Environmental Entomology, XX(X), 2018, 1–6
doi: 10.1093/ee/nvy165
Research

Quantifying Temporal Variation in the Benefits of Aphid Honeydew for Biological Control of Alfalfa Weevil (Coleoptera: Curculionidae)

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Subject Editor: Zsofia Szendrei

Received 31 August 2018; Editorial decision 22 October 2018

Abstract

Sugar feeding by biological control agents, such as parasitoid wasps, may enhance their ability to control crop pests, although its importance is likely to vary greatly through space and time. Here we quantified temporal variation in the potential importance of sugar resources associated with honeydew secreted by the pea aphid (Acyrthosiphon *pisum* (Harris) (Hemiptera: Aphididae)) in determining levels of parasitism of the alfalfa weevil (*Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae)) by its dominant parasitoid, *Bathyplectes curculionis* (Thomson) (Hymenoptera: Ichneumonidae) across irrigated alfalfa fields in Montana, United States over 5 yr. A positive association between parasitism of *H. postica* and *A. pisum* densities at the across-site scale was observed in 2 of 5 yr, with parasitism increasing twofold to fourfold over gradients in *A. pisum* density. The relationship was strongest in the 2 yr of lowest parasitoid relative to host densities, when increases in per capita effects of individual parasitoids would be expected to be particularly important. *Acyrthosiphon pisum* densities were at their lowest in these same years, suggesting that they may generally be sufficiently abundant that parasitoids are not limited by sugars in most years. This conclusion is supported by results of anthrone tests which revealed a high level of sugar-fed parasitoids (>50%) in a year of high aphid abundance. More studies, such as this one, that explore the frequency with which increasing sugar resource availability actually enhances parasitism levels in the field will be critical to gauge the broader potential of sugar resource addition (e.g., through flowering strips, banker plants or sugar sprays) to bolster biological control.

Key words: biocontrol, carbohydrate, fructose, sucrose, sugar limitation

Plant-derived sugars, such as those acquired from floral and extrafloral nectar, are known to be important resources for many beneficial natural enemies (Wäckers 2005). Increases in lifespan and or fecundity resulting from sugar feeding by biological control agents may in turn enhance their ability to control important crop pests (Jervis et al. 1996, Heimpel and Jervis 2005, Lundgren 2009, Benelli et al. 2017). Unfortunately, such non-host resources are often limiting in highly simplified and disturbed cropland monocultures, within which the cover and diversity of non-crop vegetation is low. Restoring non-host resources, particularly flowering plants, within these simplified agricultural landscapes has thus become a focus of conservation biological control efforts (Wratten and van Emden 1995, Landis et al. 2000, Gurr et al. 2004, Wäckers 2005). Honeydew associated with phloem-feeding Homoptera is also recognized as an important source of nutrition for parasitoids (Evans 1994, Wäckers et al. 2008) that has been perhaps less studied in the context of conservation biological control. Although it may be an inferior resource relative to floral nectar for some species, it is the most available exogenous carbohydrate source in many habitats, including agroecosystems (Wäckers 2005, Wäckers et al. 2008, Tena et al. 2013). Furthermore, for some parasitoid species, feeding on honeydew can enhance performance to the same or a greater extent as sugars associated with nectar (reviewed in Wäckers et al. 2008, Benelli et al. 2017). One of the best-studied cases is the alfalfa weevil parasitoid, *Bathyplectes curculionis* (Thomson), which benefits from honeydew secreted by the pea aphid, *Acyrthosiphon pisum* (Harris), in alfalfa, *Medicago sativa* (Evans and England 1996, England and Evans 1997, Jacob and Evans 1998) and is the focus of this study.

Sugar limitation of parasitoids is often inferred from studies correlating sugar resource abundance with parasitoid performance or parasitism levels. However, a more direct way of gauging the extent to which parasitoids are potentially sugar limited is by analyzing sugars in field-collected individuals (Heimpel and Jervis 2005). Sugar levels in these individuals can then be compared to

