

RESEARCH ARTICLE

An acquired distaste: sugar discrimination by the larval parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae) is affected by prior sugar exposure

J. K. Makatiani¹, H. K. Le^{1,*}, D. M. Olson², F. L. Wäckers³ and K. Takasu^{4,‡}

ABSTRACT

We examined gustatory responses of the larval parasitoid *Microplitis croceipes* to determine whether the adults discriminate among common sugars, including fructose, glucose, maltose and sucrose, found in plants. When given single sugar solutions of sucrose, glucose, fructose and maltose at concentrations of 0.008–2.0 mol l⁻¹, the estimated concentrations at which 50% of wasps initiated feeding ranged between 0.054 and 0.085 mol l⁻¹ for sucrose, glucose and fructose, which was significantly lower than for maltose. Wasps showed a strong decrease in feeding time for maltose or fructose following a brief exposure to other sugars, suggesting that wasps can distinguish maltose and fructose from the other sugars tested. The higher acceptance threshold and short feeding time in the case of maltose appears adaptive in light of the relatively poor nutritional quality of the sugar in the longevity trial. The pronounced feeding inhibition seen for fructose following exposure to other sugars is not linked with lower nutritional performance. This feeding inhibition was even seen in wasps that had fed on glucose at the lowest acceptance threshold (0.031 mol l⁻¹) and persisted for 24 h. This study is the first to show feeding inhibition of otherwise phagostimulant sugars such as maltose and fructose after gustatory stimulation on other sugars.

KEY WORDS: Feeding inhibition, Gustatory responses, Sugar feeding

INTRODUCTION

There is a large variation in sugar composition among natural sugar sources, such as nectar and honeydew (Percival, 1961; Kunkel and Kloft, 1977; Baker and Baker, 1983; Nemeč and Starý, 1990; Wäckers, 2005). Floral nectars have been categorized according to their nectar composition as sucrose-rich or hexose (glucose/fructose)-rich (Baker and Baker, 1983), and insect groups often show a tendency to visit nectars of a certain carbohydrate composition (Baker and Baker, 1982). However, in many groups of nectar feeders, it remains unclear whether the observed insect–flower associations are directly driven by nectar sugar preferences, or whether this is based on other factors, such as flower architecture (Jervis, 1998; Olson et al., 2005; Wäckers and van Rijn, 2012).

Parasitoid feeding responses to different nectar and honeydew sugars can vary widely; while several sugars fail to elicit any feeding

response in food-deprived parasitoids, other sugars stimulate feeding at concentrations as low or lower than 0.016 mol l⁻¹ (Wäckers, 1999; Beach et al., 2003). At equal concentrations, adult parasitoids consume larger quantities of high-quality sugars such as glucose, fructose and sucrose as compared with low-quality sugars (Wäckers, 2001; Beach et al., 2003; Williams and Roane, 2007). Because the parasitoid feeding response is often correlated with the nutritional suitability of the carbohydrates tested (Wäckers, 2001), the variation of parasitoid feeding responses to different sugars could be explained by optimal foraging theory, which predicts that foragers should choose diet items to maximize their net energy intake or net fitness benefits (Charnov, 1976; Stephens and Krebs, 1986; Sih and Christensen, 2001). Parasitoids are known to adapt their preference for hosts of different quality in accordance with their host encounter rates and their own physiological states (van Alphen and Vet, 1986). As natural sugar sources are vital for their survival and reproduction, parasitoids can be expected to have a preference for high quality of sugars and adapt their preference in accordance with feeding experiences. While parasitoid gustatory responses to individual sugars have been tested, the impact of prior experience and the relative preference for individual sugars remains unknown.

The preference of nectar feeders for various nectar components has been investigated in several vertebrates and invertebrates. The methods used to assess such preferences vary among studies. The most direct way to investigate the ability and propensity of an animal to discriminate and select foods is to present it with two or more types of food simultaneously, observe its behavior and/or measure the quantity of food eaten (Dethier, 1976). These methods require that the test organism shows repeated feeding bouts, and an inclination to sample the food source. In the case of social Hymenoptera, both of these conditions are met, as the foragers continuously collect food to cover the needs of the entire colony. This has facilitated the study of food preferences in ants and honeybees. The tests used to determine feeding preferences in honeybees typically assess the relative consumption in choice experiments (Wykes, 1952; Waller, 1972; Inouye and Waller, 1984; Alm et al., 1990). An alternative method makes use of the fact that bees exhibit a typical ‘bee dance’ to recruit additional foragers (von Frisch, 1934). As bees communicate food source quality through the turning frequency, this parameter can also be used as an indicator of how bees value a previously visited food source (Waller and Bachman, 1981; Barron et al., 2009). In the case of ants, the relative intensity with which scouts recruit nestmates to food alternatives is also used as a method to establish preferences in choice experiments. These studies typically count the number of ants visiting the different food alternatives (Lanza and Krauss, 1984; Lanza, 1988; Lanza et al., 1993; Völkl et al., 1999).

While these methods are effective in establishing preferences in social Hymenoptera, they are unsuitable for the study of food

¹Graduate School of Bioresources and Bioenvironmental Sciences, Kyushu University, Fukuoka 812-8581, Japan. ²Crop Protection and Management Unit, USDA-ARS, Tifton, GA 31793-0748, USA. ³Centre for Sustainable Agriculture, Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster LA1 4YQ, UK. ⁴Faculty of Agriculture, Kyushu University, Fukuoka 812-8581, Japan.

*Present address: Nong Lam University, Ho Chi Minh City, Vietnam.

‡Author for correspondence (takasu@bri.kyushu-u.ac.jp)

Received 5 June 2013; Accepted 22 January 2014

preferences in solitary organisms, such as adult parasitoids. Moreover, parasitoids can consume sugar meals of up to a third of their body mass at a time during *ad libitum* feeding, which restricts the number of their feeding events. Upon encountering a food source of sufficient quantity and quality, a food-deprived parasitoid will typically fill its gut, rather than sample the food site and continue foraging for alternative sugar sources. Few methods have been developed to investigate sugar preferences for solitary arthropods or individuals of social species. Some studies have inferred sugar preferences on the basis of sugar consumption when provided access to one particular sugar source (Barker and Lehner, 1974; Boevé and Wäckers, 2003). However, this method only provides an indirect measure of preference, as insects are not experiencing alternatives. In several butterflies, preferences for nectar constituents have been investigated by dipping the tip of the proboscis alternately into two drops of alternative test solutions. A preference can be inferred if butterflies imbibe one solution after rejecting the previously experienced alternative (Erhardt, 1992; Romeis and Wäckers, 2000). Bachman and Waller (Bachman and Waller, 1977) conducted a similar test for individual honeybees. They alternately offered bees small amounts of two sugar solutions. A preference was observed only when bees accepted one solution after rejecting the other solution.

Here we use a different method to determine feeding preferences in solitary organisms. In this method, an individual wasp is first briefly exposed to one food source, before being allowed to feed *ad libitum* on the alternative. The total amount of consumption is then compared with that of a control group, whose initial experience involved the same sugar as the one offered in the *ad libitum* feeding. As this method allows individuals from the first group to modify their sugar intake based on complete information on both alternatives, it could provide a more appropriate assessment of preference.

