse effects of pesticides on parasitoid performance, we worked in vineyards that were not treated with either insecticides or fungicides. To characterize spatial and temporal variation in sugar availability in the field, we sampled parasitoids in four vineyards throughout the day, and along the season and applied a simple chemical test to detect the presence of sugar in the abdomens of sampled individuals. We further investigated the possible consequences of sugar feeding for the realized reproductive success of adult females in the field. *Anagrus* spp. are proovigenic, i.e., females emerge with their full egg load, and do not resorb eggs (Jepsen et al., 2007b). Hence, feeding on sugar cannot affect egg maturation rates; rather, it could only enhance reproductive success via increased lifespan and/or oviposition rate. To measure oviposition success, we dissected samples of naturally foraging females and counted the number of eggs remaining in their ovaries. Since adult females do not mature or resorb eggs, their egg load changes only through oviposition, providing a more direct estimate of oviposition success than can readily be achieved for synovigenic parasitoids (Casas et al., 2000; Lee and Heimpel, 2008). We show that sugar availability varies both spatially and temporally and provide possible evidence for a weak effect of sugar feeding on reproductive success.

2. Materials and methods

2.1. Parasitoids and host insects

We studied two species of parasitoid wasps of the genus *Anagrus*, *A. daanei* Triapitsyn and *A. erythroneurae* Triapitsyn Chiappini (Hymenoptera, Mymaridae), which parasitize eggs of leafhoppers in the genus *Erythroneura* (Homoptera: Cicadellidae), major herbivores of grapes in California (Doutt and Nakata, 1973; Kido et al., 1983). *Anagrus* spp. complete their entire development (egg to adult) inside the leafhopper egg, consuming the egg as they develop. These parasitoids are solitary (develop singly inside the leafhopper), proovigenic (emerge with their full egg load), and do not resorb eggs (Jepsen et al., 2007b). Emergence of parasitoids occurs mainly during the early morning hours. *Erythroneura* spp. females deposit eggs singly or in clusters under the leaf epidermal tissue or along leaf veins. Each egg is minute, about 0.8 mm long. The freshly deposited egg is transparent. When eggs are parasitized, they become brown or red. We found three species of leafhoppers in our field sites: (i) the western grape leafhopper *Erythroneura elegantula* Osborn, which is native to California; (ii) the variegated leafhopper *Erythroneura variabilis* Beamer; and (iii) the Virginia creeper leafhopper *Erythroneura ziczac* Walsh.

2.2. Field sites

We worked in agricultural fields that were not treated with either insecticides or fungicides. In particular we avoided fields

that were sprayed with sulfur, which has been shown to be harmful to the wasps (Jepsen et al., 2007a; Martinson et al., 2001). We chose one vineyard in a private farm in Solano County, south of Davis [38.498181, -121.696683]; one vineyard that is grown for non-commercial purposes in Village-Homes neighborhood in Davis [38.549737, -121.782278]; one experimental field within the UC Davis campus [38.52826, -121.80263]; and one commercial field in Pope Valley [38.651349, -122.466788]. All vineyards received irrigation, fertilization and weed suppression.

2.3. Parasitoid survival in the laboratory

To characterize parasitoid longevity in the laboratory, we brought fresh leaves with leafhopper damage from the Village Homes field site to the laboratory on 4 September, 2010 and placed them inside emergence cages. The cages were carton containers with a transparent funnel and a vial on top. Emerging parasitoids were attracted to the light and were collected from the vials daily. Parasitoids were randomly assigned to one of two treatments: (1) *Unfed* parasitoids were placed in individual glass vials without any food ($N = 14$); and (2) and *Fed* parasitoids were placed in individual glass vials with a tiny drop of honey smeared on the side ($N = 15$). In preliminary tests parasitoids were often observed feeding on honey presented to them in this manner. All wasps were held at room temperature (23 °C) and observed daily until they were found dead. For this experiment we used male and female parasitoids of both species (*A. daanei* and *A. erythroneuræ*).