individuals that have been starved for a given amount of time (e.g., Seagraves et al. 2011) or among individuals exposed to different treatments or resource gradients. Despite its potential utility (Tena et al. 2015), analysis of sugars in parasitoids collected across environmental gradients in resource availability remains uncommon. Results from existing studies are variable. For example, Kishinevsky et al. (2018) found that the frequency of sugar-feeding in field-collected parasitoids was higher in natural habitats than in neighboring pomegranate orchards, consistent with the theory that simplified agricultural systems are lacking sugar resources for parasitoids. Similarly, Winkler et al. (2009) found that sugar levels in *Diadegma semiclausum*, an important parasitoid of the Diamond back moth, *Plutella xylostella*, were significantly higher in individuals collected from Brussels sprout plots bordered by flowers than grass-bordered controls. However, the proportion of sugar-fed individuals did not significantly differ across these plot types. Finally, Lee et al. (2006) found no consistent influence of floral borders on the proportion of sugar-fed *Diadegma insulare*, another parasitoid of *P. xylostella*, in cabbage plots over 4 yr.

Variability in the importance of sugar resources in enhancing sugar feeding by parasitoids, and ultimately promoting biological control, will in part be determined by environmental context. For example, Heimpel and Jervis (2005) argue that increased lifespan associated with sugar feeding will lead to higher parasitism levels under many field conditions, since more time is available for host location and egg maturation. However, they point out that sugar feeding may be less important if parasitoids are able to realize maximum fecundity before starvation occurs. The probability of the latter outcome could increase when host densities are high, since this would reduce the amount of search time necessary for individual wasps to locate hosts. On the other hand, very high parasitoid densities could result in high levels of parasitism, regardless of the lifespan or fecundity of individual wasps, decoupling sugar resource levels from parasitism levels (e.g., when numerical influences dominate over per capita influences). Thus host density may modulate the importance of sugar resources for biological control in complex ways (Heimpel and Jervis 2005). The availability and importance of host and non-host resources is likely to fluctuate greatly through space and time. For example, Kishinevsky et al. (2018) found that the proportion of sugar-fed parasitoid individuals approximately doubled between 2 yr of their study, and similarly large shifts have been observed across seasons (Segoli and Rosenheim 2013, Tena et al. 2013). Thus assessing the potential importance of sugar resources for biological control would benefit from a more explicit examination of its spatial and temporal variability.

In this study, we examined the potential importance of sugar resources in determining levels of parasitism of the alfalfa weevil (*Hypera postica* (Gyllenhal)) by its dominant parasitoid, *B. curculionis*, across irrigated alfalfa fields in Montana, United States. *Bathyplectes curculionis* is non-host feeding as an adult and synovegenic (i.e., maturing its eggs over time after eclosion of the adults) both traits which make it particularly likely to benefit from sugar resources in the field (Dowell 1978). Sugar, and more specifically, honeydew, feeding by this parasitoid increases both the longevity (by ~50%) and egg load (11–15%) of *B. curculionis* adults in laboratory studies (Dowell 1978, England and Evans 1997). Such increases in adult lifespan and fecundity could increase the per capita impacts of the parasitoid on its host. This prediction is supported by field studies showing increased parasitism levels associated with higher pea aphid densities in caged alfalfa plots, where aggregative numerical responses resulting from parasitoids attraction to areas of high aphid abundance would have been prevented (Evans and England 1996). However, whether similar effects of *A. pisum* density potentially scale up to influence patterns of parasitoid sugar feeding and weevil parasitism across fields, and how this relationship potentially varies among years, has not been previously investigated. In this study, we conducted field surveys to quantify the abundance of pea aphids (a source of honeydew), alfalfa weevil larvae (the host for this parasitoid) and parasitoid adults across alfalfa fields over 5 yr. Surveys were combined with rearing of alfalfa weevil larvae (to quantify parasitism levels) and direct analysis of sugar contents of field-collected wasps, in order to assess the potential importance of aphid honeydew in promoting the biological control of alfalfa weevil. In particular, we set out to address the following questions:

1. Are pea aphid densities positively related to parasitism of the alfalfa weevil across alfalfa fields (independent of variation in host densities), and is the effect consistent across years?
2. Is there evidence that parasitoids are sugar limited in the field, and does the proportion of sugar-fed wasps increase with increasing resource, either pea aphid or flower, density at the field scale?