Microplitis croceipes (Cresson 1872) (Hymenoptera: Braconidae) is an endo-larval parasitoid of *Helicoverpa* and *Heliothis* species (Lewis and Burton, 1970; King and Coleman, 1989; Knippling and Stadelbacher, 1983; Hoang and Takasu, 2005). *Microplitis croceipes* was chosen for this study because it does not host feed (Takasu and Lewis, 1993) or feed on pollen (Jervis, 1998), and therefore it represents the large group of parasitoids whose diet is restricted to sugar sources, such as nectar and honeydew (Takasu and Lewis, 1993; Takasu and Lewis, 1996; Stapel et al., 1997; Röse et al., 2006; Wäckers et al., 2006; Zhou et al., 2012). Additionally, this parasitoid had been used in previous sugar feeding studies (Wäckers et al., 2002; Nafziger and Fadamiro, 2011), and both male and female adults can also learn to associate odors with various sugars and subsequently respond to the learned odors (Lewis and Takasu, 1990; Takasu and Lewis, 1995; Takasu and Lewis, 1996; Stapel et al., 1997; Wäckers et al., 2002; Wäckers et al., 2006). In associative conditioning of food odors by *M. croceipes*, glucose, sucrose, fructose and melezitose are effective as unconditional stimuli (Wäckers et al., 2006). Here we examined innate feeding responses of food-naïve and food-experienced male and female adults of *M. croceipes* to four major sugars found in plants: sucrose, glucose, fructose and maltose. This was to determine whether the parasitoids are able to discriminate between individual sugars. Subsequently, we examined whether feeding responses to sugars changed after a feeding experience with the same or other sugars. Differences in sugar feeding responses between our laboratory strain that had been maintained for more than 10 years in Japan (Kyushu strain) and its stock culture from the USA (Tifton strain) were also tested. The changes of feeding response are discussed in terms of sugar nutritional suitability, i.e. their ability to sustain adult longevity.

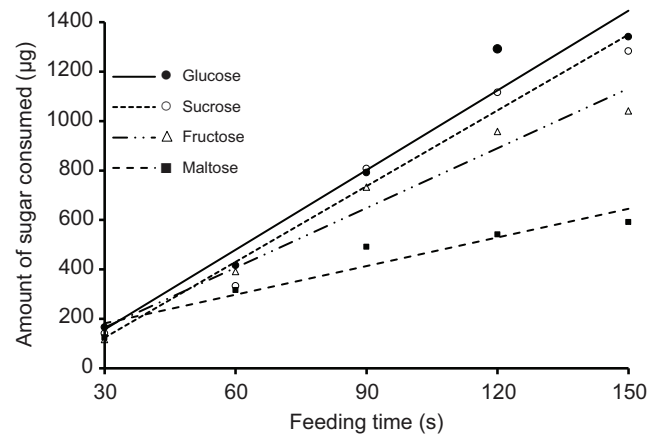


Fig. 1. Relationship between feeding time and amount of a 1 mol l⁻¹ sugar solution consumed by *Microplitis croceipes* females. Linear regression results are as follows: sucrose $y = -183 + 10.2x$, $R^2 = 0.7272$, $P < 0.0001$; glucose $y = -166 + 10.8x$, $R^2 = 0.6225$, $P < 0.0001$; fructose $y = -77 + 8.1x$, $R^2 = 0.8621$, $P < 0.0001$; maltose $y = 66 + 3.9x$, $R^2 = 0.6170$, $P < 0.0001$.

RESULTS

Effect of sugars on feeding time and sugar intake

As feeding time increased, the amount of sugar consumed increased. Linear relationships were found between feeding time (s) and the amount (µg) of sugar consumed for the four sugars (Fig. 1). However, there was a significant difference among slopes for the four sugars (ANCOVA: interaction between feeding time and sugar type, $F_{3,220} = 5.15$, $P < 0.0001$). The slopes of linear regressions for sucrose, glucose and fructose were not significantly different ($F_{2,165} = 1.15$), but each was significantly different from that for maltose ($F_{1,1230} = 11.89$; Bonferroni procedure for multiple comparisons, $P < 0.05$).

Innate feeding response to single sugars

As the concentration of the sugar solution decreased, the percentage of wasps that accepted the sugar solution decreased (Fig. 2). All of the wasps tested rejected glucose at 0.016 mol l⁻¹ and maltose at 0.063 mol l⁻¹, and the two other sugars were rejected at 0.008 mol l⁻¹ (Fig. 2). The Probit analysis estimated that the EC₅₀ (the effective concentration at which at least 50% of the wasps tested accepted a sugar) was significantly higher for maltose compared with sucrose, glucose and fructose (Table 1). Analysis of covariance using sugar as the group variable and log concentration as a covariate showed

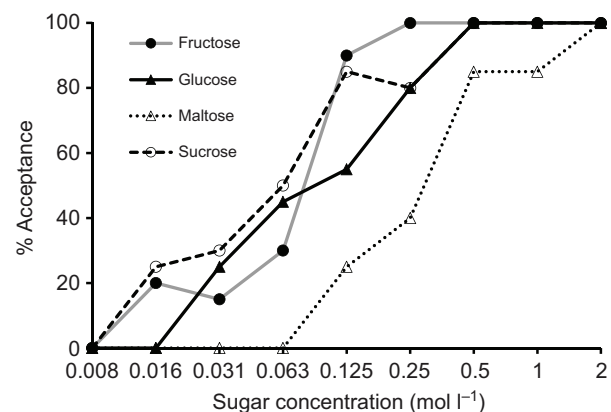


Fig. 2. Feeding acceptance by *Microplitis croceipes* females toward four sugar solutions at nine different concentrations.

Table 1. Probit analyses of innate feeding responses to four sugar solutions by *Microplitis croceipes* females

Sugar	Regression	Chi-square (d.f.=7) (<i>P</i> -value)	EC ₅₀ (mol l ⁻¹) (95% CI)
Sucrose	$y=0.858x+7.501$	7.1 (0.418)	0.054 (0.039–0.073) ^a
Fructose	$y=1.196x+8.404$	13.8 (0.054)	0.058 (0.045–0.075) ^a
Glucose	$y=1.019x+7.505$	5.5 (0.598)	0.085 (0.065–0.113) ^a
Maltose	$y=1.173x+6.486$	5.4 (0.617)	0.282 (0.217–0.366) ^b

y, probit acceptance; *x*, natural log of sugar concentration.

Values with the same superscripted letters within the EC₅₀ column are not significantly different (likelihood ratio test: *P*=0.05).

that there was a significant difference among the sugars in the percentage of wasps that accepted the sugars (ANCOVA: $F_{3,31}=68.06$, $P<0.0001$).