2.4. Parasitoid sampling

To characterize egg loads and sugar availability for actively foraging parasitoids from the field, we shook grape canes above a white plastic cafeteria tray (25 × 36 cm) at each site several times during the season (July to September 2010). We sampled four times in the site south of Davis (8 July; 4–5 August; 13 and 28 September); three times in Village Homes (7 July; 9 September; 18 October); twice in UC Davis campus (20–21 July; 31 August); and twice, over several days, in Pope Valley, where parasitoid densities were particularly low (14–15 July; 3–8 September). Sampling was performed for 30 min for every hour from 08:00–16:30 h. Parasitoids were held at -30 °C prior to sugar assays. Sample sizes

(Table 1) were highly unequal across dates and sites because of strong variation in population density.

2.5. Parasitoid abundance in the field

The number of cane shakes per half hour of sampling depended on the number of parasitoids falling on the trays (if many individuals were found, it took longer to put them in vials, and consequently the number of shakes was reduced). Therefore, we recorded the number of shakes per half hour for a sample of bouts by one of the samplers ($N = 144$ bouts, 15–17 for each time frame). This allowed us to estimate the number of parasitoids captured per shake as our primary measure of parasitoid abundance.

2.6. Sugar availability in the field

All of the males and a sample of the collected females were assayed with a cold anthrone test to detect the presence of sugar in their guts (van Handel, 1984). We placed an individual *Anagrus* inside a drop of the anthrone reagent on a slide. We covered it with a cover slip and pushed down gently to liberate the gut contents. The presence of a sugar meal in the gut of an individual parasitoid was signaled by the appearance of a blue–green spot around the insect's gut within an hour (Heimpel et al., 2004). Female *A. erythroneuræ* and *A. daanei* were distinguished using a phase-contrast microscope (Triapitsyn, 1998) while on the slide with the anthrone reagent. We could not distinguish between males of the two species. To confirm the reliability of the anthrone test, we also tested a group of newly emerged individuals that were kept in a vial with honey for an hour ($n = 18$) or that were kept unfed ($n = 16$).

2.7. Egg loads of females from the field

We dissected the remaining females in a drop of water under a dissecting microscope to count the number of eggs in their abdomen. We then slide mounted females in Hoyer's solution to measure the length of their hind tibia and to identify the species. In addition, we estimated the number of eggs laid by females. This was calculated by using an estimate of a female's initial egg load (based on her body size, according to a previously established

Table 1

Sample sizes of live female *A. daanei* and *A. erythroneuræ* that were either dissected or used for anthrone tests.

Location	Date collected	Anthrone test		Dissections	
		Sex	N	Species	N
South Davis	7-Jul	females	23	<i>A. daanei</i>	26
		males	22	<i>A. erythroneuræ</i>	15
	4-Aug	females	16	<i>A. daanei</i>	17
		males	22	<i>A. erythroneuræ</i>	5
	13-Sep	females	5	<i>A. daanei</i>	23
		males	23	<i>A. erythroneuræ</i>	1
28-Sep	females	6	<i>A. daanei</i>	14	
	males	14	<i>A. erythroneuræ</i>	0	
Davis Campus	20-Jul	females	20	<i>A. daanei</i>	65
		males	29	<i>A. erythroneuræ</i>	5
	31-Aug	females	4	<i>A. daanei</i>	8
		males	5	<i>A. erythroneuræ</i>	3
Pope Valley	14-Jul	females	1	<i>A. daanei</i>	2
		males	1	<i>A. erythroneuræ</i>	6
	3-Sep	females	6	<i>A. daanei</i>	10
		males	7	<i>A. erythroneuræ</i>	11
Village Homes	7-Jul	females	9	<i>A. daanei</i>	9
		males	18	<i>A. erythroneuræ</i>	4
	3-Sep	females	7	<i>A. daanei</i>	22
		males	46	<i>A. erythroneuræ</i>	7
	18-Oct	females	0		
	males	15			

relationship; Segoli and Rosenheim, in press), and subtracting the female's egg load upon capture.