Materials and Methods

Sampling Alfalfa Insects to Quantify Insect Density and Parasitism

Insect abundances and parasitism levels were monitored in alfalfa, just prior to the first cutting of the season between 7 and 16 June in each year, by sampling between 14 and 26 alfalfa fields in each of 5 yr: 2009, 2010, 2012, 2013, and 2014. Insect sampling was carried out between 10:00 and 4:00 p.m. on clear to partly cloudy days, when temperatures were ≥15°C, and wind speeds were ≤24 km/h as these factors can affect the efficacy of sweep sampling in alfalfa (Kieckhefer et al. 1992). In 2009 through 2013, 10 samples consisting of fifty 180° sweeps with a 38 cm diameter sweep net were taken in each field. Each 50-sweep sample was done along a 50-m transect running parallel to the field edge. To avoid edge effects, the first transect was started ≥15 m from the field edge; each subsequent transect was located 10 m farther into the field from the previous one.

In 2014, we streamlined our design. Sampling was reduced to four 50-sweep samples per field, and the transect design was altered slightly to maximize efficiency. Two sets of 50-sweep samples were done along two 100-m transects. The first transect was initiated 15 m in from the field edge running perpendicular to it (directly into the field). A second set of two 50-sweep samples were taken while returning to the field edge along the second 100-m transect initiated 50 m over from the first. Extra sweeps were done as necessary to bolster sample sizes for weevil rearing to determine parasitism levels in fields with very low weevil densities, with the aim of rearing at least 50 larvae per field. Each 50-sweep sample was emptied into a 7.57-liter, sealable, plastic bag containing a paper towel to absorb extra moisture. Bags were sealed and stored in coolers during collection and then in a laboratory refrigerator at ~2°C until processed (see Rearing Alfalfa Weevils to Quantify Parasitism).

Sampling Parasitoid Adults for Sugar Analyses

In 2014, sweep sampling was additionally carried out 6–10 June, a week to 10 d before the yearly sampling described above, to collect parasitoid adults for sugar analysis (n = 12 sites). Sampling was carried out under the same environmental criteria described above, but within a more restricted time window, 11:00 a.m. to 2:00 p.m. on sunny days, to ensure insects would be actively foraging. We carried

out four 50-sweep samples along two 100-m transects 50 m apart, as described above, to again quantify insect densities.

To quantify the potential abundance of floral resources, we sampled vegetation in 10, 0.5 × 0.5 m quadrats placed approximately every 10 m along a 100-m transect. This transect was placed between the two quantitative sweep sampling transects. In each quadrat, we counted the number of flowering inflorescences of non-crop plant species (mostly weedy Brassicaeae: *Descunaria* sp., *Sisymbrium* sp. and *Capsella* sp. and Asteraceae: *Taraxacum* sp.) following the approach of Delaney et al. (2015).

Additional sweep samples were taken in between our two quantitative sampling transects to collect adult parasitoids for sugar analyses. After every 50-sweep sample was taken, we inverted the contents into a 7.57-liter, sealable plastic bag. All *B. curculionis* adults were then removed from bags with a handheld aspirator. Sampling continued until we reached desired sample sizes (≥25 individuals). For each sampled site, 5–10 *B. curculionis* individuals were aspirated into a 33-ml vial, transported to the laboratory in field coolers, placed into 1.9-liter cages (Bioquip #2845) containing a piece of dampened filter paper, and starved for 24 h. Those individuals still alive after 24 h were removed and stored at −80°C until transported to the Blue Dasher Farm, in Estelline SD, for sugar analyses (see Sugar (Anthrone) Analysis). These individuals served as our unfed controls. The remaining individuals collected at a site were aspirated into a separate 33-ml vial, placed on dry ice in a field cooler within 15 min of collection, transported to the lab, and again stored at −80°C until analyzed for sugars.

Rearing Alfalfa Weevils to Quantify Parasitism

Larval alfalfa weevils collected in yearly sweep samples were removed from bags within 24 h of collection, and reared individually in 29.6-ml cups (2009–2010) or in groups (maximum of 50) in paper grocery bags lined with paper towels (2012–2014). When densities were high, we removed the first 10–25, third-fourth instar individuals encountered from each successive sweep sample until a maximum sample size of 100 adult weevils was reached. In years when larval weevil densities were low, all third–fourth instars were removed. Rearing containers/bags were kept at room temperature (20–22°C), adding fresh alfalfa foliage two to three times per week, for 4–6 wk. The weevils were then removed from containers or bags, and foliage, paper towels and the inside of bags scanned carefully for parasitoids (cocoons, larvae, and adults) and weevil pupae or adults.