Feeding time for all sugars decreased as sugar concentration decreased (Fig. 3). Feeding time on maltose was significantly lower than on other sugars at 0.5 mol l⁻¹ (Steel–Dwass test: d.f.=3, $H=36.50$, $P<0.0001$), 0.25 mol l⁻¹ (Steel–Dwass test: d.f.=3, $H=28.71$, $P<0.0001$) and 0.125 mol l⁻¹ (Steel–Dwass test: d.f.=3, $H=18.23$, $P=0.0004$; Fig. 3).

Effect of previous experience on feeding response to sugars

Prior feeding by male and female *M. croceipes* (Kyushu strain) on 1 mol l⁻¹ solutions of glucose or sucrose did not affect their subsequent feeding time on either of those sugar solutions (Steel–Dwass tests: female, d.f.=3, $H=4.73$, $P=0.193$; male, d.f.=3, $H=6.50$, $P=0.089$; Fig. 4A). However, prior feeding on the glucose or sucrose solutions significantly reduced subsequent feeding time on the maltose solution (Steel–Dwass tests: glucose, female, d.f.=3, $H=56.11$, $P<0.0001$; male, d.f.=3, $H=48.15$, $P<0.0001$; sucrose, female, d.f.=3, $H=61.13$, $P<0.0001$; male, d.f.=3, $H=71.49$, $P<0.0001$; Fig. 4B,C).

Similarly, feeding time on the 1 mol l⁻¹ fructose solution was significantly reduced after wasps had fed on the glucose solution (Steel–Dwass tests: female, d.f.=3, $H=61.69$, $P<0.0001$; male, d.f.=3, $H=71.06$, $P<0.0001$; Fig. 4D) or on sucrose (Steel–Dwass tests: female, d.f.=3, $H=58.91$, $P<0.0001$; male, d.f.=3, $H=63.97$, $P<0.0001$; Fig. 4E).

Prior feeding by male and female *M. croceipes* on a 1 mol l⁻¹ fructose solution did not affect subsequent feeding on the maltose solution, but prior feeding by *M. croceipes* males on the maltose solution significantly reduced subsequent feeding time on the fructose solution (Steel–Dwass test: d.f.=3, $H=21.34$, $P<0.0001$; Fig. 4F). Feeding time on the fructose solution by *M. croceipes* females after feeding on the maltose solution was not different from feeding time on the maltose solution (control) alone, but was significantly lower than feeding time on the fructose solution alone (control) (Steel–Dwass test: d.f.=3, $H=27.58$, $P<0.0001$).

The Tifton strain showed similar results. Here as well we see that prior feeding on a 1 mol l⁻¹ sucrose, glucose or maltose solution significantly reduced subsequent feeding time on the fructose solution (Steel–Dwass tests: sucrose, d.f.=3, $H=28.15$, $P<0.0001$; glucose, d.f.=3, $H=47.24$, $P<0.0001$; maltose, d.f.=3, $H=21.28$, $P<0.0001$; Fig. 5).

Persistence of fructose feeding inhibition following glucose experience

When wasps were provided with 1 mol l⁻¹ glucose and fructose solutions in sequence, feeding time on fructose increased gradually as the interval between exposures to those sugars increased (Fig. 6A). Nevertheless, feeding time on the fructose solution following 5 s feeding on the glucose solution was significantly reduced relative to the control treatments for all time intervals (Steel–Dwass tests: 30 s, d.f.=3, $H=46.02$, $P<0.0001$; 30 min, d.f.=3, $H=49.02$, $P<0.0001$; 1 h, d.f.=3, $H=45.71$, $P<0.0001$; 6 h, d.f.=3, $H=46.08$, $P<0.0001$; 12 h, d.f.=3, $H=39.14$, $P<0.0001$; Fig. 6). Similarly, feeding time on the fructose solution 18 h after feeding on the glucose solution for 50 s was significantly reduced relative to the control treatments (Steel–Dwass test: d.f.=3, $H=46.69$, $P<0.0001$; Fig. 6B).

Effect of glucose concentration on subsequent fructose feeding inhibition

When female wasps were provided with various concentrations of glucose, their subsequent feeding time on 1 mol l⁻¹ fructose was significantly reduced at all concentrations of glucose tested (Steel–Dwass tests: 0.5 mol l⁻¹, d.f.=3, $H=41.90$, $P<0.0001$; 0.25 mol l⁻¹, d.f.=3, $H=55.00$, $P<0.0001$; 0.125 mol l⁻¹, d.f.=3, $H=45.02$, $P<0.0001$; 0.063 mol l⁻¹, d.f.=3, $H=41.24$, $P<0.0001$; 0.031 mol l⁻¹, d.f.=3, $H=36.33$, $P<0.0001$; Fig. 7).

Effect of sugars on longevity

Survival curves for both sexes differed significantly among the four 2 mol l⁻¹ sugar solutions (female: d.f.=4, $\chi^2=126.02$, $P<0.0001$;

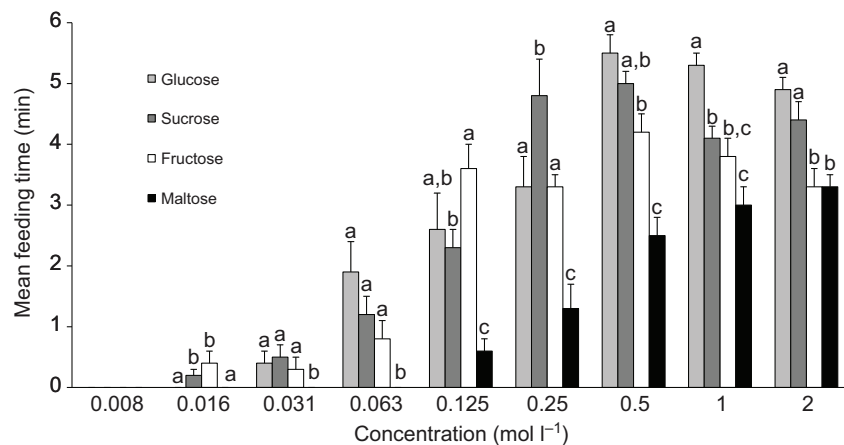


Fig. 3. Feeding time by *Microplitis croceipes* females on four sugar solutions at nine concentrations. Error bars represent \pm s.e.m. Bars with the same letter within the same concentration (mol l⁻¹) are not significantly different (Steel–Dwass test: *P*=0.05).