2.8. Statistical analysis

We used survival analysis with a log rank test to compare parasitoid survival between the two treatment groups (fed and unfed) in the longevity study in the lab. To confirm the reliability of the slide-press anthrone assay, Fisher's exact test was used to compare the number of parasitoids that scored positive or negative in the anthrone test among positive (fed) and negative (unfed) controls. We used a general linear model to test the effect of hour, field site and date on the number of parasitoids captured per vegetation-shake in the field. We used multiple logistic regression to test the effect of hour, field site and date on the likelihood of a field-captured individual parasitoid scoring positive in the anthrone test. Finally, we used a general linear model to test the effects of hour, species, hind tibia length and sugar availability (proportion of individuals that scored positive in the anthrone test at a certain collection) on the egg loads or the estimated number of eggs laid by field-captured females. Because each collection was characterized by a single value of sugar availability, we ran an additional model with collection (a nominal factor) instead of food availability (a continuous variable). We compared AICc values between these two alternative models.

3. Results

3.1. Parasitoid survival in the lab

Parasitoids that had access to honey survived longer (*Median* = 2 days, *n* = 15) than parasitoids that had no access to honey (*Median* = 0, i.e., less than a day, *n* = 14; Fig. 1, Survival analysis, log-rank test, $\chi^2 = 19.99$, $P < 0.001$).

3.2. Positive and negative controls for cold anthrone test

All 16 newly emerged individuals that were not fed with honey scored negative in the anthrone test. In contrast, 15 of 18 individuals that were given access to honey scored positive (Fisher's exact test, $P < 0.001$). The negative results for the three fed individuals could indicate that they did not feed successfully on the honey in the vial during their first hour of access, or that the anthrone test is not fully reliable, resulting in a small proportion of false-negative results for the presence of sugar.

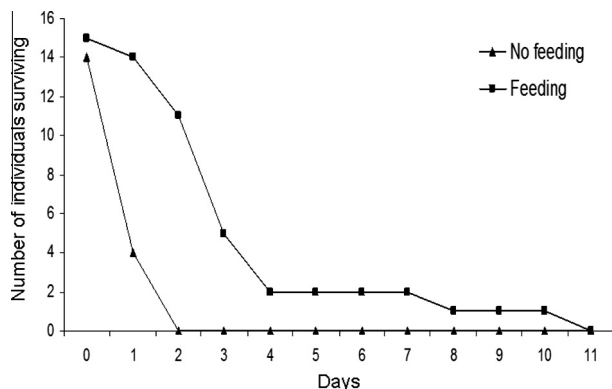


Fig. 1. Survival of parasitoids that were fed (squares) or that were not fed with honey (triangles).

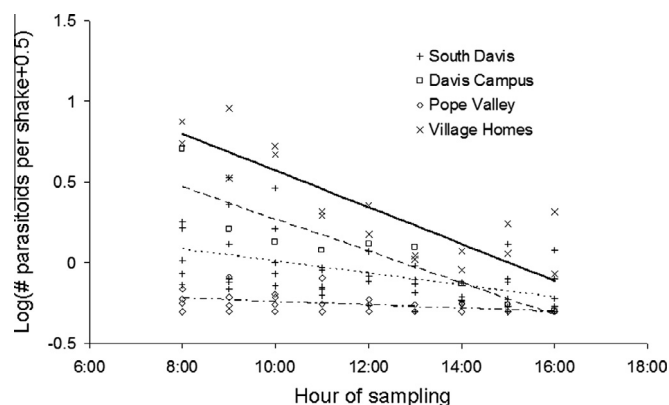


Fig. 2. Number of parasitoids captured per vegetation-shake at different hours of the day at the different field sites. *N* = 144 sampling bouts (15–17 for each hour interval). Data are transformed: $\text{Log}(x + 0.5)$.