Sugar (Anthrone) Analysis

Total sugars and fructose were quantified per wasp using the hot and cold anthrone reactions, respectively (Van Handel 1985, Olson et al. 2000). The presence of fructose is regarded as indicative of plant feeding, since it is not synthesized within insects, while sucrose is reflective of sugar consumption more generally. All wasps were surface washed with water to remove sugar contaminants, and female wasps from each site (n = 10–25 wasps per site) were analyzed for their sugar contents. Wasps were ground with a pestle in 50 µl of phosphate buffered saline solution; wasps were each vortexed in 450 µl of methanol:chloroform (2:1, v:v), and solids were centrifuged out at 21,300 g for 4 min. The supernatant was separated equally into two glass tubes, and then heated to 90°C for 15 min, or until a final volume (approximately 25 µl) was reached. Anthrone reagent (975 ml; 750 mg anthrone reagent (Product #319899; Sigma-Aldrich), in 380 ml of sulfuric acid) was added to each tube, and the mixture was vortexed for 10 s. The subsample destined for fructose analysis (cold anthrone reaction) was incubated for 1.5 h at 34°C. Total sugars were quantified by heating the subsample for 15 min at 90°C. A plate-specific set of standard curves was established on each microplate for use in quantifying the amount of sugars. Sucrose and fructose were diluted to attain aqueous solutions of 0, 0.001, 0.01, 0.1, 0.25, 0.5, 1, and 2 g/ml, and the lines that were fit to the resulting data were used to calculate the weight of sucrose and fructose per unit weight of wasp. Absorbance was read on a microplate reader at 625 nM.

The levels of sugars in control wasps that had been starved for 24 h (n = 79 across the 12 sites) were used as a baseline to calculate the proportion of sugar-fed wasps at each site, following a threshold approach used in previous studies (Lee et al. 2006, Seagraves et al. 2011). Any individual in which fructose or sucrose levels exceeded mean level of starved (control) individuals plus three standard deviation units (0.0448 and 0.0424 g of sugar per gram of wasp tissue for fructose and sucrose, respectively) were considered to be positive for that sugar. In addition, for those wasps that were positive for a particular sugar, we calculated mean sugar levels across individuals at a given site.

Statistical Analyses

All statistical analyses were carried out in JMP 13 (SAS Institute Inc. 1989–2013). We ran a cumulative generalized linear model (binomial distribution with a logit link) across all years of data, to examine the effects of year, *A. pisum* density (source of honeydew) and *H. postica* density (host) and the interaction between insect densities and year on proportion parasitism. Insect densities were natural log-transformed to normalize distributions. The initial model revealed significant interactions between year and *A. pisum* density, indicating that the strength or direction of the relationship *A. pisum* density and proportion parasitism differed across years. Thus, to further explore these relationships, we ran similar generalized linear models for each year separately.

A generalized linear model (binomial distribution, logit link) was additionally run to examine the potential influences of *A. pisum* density and inflorescence density on the proportion of sucrose or fructose positive *B. curculionis* adult females in 2014. A similar model (normal distribution, identity link) was run to examine whether these same predictor variables influenced the average sugar levels in those individuals that were determined to have sugar fed.

Results

The cumulative, across-year, model revealed significant interactions between both year and *H. postica* larval density (df = 4,70; Likelihood Chi-square = 18.9056; P = 0.0008) and year and *A. pisum* density (df = 4,70; Likelihood Chi-square = 11.2241; P = 0.0242), suggesting that the strength and/or direction of the influence of alfalfa weevil larval and pea aphid densities on parasitism differed across years.

When years were examined separately (Table 1), we found that *A. pisum* density was significantly positively related to the proportion parasitism across sites in 2 yr, 2010 and 2012 (Fig. 1; Table 1). In 2013, we found that parasitism was significantly negatively related to *H. postica* larval density (Table 1). There were no significant independent effects of either insect density variable (*H. postica* larvae or *A. pisum*) on the proportion of parasitized *H. postica* larvae in two of the five sampling years (2009 and 2014). Densities of all three insects (*H. postica, A. pisum, and B. curculionis*) as well as parasitism levels varied considerably across years (Fig. 2).