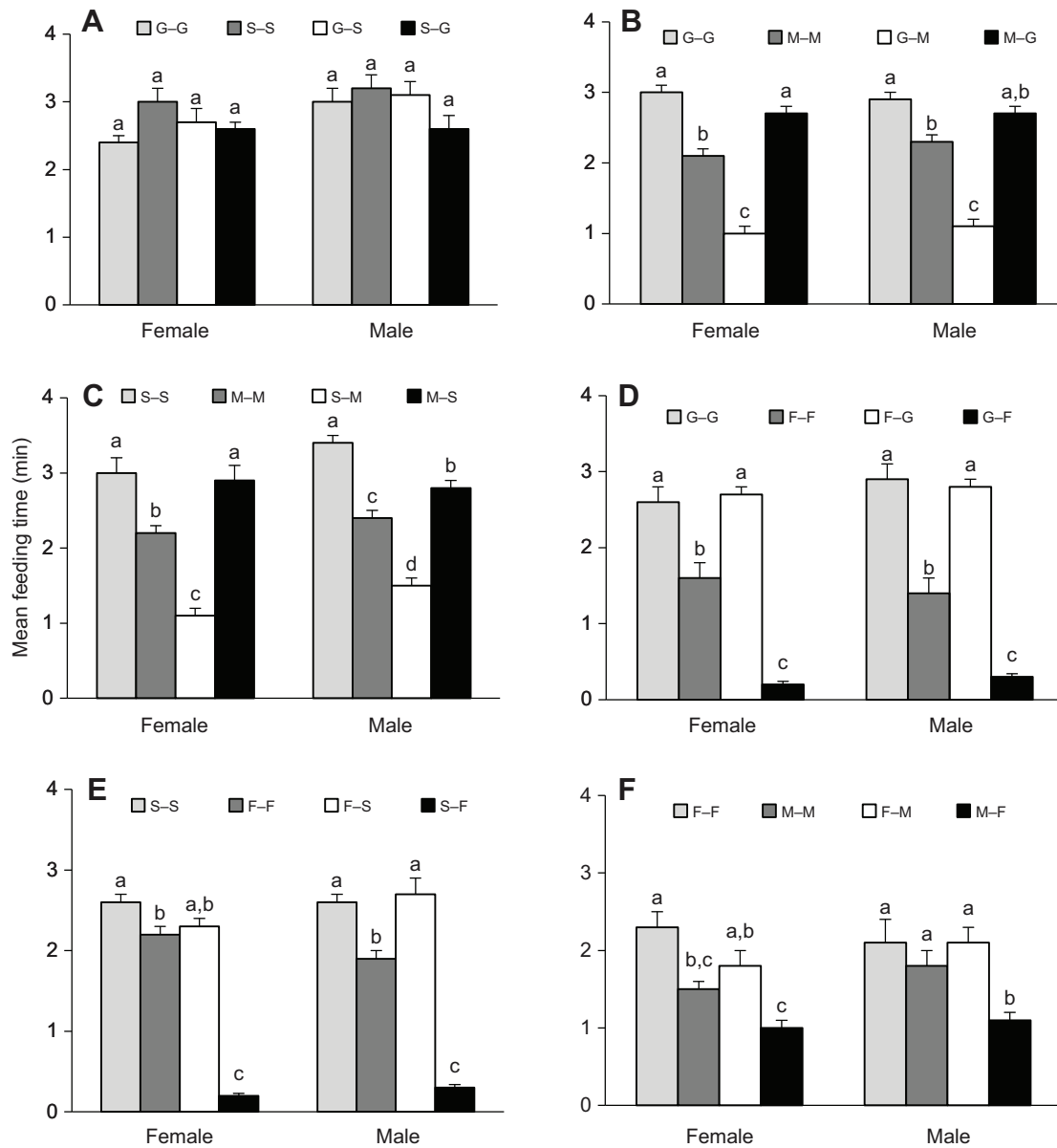


Fig. 4. Effect of previous sugar feeding on feeding time on four 1 mol l^{-1} sugar solutions in *Microplitis croceipes* (Kyushu strain). The combinations of two sugars given to individual wasps were: (A) glucose and sucrose, (B) glucose and maltose, (C) sucrose and maltose, (D) glucose and fructose, (E) sucrose and fructose and (F) fructose and maltose. S, sucrose; F, fructose; G, glucose; M, maltose. Error bars represent \pm s.e.m. Bars with the same letter within a sugar combination are not significantly different (Steel-Dwass test: $P=0.05$).

male: d.f.=4, $\chi^2=172.39$, $P<0.0001$; Fig. 8). For both sexes, longevity of wasps that fed on the maltose solution was significantly shorter than that of those that fed on the other sugar solutions. Longevity of females given different sugar solutions was significantly higher than that of males (d.f.=1, $\chi^2=24.36$, $P<0.0001$).

DISCUSSION

Relationship between sugar feeding time and consumption

Feeding time is often recorded as a measure of insect gustatory response and food consumption (Wäckers et al., 2006), with the assumption that there is a positive relationship between sugar feeding time and consumption. However, feeding time on a sugar solution may not always reflect consumption of the solution because of different feeding speeds due to different viscosities of the sugars (Siekmann et al., 2001; Wäckers, 2001; Wäckers et al., 2006; Faria

et al., 2008; Wyckhuys et al., 2008). In the present study, we found positive linear relationships between feeding time and consumption for 1 mol l^{-1} in all four sugars, suggesting that at this concentration, feeding time is a good indicator of gustatory responses and consumption in all sugars tested.

However, the slope of the regression for maltose was significantly steeper than those of the other three sugars. This suggests that feeding speed is lower for maltose solutions than for the other sugar solutions. Siekmann et al. (Siekmann et al., 2001) and Wyckhuys et al. (Wyckhuys et al., 2008) suggest that the higher viscosity of maltose may reduce feeding speed relative to the other sugars, but Nithiyanantham and Palaniappan (Nithiyanantham and Palaniappan, 2013) and Chirife and Buera (Chirife and Buera, 1997) reported equal viscosity of sucrose and maltose. Thus, it remains unclear what properties of maltose reduce the feeding time in *M. croceipes*.

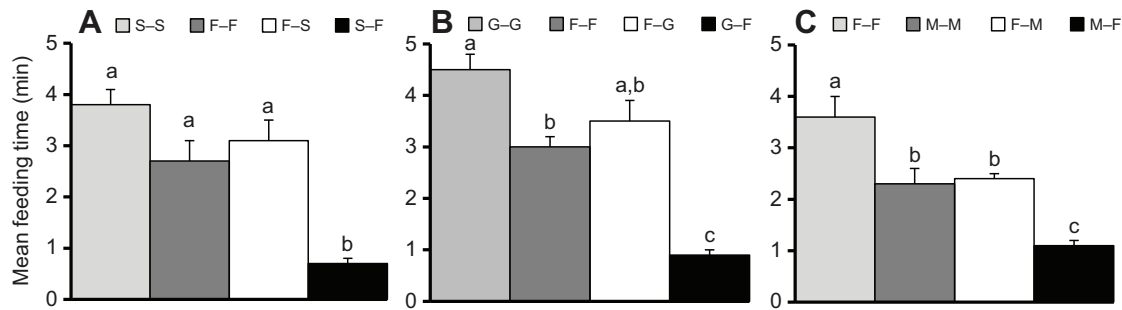


Fig. 5. Effect of previous sugar feeding on feeding time on four 1 mol l^{-1} sugar solutions in *Microplitis croceipes* females (Tifton strain). The combinations of two sugars given to individual wasps were: (A) sucrose and fructose, (B) glucose and fructose, and (C) fructose and maltose. S, sucrose; F, fructose; G, glucose; M, maltose. Error bars represent \pm s.e.m. Bars with the same letter within a sugar combination are not significantly different (Steel–Dwass test: $P=0.05$).