3.3. Parasitoid abundance in the field

The number of parasitoids captured per cane shake decreased throughout the day, and differed among field sites, but was not significantly affected by the date of sampling (Fig. 2, GLM, $F_{3,151} = 36.52$, $P < 0.001$ for field site; $F_{1,151} = 37.69$, $P < 0.001$ for hour; and $F_{1,151} = 1.74$, $P = 0.19$ for sampling date).

3.4. Sugar availability in the field

The proportion of individuals with sugar in their guts did not differ between male and female parasitoids ($\chi^2 = 0.11$, $P = 0.74$, *N* = 202 males and 97 females). Among females, feeding rate was not affected by species ($\chi^2 = 0.07$, $P = 0.80$, *N* = 61 *A. daanei* and 20 *A. erythroneuræ* females). Thus, we combined individuals of both genders and species in a global analysis, including 16 females for which species could not be determined with certainty. Overall, the proportion of individuals with positive anthrone tests was low (mean \pm SE: 0.14 ± 0.04 , *N* = 11 collections). The likelihood of scoring positive for a sugar meal increased later in the day (Fig. 3, Logistic regression, $\chi^2 = 33.01$, $P < 0.001$ for time of sampling). In addition, the proportion of individuals with sugar in their guts differed among field sites ($\chi^2 = 16.46$, $P < 0.001$) and decreased as the season progressed ($\chi^2 = 10.34$, $P = 0.001$ for date of sampling). The seasonal change was especially obvious for parasitoids that were captured in the afternoon, where the proportion of sugar-fed individuals decreased from ~ 0.30 in July to ~ 0.08 in October, but not for those captured in the morning hours, where this proportion was small throughout the season (Fig. 4).

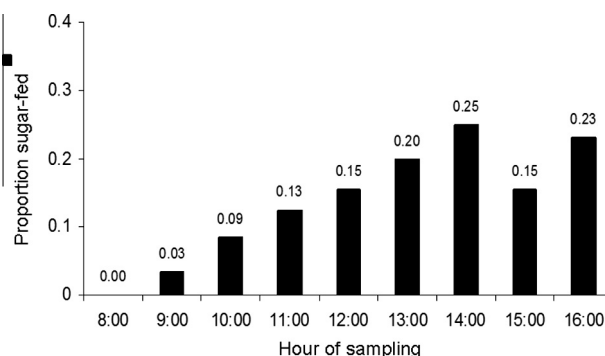


Fig. 3. The proportion of parasitoids that scored positive in the anthrone test at different hours of the day (sites and dates pooled).

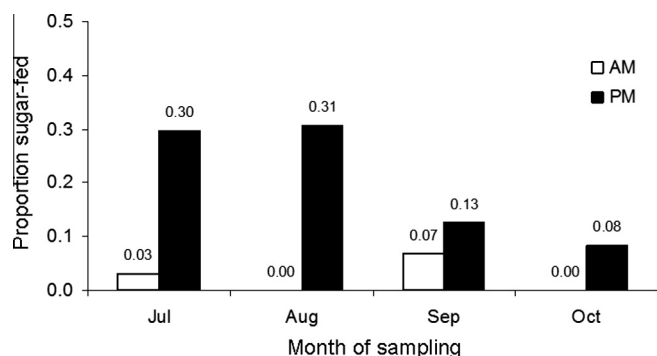


Fig. 4. The proportion of parasitoids captured in the morning hours (white bars) and in the afternoon hours (black bars) that scored positive in the anthrone test at different months (sites pooled).