The proportion of wasps that tested positive for sugars was high overall, and varied considerably across sites (fructose: mean = 53%,

Table 1. Results of generalized linear models examining the relationship between insect densities and proportion parasitism of the alfalfa weevil, *Hypera postica*, by its dominant parasitoid in the study region, *Bathyplectes curculionis*

Year	Source	df	Likelihood-ratio Chi-square	*P*
2009	Alfalfa weevil larval density (ln)	1,12	0.08	0.7802
	Aphid density (ln)	1,12	0.01	0.9307
2010	Alfalfa weevil larval density (ln)	1,23	1.15	0.2833
	Aphid density (ln)	1,23	4.71	**0.0299**
2012	Alfalfa weevil larval density (ln)	1,11	0.21	0.6439
	Aphid density (ln)	1,11	7.90	**0.0049**
2013	Alfalfa weevil larval density (ln)	1,13	11.39	**0.0007**
	Aphid density (ln)	1,13	0.48	0.4895
2014	Alfalfa weevil larval density (ln)	1,11	0.15	0.6999
	Aphid density (ln)	1,11	0.00	0.9721

Significant effects ($P \leq 0.05$) indicated in bold text.

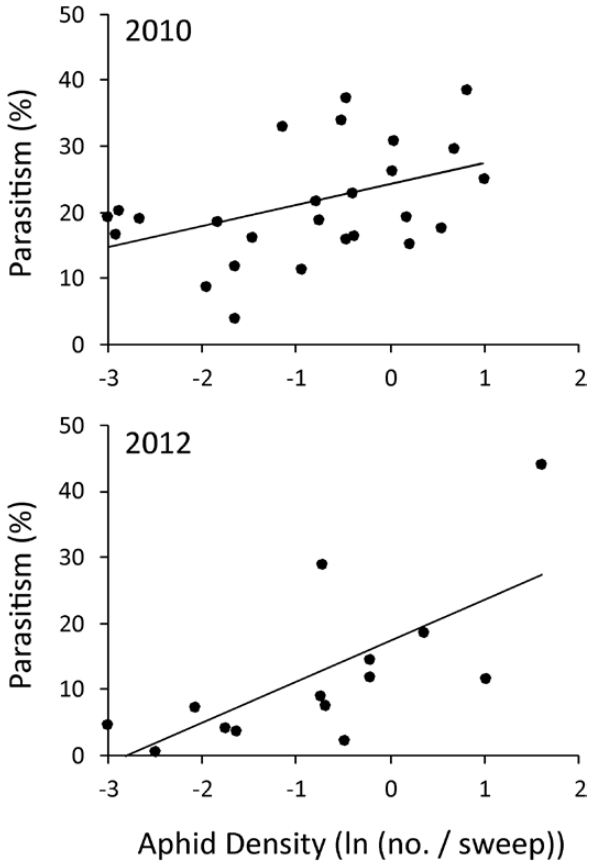

Fig. 1. Relationship between pea aphid (*Acyrthosiphon pisum*) density (ln transformed) and percent parasitism of alfalfa weevil (*Hypera postica*) by the parasitoid *Bathyplectes curculionis*, across sites in the 2 yr where a significant relationship was observed over the 5-yr study.

range = 20–100%; sucrose: mean = 59%, range = 30–82%). The levels of sugars detected in sugar-fed individuals also varied to some extent across sites (fructose: mean = 0.0762, range = 0.0587–0.0952 g per gram of wasp tissue; sucrose: mean = 0.0843, range = 0.0673–0.0960 g per gram of wasp tissue). However, neither the proportion of sugar-fed wasps, nor mean sugar levels for those wasps that tested positive for sugars, was significantly related to either inflorescence density (range: 0.0–5.5 per 0.25 m^2) or *A. pisum* density (range: 0.7–8.3 per sweep) at a site in 2014 (Table 2).

Discussion

The pea aphid, *A pisum*, is seldom a pest in alfalfa in the study region, with levels observed across the 5 yr of our study never exceeding economic thresholds at any site. On the contrary, our results suggest that increases in *A. pisum* density at the field scale potentially benefits crop production by increasing parasitism levels of *H. postica*, the dominant insect pest of alfalfa, under some conditions. A positive association between parasitism of *H. postica* and *A. pisum* densities was observed in 2 of 5 yr (Table 1). In the year where the relationship was the strongest, 2012, parasitism more than quadrupled over the gradient in *A. pisum* density (Fig. 1). Our results are consistent with previous work showing that honeydew associated with *A. pisum* increases *B. curculionis* longevity and fecundity, which can lead to an increase in the biological control pressure on *H. postica* over smaller, within field, spatial scales as aphid densities increase (Evans and England 1996, England and Evans 1997). They also are consistent with studies in other systems that have found a positive relationship between sugar feeding by parasitoids and densities of honeydew-producing insects at within-field spatial scales (Tena et al. 2013, Dieckhoff et al. 2014). However, our results suggest that the importance of honeydew-producing aphids, and associated sugar resources, for biological control is context dependent, varying considerably across years.