Innate gustatory response to single sugars

Gustatory responses by feeding-inexperienced hymenopteran parasitoids are known to vary between sugars (Wäckers, 1999; Beach et al., 2003). In the present study, the acceptance thresholds for sucrose, glucose and fructose, i.e. the lowest concentration at which these sugars evoke a feeding response, were in the range of 0.016 and 0.031 mol l^{-1} (Fig. 6). This is comparable to the acceptance thresholds for these sugars as reported for other parasitoids (Wäckers, 1999; Beach et al., 2003) and far below the concentrations at which those sugars naturally occur in nectars or honeydews (Baker and Baker, 1983). This suggests that *M. croceipes* should have no problem detecting these sugars in nectars and honeydews.

However, the innate response by *M. croceipes* females to maltose is less sensitive than their response to sucrose, glucose and fructose. The EC_{50} was higher for maltose than for the other three sugars. At 1 mol l^{-1} or lower concentrations, wasps fed for a significantly shorter time on maltose when compared with the other sugars. This may reflect the fact that this sugar is less common in nectar and nutritionally less suitable. Previous parasitoid studies have shown that innate gustatory sugar responses are positively correlated with the relative nutritional quality of the sugar in terms of metabolic utilization and fitness (Wäckers, 1999; Wäckers, 2001; Williams and Roane, 2007; Luo et al., 2013). Similarly, in *M. croceipes*, the lower nutritional value of maltose is expressed in the substantially reduced longevity of parasitoids fed this sugar, as compared with the other sugars.

Gustatory discrimination between sugars

A pre-exposure to sucrose and glucose significantly reduced feeding time in the case of maltose or fructose. This suggests that *M. croceipes* adults can discriminate maltose or fructose from the other three sugars. Although feeding discrimination against a mixture of sugars is known in a few parasitoids (Wäckers, 1999; Beach et al., 2003; Vollhardt et al., 2010), this is the first demonstration of the ability of parasitic wasps to discriminate between single sugars.

While innate feeding responses were weaker for maltose than the other sugars, prior feeding on glucose or sucrose further inhibited feeding responses to maltose. Optimal foraging theory predicts that when an organism is provided a choice between two types of food that differ in quality, they should choose the higher quality food, and reduce consumption of the lower quality food (Pulliam, 1974; Charnov, 1976; Sih and Christensen, 2001). In the present study, feeding inhibition to maltose following previous feeding on other sugars could be explained in these adaptive terms, with parasitoids refraining from feeding on the nutritionally inferior maltose, when having experienced nutritionally superior sugars. This acquired feeding inhibition implies that in a patch containing superior sugars such as glucose, sucrose or fructose, parasitoids rapidly give up the lesser quality food in favor of foraging for the nutritionally superior sugars. Similarly, parasitoids effectively discriminate between hosts in and among patches containing different qualities of hosts, and they change host acceptance based on previous experience (Papaj and Vet, 1990; Poolman Simons et al., 1992; Geervliet et al., 1998; Duan and Messing, 1999; Fujiwara et al., 2000).

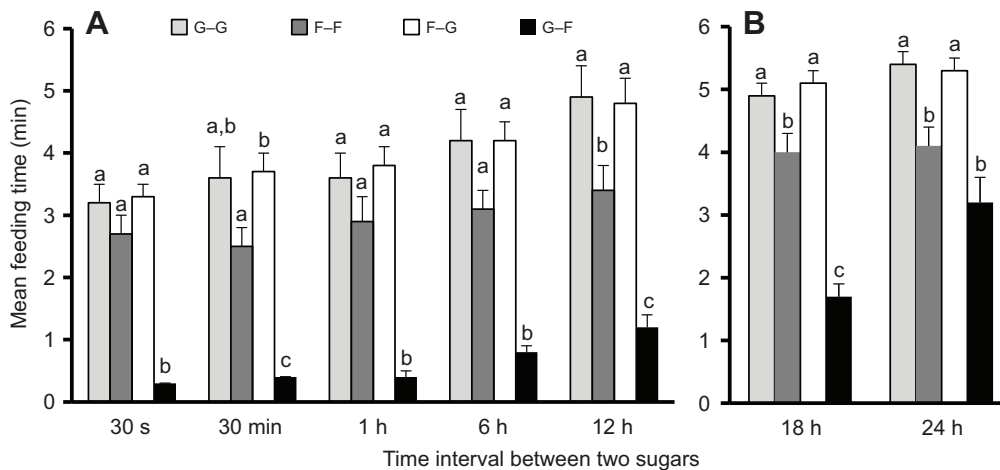


Fig. 6. Effect of time lapse after feeding on the first sugar solution on feeding time on the second sugar solution by *Microplitis croceipes*. (A) Wasps were given the second 1 mol l^{-1} sugar solution 30 s to 12 h after feeding on the first 1 mol l^{-1} sugar solution for 5 s. (B) Wasps were given the first 1 mol l^{-1} sugar solution for 50 s. S, sucrose; F, fructose; G, glucose; M, maltose. Error bars represent \pm s.e.m. Bars with the same letter within a combination of two sugars are not significantly different (Steel–Dwass test: $P=0.05$).

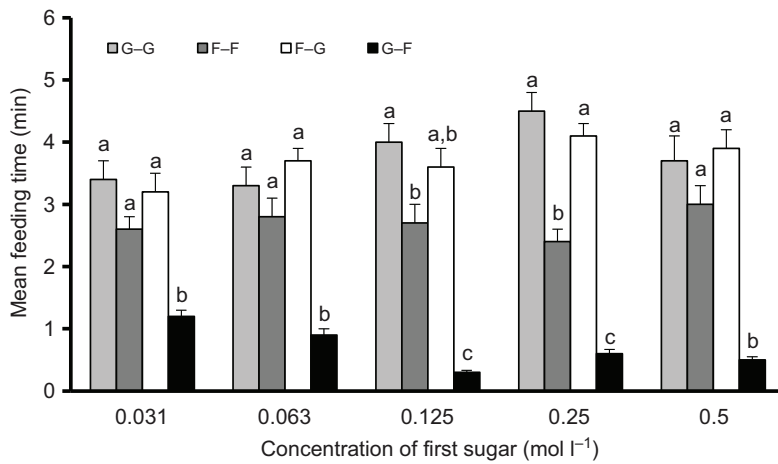


Fig. 7. Effect of glucose concentration on subsequent fructose feeding by *Microplitis croceipes*. S, sucrose; F, fructose; G, glucose; M, maltose. Error bars represent \pm s.e.m. Bars with the same letter within a concentration (mol l^{-1}) of first sugar are not significantly different (Steel–Dwass test: $P=0.05$).