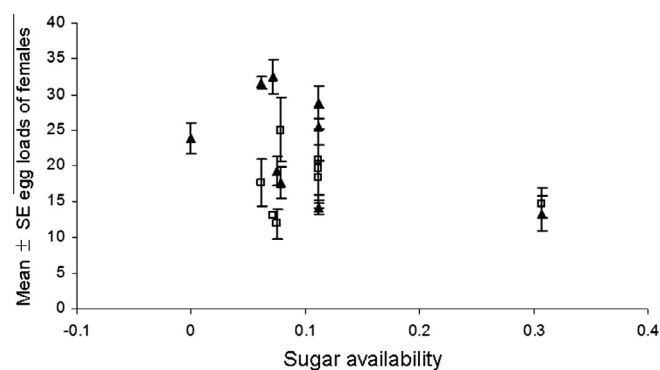


Fig. 5. Mean \pm SE egg loads of *A. daanei* (black triangles) and *A. erythroneurae* (empty squares) females vs. estimated sugar availability at the time of sampling (the proportion of individuals that scored positive for the presence of a sugar meal in their gut at a certain site and date).

3.5. Egg loads of parasitoids from the field

We excluded one collection where only two individuals were used for the anthrone test and hence the estimate of sugar availability was not reliable (Pope Valley, 15 July, 2010). GLM analysis revealed that egg loads were affected by parasitoid species, decreased as the day progressed, and were positively affected by hind tibia length (Table 2). In addition, sugar availability, estimated as the proportion of individuals that tested positive for the anthrone test at a certain collection (month and location), had a negative effect on the egg loads of females (Fig. 5, Table 2). This may suggest that females laid more eggs when more sugar resources were available to them. However, a similar model including ‘collection’ as a nominal factor instead of food availability as a continuous factor had a lower AICc score than the model including food availability (Table 2). As expected, the estimated number of eggs laid by females before they were captured was higher at sites with greater food availability ($P = 0.049$, full analysis not shown). AICc values were again lower when including ‘collection’ as a nominal factor (AICc = 1746) instead of food availability as a continuous factor (AICc = 1785). Thus, we cannot distinguish the effect of food availability *per se* on egg laying from the causal effect of other site-date specific factors.

Table 2

Results of a general linear model testing the effect of parasitoid species, time of the day and collection (site and date) on egg loads of *Anagrus* spp. Model 1 includes sugar availability (the proportion of parasitoids of a certain collection that scored positive for sugar in their guts) as a continuous variable, and Model 2 includes collection as a nominal factor instead.

	DF	Sum of squares	F ratio	P value
<i>Model 1</i>				
Hour	1	460.9	6.7	0.003
Species	1	671.9	9.8	0.010
Hind tibia length	1	6754.9	98.4	0.002
Sugar availability	1	933.4	13.6	<0.001
Error	240	16482.0		
Total	244			
R ²	0.42			
AICc	1739			
<i>Model 2</i>				
Hour	1	390.6	7.3	0.0073
Species	1	222.2	4.2	0.0423
Hind tibia length	1	4036.9	75.7	<0.0001
Collection	8	4994.3	11.7	<0.0001
Error	233	12421.0		
Total	244			
R ²	0.55			
AICc	1685			

4. Discussion

We tested basic assumptions underlying the feasibility of floral supplementation to enhance biological control by *Anagrus* parasitoids in vineyards. Feeding rates differed significantly among field sites suggesting spatial variability in the availability of sugar resources. However, overall sugar availability was low in the sampled fields, with 0–30% of the individuals acquiring a sugar meal. These low feeding rates are comparable to those found for several parasitoid species in agricultural systems (e.g., 0–20% for *Macrocentrus grandii* in corn fields; 13–30% for *Aphelinus albipodus* in soybean fields and 4% for *Aphytis aonidia* in almond orchards), but were notably lower than those observed in other systems (e.g., 30–90% for *Diadegma insulare* and 70–80% for *Cotesia glomerata* in cabbage plots with or without flowering buckwheat, and nearly 100% for *Venturia canescens* foraging within botanical gardens; (Casas et al., 2003; Heimpel and Jervis, 2005; Heimpel et al., 2004; Lee et al., 2006). Higher feeding rates were also previously documented for *Anagrus erythroneurae* (0.30–0.85, J.A. Rosenheim and J. Hodgen, unpublished data, reviewed in (Heimpel and Jervis, 2005). The low feeding rates observed in our study suggest that *Anagrus* parasitoids in the sampled vineyards were frequently limited by the availability of sugar resources. Thus, supplementation of sugar resources appears to hold the potential for enhancing *Anagrus* survival and performance.