Insect densities varied dramatically across years, with the peak in adult parasitoid densities lagging a year behind the peak in host density, and very high parasitoid-host ratios (and parasitism levels) resulting from the rapid declines in host density in the years following its peak in 2012 (Fig. 2). The strongest relationship between *A. pisum* density and parasitism of *H. postica* was found in 2012, an outbreak year for the weevil (Fig. 2). High host density combined with low numbers of parasitoid adults, resulted in the lowest overall levels of parasitism observed across years (averaging 12%). A weaker, but still significant, relationship between *A. pisum* density and parasitism was also observed in 2010, a year with much lower weevil densities, but in which adult parasitoid densities and parasitism levels were similarly low. Thus, sugar resources may be particularly important in years of low parasitoid adult abundance, when increases in per capita impacts of individual wasps due to increased lifespan would be expected to be particularly important in augmenting parasitism rates, as observed. In contrast, in years of high parasitoid relative to host densities (2009 and 2014), a very high percentage of weevil larvae were parasitized across sites (Fig. 1); under such saturating conditions the enhanced performance of individual wasps associated with sugar feeding may not translate into

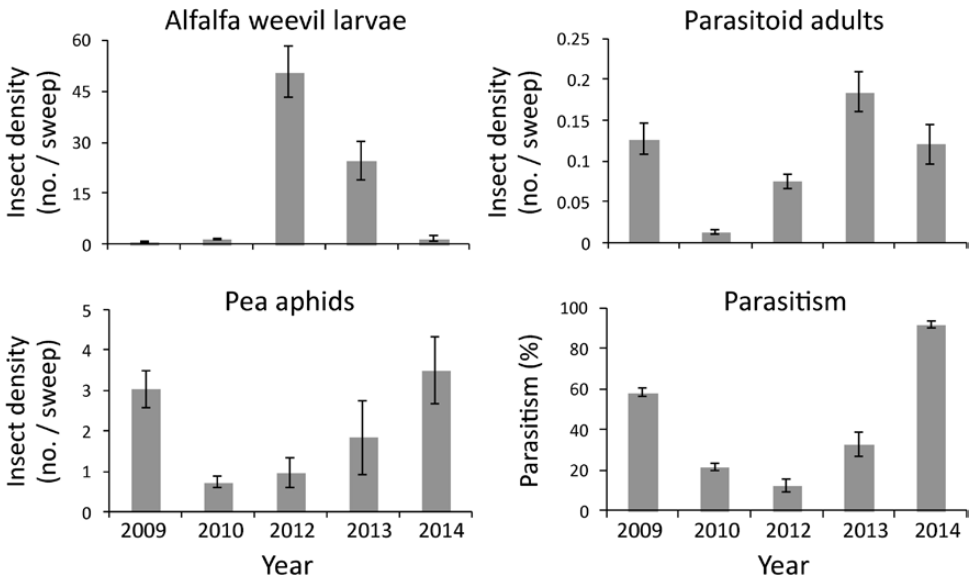

Fig. 2. Mean ± SE (across sites) insect densities and percent parasitism of alfalfa weevil over the 5 yr of the study.

Table 2. Results of generalized linear models examining the relationship between resource availability (*Acyrthosiphon pisum* and inflorescence density) and the proportion of sugar-fed wasps and mean sugar levels (grams of sugar per gram of wasp tissue) in individuals that had fed in 2014