Surprisingly, also in the case of fructose we see a pronounced reduction in feeding time following exposure to glucose or sucrose; this phenomenon was consistently observed in multiple experiments and with both colonies of *M. croceipes*. Following exposure to the other sugars, the wasps showed a pronounced change in behavior when exposed to the fructose. They exhibited frequent rejections followed by repeated return visits to the fructose solution, suggesting that they were still hungry. Upon each contact with a fructose droplet, the wasps repeatedly touched the fructose solution with their mouthparts, antennae and front legs, and made several quick turns while walking around the sugar droplet. This behavior was not observed when wasps encountered the other sugars. Moreover, this behavior was also not seen in wasps encountering fructose without prior feeding on another sugar. Unlike the example

of maltose, the feeding inhibition in the case of fructose following experience with glucose or sucrose is not explained by a lower nutritional suitability of fructose. Actually, fructose supported adult survival equally as well as glucose and sucrose. In addition, we did not find any difference in oviposition behavior between fructose-fed females and glucose- or sucrose-fed females (K.T., unpublished). Fructose, as well as glucose and sucrose, is also an effective unconditional stimulus for associative learning of food odors (Wäckers et al., 2006). All of these facts suggest that fructose is nutritionally equivalent to glucose or sucrose under the experimental conditions. However, we do not know the effect of feeding on only fructose on reproductive performance by *M. croceipes* adults in the natural conditions. The possible function for the inhibition of fructose feeding following exposure to the other sugars therefore

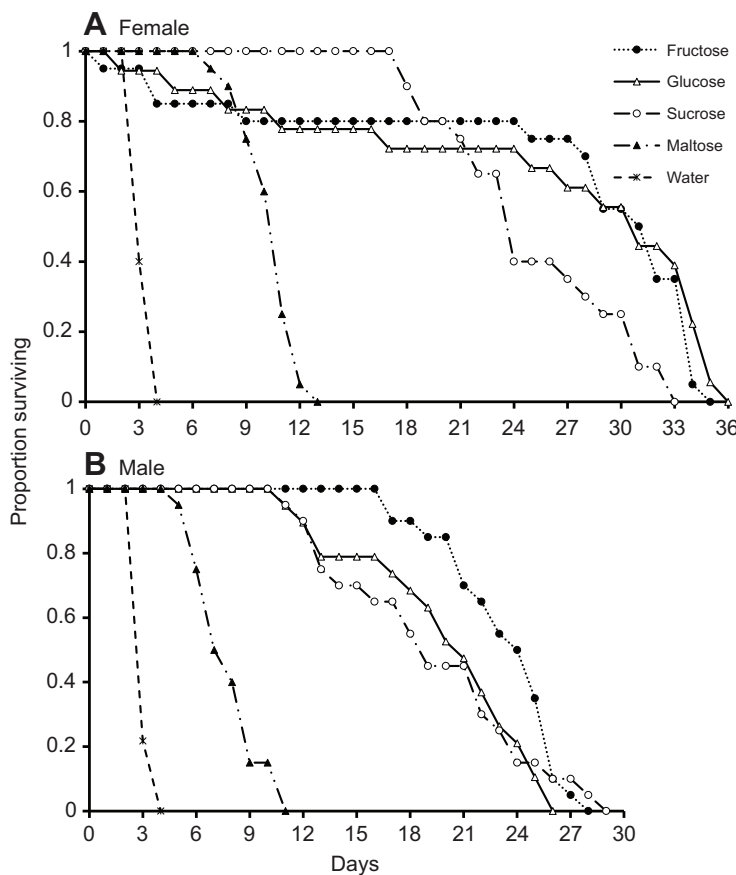


Fig. 8. Survivorship curves for both sexes of *Microplitis croceipes* provided with 2 mol l^{-1} solutions of four sugars and water.

remains to be investigated. It would be interesting to test the wasps' responses to fructose after feeding on a mixture of glucose, sucrose and fructose because multiple sugars are typically present in plant nectars and honeydew.

Although it is known that the acceptance of phagostimulatory sugars in mixtures can be inhibited by phagodeterrent sugars, Wieczorek and Wolff (Wieczorek and Wolff, 1989) demonstrated an inhibition of blowfly feeding on fructose when mixed with mannose (monosaccharide) and raffinose (trisaccharide). Wäckers (Wäckers, 1999) found that sucrose mixed with mannose or raffinose inhibited feeding by the parasitic wasp *Cotesia glomerata* (Hymenoptera: Braconidae) relative to pure sucrose, and Beach et al. (Beach et al., 2003) found that rhamnose (monosaccharide) mixed with maltose inhibited acceptance of the latter by the parasitoid *Anaphes iole* (Hymenoptera: Mymaridae). Such discrimination abilities have been attributed to differences in response by insect gustatory receptor cells or to nutrient unsuitability of those compounds (Stoffolano, 1973; Blaney et al., 1986; Bernays, 1995; Wäckers, 2001). However, to our knowledge, our results present the first example of an inhibitory interaction between two phagostimulant sugars. Future studies will have to address the physiological mechanism(s) and ecological implications.

MATERIALS AND METHODS

Parasitoids

Microplitis croceipes were reared at the laboratory of Bioresource and Management, Kyushu University, Japan. The parasitoid stock culture originated from the USDA-ARS Crop Protection and Management Research Unit (CPMRU) in Tifton, GA, USA, and has been reared at Kyushu University since June 2001, with no infusion with feral wasps. The parasitoids were reared on *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae with honey as food under a 16 h:8 h light:dark photoperiod at 25°C (Hoang and Takasu, 2005). For the experiment, newly emerged adults were kept in a plastic rearing cage (30×30×25 cm, length × width × height) and provided with distilled water only. *Microplitis croceipes* that were tested at CPMRU had been reared on *Helicoverpa zea* (Lepidoptera: Noctuidae) larvae with honey as food. Two-day-old adults were used in all the experiments. Individual wasps were provided with a droplet of water and kept individually in glass vials (diameter 1.7 cm, height 10 cm) for 30 min before each test.

Sugars tested

The sugars used in the experiments were the disaccharides sucrose and maltose, and the monosaccharides D-(+)-glucose and D-(-)-fructose (Levulose) (all from Nacalai Tesque, Inc., Kyoto, Japan). Based on their molecular weights, the sugars were diluted with distilled water to 1 mol l⁻¹. Fresh sugar solutions were prepared for each of the experiments.

In all experiments, a 10 µl droplet of a sugar solution was pipetted at the center of a truncated cone-shaped plastic cup (diameter: top 12 cm, bottom 10 cm; height 6 cm). An individual wasp was allowed to walk from the glass vial to the droplet of solution in the cup. After the wasp moved to the cup, the cup was placed upside down on wet filter paper to avoid a concentration increase in the test solution due to evaporation. The wasp's feeding time on the solution was observed and recorded. Feeding time (s) was recorded as time during which the wasp's mouthparts were seen to be in continuous contact with the sugar solution. The wasps never paused once they initiated feeding.