4.1. Sugar resources

Floral nectar is likely to be the main sugar resource for *Anagrus* parasitoids. This is consistent with the larger proportion of parasitoids with sugar in their guts that were captured early in the season (July–August), when flower resources are generally more abundant (Nicholls et al., 2008). Additional resources might have included homopteran honeydew or damaged fruits, but these are generally considered less common or of a lower quality as sugar resources for parasitoids (Heimpel and Jervis, 2005), and are likely to be more abundant towards the end of the season, when the observed feeding rates were lower. Although we did not quantify flower abundance in this study, we observed flowers such as field bindweed (*Convolvulus arvensis*) and yellow starthistle (*Centaurea solstitialis*) inside or near the vineyards at the beginning, but not towards the end, of the season, when ground vegetation had mostly dried out. In addition, we have observed *Anagrus* parasitoids in the laboratory to feed on nectar when given access to flowers collected from vineyards. Due to their very small size, *Anagrus* are difficult to observe in the field, and thus documenting nectar feeding in nature is likely to be very difficult, but other larger mymarids (*Anaphes* sp., *Gonatocerus* sp.) have been observed visiting flowers, presumably

to obtain nectar (Jervis et al., 1993). Hence, the variability in feeding rates observed in this study is likely to be the result of differences in the abundance of floral resources inside or in proximity to the vineyards across study sites and dates.

4.2. Parasitoid survival

To our knowledge, there is only a single study demonstrating a positive effect of feeding on survival in the field. *Diadegma insulare* parasitoids from supplemented cabbage plots and those from plots with higher feeding rates, were shown to have more broken wing hairs, indicating that they had reached an older age (Lee and Heimpel, 2008). The potential effect of feeding on survival in the field depends on the realized lifespan of parasitoids and on the relative importance of additional mortality factors. We found that *Anagrus* abundance dropped sharply as the day progressed, suggesting that most individuals survived for less than a day in the field. This interpretation is supported by previous investigations in which dead *Anagrus* falling from grape-vines were collected in large numbers in the early afternoon, but only in very small numbers during either the early morning or late afternoon (J.A. Rosenheim unpublished results). The observed survival in the field is comparable to that of starved parasitoids in the laboratory and hence, in combination with the low feeding rates, is consistent with widespread mortality due to starvation. Although we avoided fields that were treated with pesticides, other mortality factors such as predation and adverse weather conditions might have removed some parasitoid individuals from the population before they experienced starvation. Feeding on honey or nectar extended *Anagrus* parasitoid longevity in the laboratory substantially, in this and in previous studies (English-Loeb et al., 2003; Zhu et al., 2013). A positive effect of feeding on lifespan in the field is a candidate cause for the larger proportion of sugar-fed parasitoids that were captured later in the day. These parasitoids might have survived longer due to the nutritional advantages conferred by the sugar meal that they had obtained. However, the observed pattern might have also resulted from longer-lived parasitoids simply having more time to locate scarce sugar resources. Additional work and a better understanding of the time-course of sugar-meal digestion in the gut of *Anagrus* parasitoids after feeding is required to assess the relative importance of these two non-mutually exclusive explanations.