Response	Source	df	Likelihood-ratio Chi-square	*P*
Proportion fructose positive	Aphid density (ln)	1,9	0.81	0.3675
	Inflorescence density	1,9	0.05	0.8242
Proportion sucrose positive	Aphid density (ln)	1,9	0.00	0.9468
	Inflorescence density	1,9	0.01	0.9219
Mean fructose level	Aphid density (ln)	1,9	0.23	0.6334
	Inflorescence density	1,9	2.60	0.1068
Mean sucrose level	Aphid density (ln)	1,9	3.04	0.0812
	Inflorescence density	1,9	0.26	0.6069

large increases in parasitism levels (i.e., because of the high densities of adults, which are driven by extrinsic factors at this scale, most hosts are parasitized regardless of potential increases in lifespan of individual wasps). Thus the lack of a significant relationship between parasitism and *A. pisum* densities in those years is not surprising. *Acyrthosiphon pisum* densities also varied considerably across years, and may be an additional factor contributing to year to year differences in the relationship between *A. pisum* density and parasitism across sites. Aphid densities were at their lowest levels in 2010 and 2012, and thus aphid honeydew may have been particularly limiting in those years, resulting in a strong signature of increased parasitism associated with increasing *A. pisum* density across sites. In contrast, in years of high *A. pisum* densities, parasitoids were likely not limited by sugars produced by this insect. Previous studies have shown that the degree of sugar limitation in parasitoids can be strongly related to seasonal variation in the availability of honeydew-producing hosts (Tena et al. 2013), including in the aphid-alfalfa weevil system (Evans and England 1996). Our results suggest that similar fluctuations in the availability and importance of honeydew may be common across years.

In 2014, the year that sugar analyses were conducted, neither the proportion of sugar-fed parasitoids, nor sugar levels in parasitoids that had fed, were significantly related to either the abundance of pea aphids or flowers in a field. This contrasts with other studies which have documented increases in sugar content, or the proportion of sugar-fed individuals, associated with increases in resource availability such as proximity to flowering field margins or higher densities of honeydew-producing homopterans (Winkler et al. 2009, Tena et al. 2013). Variability in sugar feeding across sites in our study could reflect use of some other, unmeasured sugar source in the field in that year. Overall, a high percentage of individuals tested positive for sugars that are common in honeydew and nectar, 53% for fructose and 59% for sucrose, indicating that a majority of parasitoid individuals had fed on sugars within the last 24 h. This level of sugar feeding falls within the range typically observed when resources are considered abundant (Heimpel and Jervis 2005, Tena et al. 2013), and is much higher than that thought to indicate significant sugar limitation in the field, i.e., 10–20% (Segoli and Rosenheim 2013, Tena et al. 2013). The high rate of sugar feeding observed suggests that parasitoids were not generally sugar limited in alfalfa fields in the year sugar analyses were conducted, potentially due to the high overall availability of pea aphid honeydew in that year. *Acyrthosiphon pisum* densities peaked in 2014, and were more than three times higher in that year relative to 2010 or 2012, the years when a positive association between pea aphid density and parasitism of *H. postica* was observed. The high level of sugar feeding revealed by sugar analyses thus provide an additional line of evidence that the absence of sugar limitation may in part explain the lack of an observed relationship between aphid density and parasitism across sites in the years with high aphid abundance.

Many factors potentially contribute to temporal and spatial variation in parasitism levels, including potential influences of environmental factors, both biotic and abiotic, on densities of hosts, parasitoids and non-host resources, as well as on parasitoid performance (Heimpel and Jervis 2005). Given this complexity, it is perhaps not surprising to find evidence of sugar limitation of parasitism under a limited subset of conditions, as observed in our study. Our results suggest that sugars associated with honeydew were potentially limiting parasitism in only 2 of 5 yr. Thus the systematic provisioning of sugar resources may not be an efficient means of bolstering natural pest control in our system. Our work re-enforces previous studies done at smaller scales which suggest that sugar resource addition, e.g., via sugar sprays, will only be effective when natural sources, such as aphid honeydew, are low (Jacob and Evans 1998), which appears to be relatively rare in this system. Longer-term studies such as this one are uncommon, yet critical in determining the environmental conditions under, and frequency with, which increasing sugar resource availability actually enhances parasitism levels in the field. Such information is necessary to gauge the broader potential of sugar resource addition (e.g., through flowering strips, banker plants or sugar sprays) to bolster biological control.

Acknowledgments

We thank the many individuals who helped us collect and rear alfalfa weevils, and sort samples over the years: Deb Waters, Amelia Jurkowska, Alyssa Kessell, Megan Greenwood, Joyce Wick, Anna Petroff, Lance Turner, Kayleigh Hauri, and Ellen Titus. We are additionally grateful to the alfalfa growers who allowed us to access their fields.

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