Effect of sugars and feeding time on sugar intake

Gustatory responses in insects are often assessed in terms of feeding time. However, feeding time of a food item may not accurately reflect its intake (Siekman et al., 2001; Wäckers, 2001; Azzouz et al., 2004; Wyckhuys et al., 2008). Therefore, we first examined the relationship between feeding time and intake of the sucrose, glucose, fructose and maltose solutions. Individual female wasps were placed at 5°C for 10 min to immobilize them,

and then weighed on a precision balance (GR-60 series, A & D Company Limited, Tokyo, Japan). Thereafter, each wasp was allowed to feed continuously on a droplet of a 1 mol l⁻¹ solution of each sugar for either 30, 60, 90, 120 or 150 s. Immediately after feeding, each wasp was again placed at 5°C for 10 min and re-weighed. The difference between the two weight measurements indicated the quantity of sugar ingested by each wasp. For each sugar feeding time, 16 wasps were tested.

Innate feeding response to single sugars

The effect of sugar concentration on the innate feeding response of female *M. croceipes* was examined by providing them with single solutions of sucrose, glucose, fructose and maltose. A 2 mol l⁻¹ solution of each sugar was first prepared and then serial dilutions were made in a geometric sequence of 1, 0.5, 0.25, 0.125, 0.063, 0.031, 0.016 and 0.008 mol l⁻¹. An individual wasp was allowed to feed on a droplet of solution and its feeding time (s) was recorded. If feeding lasted more than 5 s, it was regarded as an acceptance. When a wasp stop feeding on and left the sugar solution within 5 s, this was recorded as a rejection. Probit analysis was used to estimate the EC₅₀ (the effective concentration at which at least 50% of the wasps tested accepted a sugar). For each sugar concentration, 20 wasps were tested.

Effect of previous experience on sugar feeding response

This experiment was designed to determine whether male and female *M. croceipes* discriminate between two sugars. Parasitoids were given two sugar feeding bouts, either providing them with the same sugar twice (AA or BB), or offering a different sugar during the second feeding (AB or BA). The test was conducted with each pair of the four following sugars: sucrose, glucose, fructose and maltose (six pairs). Each pair was tested at the four possible sugar combinations (AA, AB, BA, BB). For each treatment, an individual wasp was allowed to feed on one solution for 50 s. Subsequently, the sugar source was removed. After a 30 s interval, the wasp was provided with the second sugar source and the duration of the *ad libitum* feeding (s) was recorded. The number of wasps tested was 20 for each of the four glucose–maltose combinations, 25 for the sucrose–maltose combinations, and 24 for the four remaining sugar combinations.

Early results with wasps of the Kyushu strain showed that they exhibit feeding inhibition to fructose after having fed on other sugars. To provide a reference for comparison, the same experiment was conducted in Tifton, using the Tifton strain of *M. croceipes*. The number of wasps tested was 20 for each of the glucose–fructose combinations, and 12 for each of the sucrose–fructose and maltose–fructose combinations.

Persistence of fructose inhibition following glucose feeding

As our first experiment showed that a brief exposure to either glucose or sucrose drastically reduced subsequent feeding on fructose, we conducted a further experiment designed to determine how long following glucose feeding the inhibition of fructose intake is maintained. An individual female wasp was first allowed to feed on a 1 mol l⁻¹ glucose solution for 5 s, and then removed from the solution. The wasp was then placed in a vial with access to water only for 30 s, 30 min or 1, 6 or 12 h. Thereafter, the wasp was provided with a 1 mol l⁻¹ fructose solution. Because 5 s feeding on a sugar could not sustain a wasp for more than 12 h, a separate experiment was carried out, in which an individual female was allowed to feed on glucose solution for 50 s. Thereafter, the wasp was placed in a vial with access to water only for either 18 or 24 h, before being provided with the fructose solution. In both experiments, control wasps were tested in a similar manner as described above except that here the wasp was first provided with fructose, followed by glucose. Another control wasp was allowed to feed on fructose or glucose first, and then provided with the same sugar solution. Feeding time (s) on the second sugar was recorded. A total of 20 wasps were tested for each combination of treatment and time interval.

Effect of glucose concentration on fructose feeding inhibition

This experiment was designed to determine the lowest concentration of glucose that could inhibit *M. croceipes* feeding on fructose. A 0.5 mol l⁻¹ glucose solution was prepared and serial dilutions were made in a geometric sequence of 0.25, 0.125, 0.063 and 0.031 mol l⁻¹. An individual female was

allowed to feed on one of the glucose concentrations for 5 s. After 30 s, the wasp was provided with a 1 mol l^{-1} fructose solution. As a control, a 0.5 mol l^{-1} fructose solution was prepared and serially diluted as above. An individual wasp was allowed to feed on one of the fructose concentrations for 5 s and after 30 s was provided with 1 mol l^{-1} glucose. Another group of control wasps was allowed to feed on a glucose or fructose concentration for 5 s, and after 30 s was provided with 1 mol l^{-1} solution of the same sugar. Feeding time (s) on the second sugar was recorded. A total of 18 wasps were tested for each treatment with each first sugar concentration.

Effect of sugars on longevity

Longevity of *M. croceipes* adults was examined for males and females provided with 2 mol l^{-1} solutions of sucrose, glucose, fructose and maltose. Newly emerged wasps were individually placed in a truncated cone-shaped plastic cup (diameter: top 12 cm, bottom 10 cm; height 6 cm) with a lid, and provided with a $10 \mu\text{l}$ of sugar solution. Control wasps were provided with $10 \mu\text{l}$ of distilled water. Sugar solutions or water were renewed every 2 days to avoid microbial growth and crystallization due to evaporation. Wasps were kept under a 16 h:8 h light:dark photoperiod at 25°C , and their survival was checked once a day. A total of 20 wasps were examined for each sugar solution.

Statistical analyses

Data on the effect of sugar and feeding time on consumption were tested by analysis of covariance (ANCOVA). As the slopes from the linear regressions for amount of sucrose, glucose and fructose consumed for different feeding times were not different, the data for these sugars were combined and a pair-wise comparison was made between the combined slopes for the three sugars and the slope for maltose. A Bonferroni correction was used ($P=0.05/k$, where $k=2$ for all sugars). After natural logarithm transformation of concentrations, EC_{50} was estimated by Probit analysis. The effect of different sugars on wasp's feeding time was analyzed by a Kruskal–Wallis test followed by Steel–Dwass tests. Longevity was analyzed using survival analysis. Survival curves (function of wasps surviving at a particular moment in time) were generated and compared using Kaplan–Meier estimates of the survival function (Lavandero et al., 2005). A Bonferroni correction was used to account for each of the pair-wise comparisons ($P=0.05/k$, where $k=10$ for all treatments in a log-rank test).

Acknowledgements

We would like to thank K. Urakawa for rearing insects. We are also grateful to two anonymous referees for improving the manuscript.

Competing interests

The authors declare no competing financial interests.

Author contributions

J.K.M. performed the experiments, analyzed the data and contributed to writing of the manuscript, H.K.L. designed the experiments, D.M.O. contributed to writing of the manuscript, F.L.W. contributed to the design of the experiments and the writing of the manuscript, K.T. conceived and designed the experiments, and contributed to writing of the manuscript.

Funding

This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI [grant number 24658052].