4.3. Parasitoid fecundity

Enhanced survival is likely to translate into reproductive success only for individuals that are limited by the time available to locate suitable hosts rather than by their finite supply of eggs, *i.e.*, are time rather than egg limited (Rosenheim, 2011). In a previous study we found that more than 80% of *Anagrus* females die prior to depleting their eggs in the field (Segoli and Rosenheim, *in press*). Therefore, a positive effect of sugar supplementation on longevity or oviposition rate may potentially enhance reproductive success in this system by allowing females to lay a larger proportion of their egg complement during their lifetime. Indeed, we found possible evidence for a positive effect of sugar availability on parasitoid oviposition success, as parasitoids from collections where sugar availability was higher had reduced egg loads, indicating that they had laid more eggs. However, the effect of sugar availability on egg load was small and could not be distinguished from other possible site-date causal effects. This is perhaps not surprising given the small proportion of parasitoids that obtained sugar in the first place. A larger number of collections made over a larger range of feeding rates will be required to demonstrate an effect of feeding on the reproductive success of females in the field more clearly. An additional attempt to demonstrate the effect of sugar feeding on parasitoid reproductive success in the field was suggestive: *D. insulare* foraging in

buckwheat-supplemented plots had fewer eggs remaining in their ovaries, and had laid more eggs than females in control plots, but the results were not significant, possibly due to low statistical power (Lee and Heimpel, 2008).

4.4. Considerations for biological control

Several studies have shown that the potential of floral resources to enhance parasitoid success depends on the ability of parasitoids to move easily between flower and pest patches (Lavandero et al., 2005; Lee et al., 2006). Although *Anagrus* parasitoids have been shown to disperse upwind over moderate distances, *e.g.*, hundreds of meters (Corbett and Rosenheim, 1996b), their small size and short lifespan may suggest that most individuals forage over modest distances during their lifetime and hence may be highly dependent on floral resources within vineyards. Such limited dispersal was demonstrated by English-Loeb et al. (2003), where the effect of floral supplementation on parasitoid abundance was apparent in the edge, but not in the center of the vineyard. Similarly, attempts to enhance biological control in vineyards by planting prune trees that provide overwintering habitat for *Anagrus* have had a limited success, as the abundance of *Anagrus* exhibited a gradual decline with increasing distance from the refuge (Corbett and Rosenheim, 1996a). Thus, floral supplementation within vineyards rather than only at their margins is more likely to be effective in enhancing parasitoid success, although the effect of ground cover on grapevine vigor should also be considered (Costello and Daane, 2003). The seasonal decrease in sugar availability observed in our study may have implications for the ability of *Anagrus* parasitoids to control leafhopper populations towards the end of the season, when leafhopper densities reach their highest levels and cause significant damage to the grape plants and generate a major nuisance for harvest workers (Daane et al., 2000). Hence, supplementation of flowers that bloom late in the season in vineyards may be recommended to enhance leafhopper control (Nicholls et al., 2008). Finally, floral supplementation is only likely to be successful in increasing parasitoid success if it can be combined with reducing the negative impact of other factors that may reduce *Anagrus* longevity. For example, sulfur, a fungicide whose use in California vineyards is nearly universal, has been shown to be highly toxic to *Anagrus* (Jepsen et al., 2007a). Under such strict constraints parasitoids may not survive long enough to experience starvation.

5. Conclusions

Our results suggest a high potential for increasing biological control by *Anagrus* parasitoids via floral supplementation in vineyards. *Anagrus* wasps seem to be highly limited by sugar resources in the field. Feeding is likely to increase parasitoid survival and oviposition success, although the magnitude of these effects should be further evaluated. Supplementation of floral resources should be implemented within fields, include flowers that bloom at different times, but especially towards the end of the season, and while avoiding other factors with adverse effects on parasitoid success.

Acknowledgments

We thank Cory Fernandez, Timothy Fong, Tierra Groff, Orly Oren, Michael Park, Moran Segoli, Corinne Stouthamer, Ian Taff, Houston Wilson, Lucinda Ye and the insect ecology group in UC Davis for technical support and fruitful discussions. We thank Beth Flory, Paul Kimsey, John Martens, Marc Rubald, and Andrew Walker for access to vineyards and permission to sample. This study was partially funded by grants from the Israel Science Foundation (ISF), the Bi-national Agricultural Research and Development Fund (BARD), and the United States National Science Foundation (NSF: DMS-1022639).

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