References

- Alm, J., Ohnmeiss, T. E., Lanza, J. and Vriesenga, L. (1990). Preference of cabbage white butterflies and honey bees for nectar that contains amino acids. *Oecologia* **84**, 53–57.
- Azzouz, H., Giordanengo, P., Wäckers, F. L. and Kaiser, L. (2004). Effects of feeding frequency and sugar concentration on behavior and longevity of the adult aphid parasitoid: *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae). *Biol. Control* **31**, 445–452.
- Bachman, W. W. and Waller, G. D. (1977). Honey bee response to sugar solutions of different compositions. *J. Apic. Res.* **16**, 165–169.
- Baker, H. G. and Baker, I. (1982). Chemical constituents of nectar in relation to pollination mechanisms and phylogeny. In *Biochemical Aspects of Evolutionary Biology* (ed. M. H. Nitecki), pp. 131–171. Chicago, IL: University of Chicago Press.
- Baker, H. G. and Baker, I. (1983). Floral nectar sugar constituents in relation to pollinator type. In *Handbook of Experimental Pollination Biology* (ed. C. E. Jones and R. J. Little), pp. 117–141. New York, NY: Van Nostrand Reinhold.
- Barker, R. J. and Lehner, Y. (1974). Influence of diet on sugars found by thin-layer chromatography in thoraces of honey bees, *Apis mellifera* L. *J. Exp. Zool.* **188**, 157–164.
- Barron, A. B., Maleszka, R., Helliwell, P. G. and Robinson, G. E. (2009). Effects of cocaine on honey bee dance behaviour. *J. Exp. Biol.* **212**, 163–168.
- Beach, J. P., Williams, L., III, Hendrix, D. L. and Price, L. D. (2003). Different food sources affect the gustatory response of *Anaphes iole*, an egg parasitoid of *Lygus* spp. *J. Chem. Ecol.* **29**, 1203–1222.
- Bernays, E. A. (1995). Effect of experience on feeding. In *Regulatory Mechanisms in Insect Feeding* (ed. R. F. Chapman and G. Boer), pp. 279–306. London: Chapman and Hall.
- Blaney, W. M., Schoonhoven, L. M. and Simmonds, M. S. J. (1986). Sensitivity variations in insect chemoreceptors; a review. *Experientia* **42**, 13–19.
- Boevé, J. L. and Wäckers, F. L. (2003). Gustatory perception and metabolic utilization of sugars by *Myrmica rubra* ant workers. *Oecologia* **136**, 508–514.
- Charnov, E. L. (1976). Optimal foraging, the marginal value theorem. *Theor. Popul. Biol.* **9**, 129–136.
- Chirife, J. and Buera, M. P. (1997). A simple model for predicting the viscosity of sugar and oligosaccharide solutions. *J. Food Eng.* **33**, 221–226.
- Dethier, V. (1976). *The Hungry Fly: A Physiological Study of the Behavior Associated with Feeding*. Cambridge, MA: Harvard University Press.
- Duan, J. J. and Messing, R. H. (1999). Effects of origin and experience on patterns of host acceptance by the opine parasitoid *Diachasmimorpha tryoni*. *Ecol. Entomol.* **24**, 284–291.
- Erhardt, A. (1992). Preferences and non-preferences for nectar constituents in *Ornithoptera priamus poseidon* (Lepidoptera, Papilionidae). *Oecologia* **90**, 581–585.
- Faria, C. A., Wäckers, F. L. and Turlings, T. C. J. (2008). The nutritional value of aphid honeydew for non-aphid parasitoids. *Basic Appl. Ecol.* **9**, 286–297.
- Fujiwara, C., Takabayashi, J. and Yano, S. (2000). Oviposition experience on a host-infested plant affects flight and antennal searching behaviour of *Cotesia kariyai* towards the host-plant complex. *Entomol. Exp. Appl.* **97**, 251–256.
- Geervliet, J. B. F., Vreugdenhil, A. I., Dicke, M. and Vet, L. E. M. (1998). Learning to discriminate between infochemicals from different plant-host complexes by the parasitoids *Cotesia glomerata* and *Cotesia rubecula* (Hymenoptera: Braconidae). *Entomol. Exp. Appl.* **86**, 241–252.
- Hoang, L. K. and Takasu, K. (2005). *Helicoverpa armigera* as an alternative host of the larval parasitoid *Microplitis croceipes* (Hymenoptera: Braconidae). *Appl. Entomol. Zool.* **40**, 679–686.
- Inouye, D. W. and Waller, G. D. (1984). Responses of honey bees (*Apis mellifera*) to amino acid solutions mimicking floral nectars. *Ecology* **65**, 618–625.
- Jervis, M. A. (1998). Functional and evolutionary aspects of mouthpart structure in parasitoid wasps. *Biol. J. Linn. Soc. Lond.* **63**, 461–493.
- King, E. G. and Coleman, R. J. (1989). Potential for biological control of *Heliothis* species. *Annu. Rev. Entomol.* **34**, 53–75.
- Knipling, E. F. and Stadelbacher, E. A. (1983). The rationale for area wide management of *Heliothis* (Lepidoptera: Noctuidae) populations. *Bull. Entomol. Soc. Am.* **29**, 29–37.
- Kunkel, H. and Kloft, W. (1977). Fortschritte auf dem gebiet der haunigttau-forschung. *Apidologie (Celle)* **8**, 369–391.
- Lanza, J. (1988). Ant preferences for *Passiflora* nectar mimics that contain amino acids. *Biotropica* **20**, 341–344.
- Lanza, J. and Krauss, B. R. (1984). Detection of amino acids in artificial nectars by two tropical ants *Leptothorax* and *Monomorium*. *Oecologia* **63**, 423–425.
- Lanza, J., Vargo, E. L., Pulim, S. and Chang, Y. Z. (1993). Preferences of the fire ants *Solenopsis invicta* and *S. geminata* (Hymenoptera: Formicidae) for amino acid and sugar components of extrafloral nectars. *Environ. Entomol.* **22**, 411–417.
- Lavandero, B., Wratten, S., Shishehbor, P. and Worner, S. (2005). Enhancing the effectiveness of the parasitoid *Diadegma semiclausum* (Helen): movement after use of nectar in the field. *Biol. Control* **34**, 152–158.
- Lewis, W. J. and Burton, R. L. (1970). Rearing *Microplitis* in the laboratory with *Heliothis zea* as hosts. *J. Econ. Entomol.* **63**, 656–658.
- Lewis, W. J. and Takasu, K. (1990). Use of learned odours by a parasitic wasp in accordance with host and food-needs. *Nature* **348**, 635–636.
- Luo, S., Michaud, J. P., Li, J., Liu, X. and Zhang, Q. (2013). Odor learning in *Microplitis mediator* (Hymenoptera: Braconidae) is mediated by sugar type and physiological state. *Biol. Control* **65**, 207–211.
- Nafziger, T. D., Jr and Fadamiro, H. Y. (2011). Suitability of some farmscaping plants as nectar sources for the parasitoid wasp, *Microplitis croceipes* (Hymenoptera: Braconidae): effects on longevity and body nutrients. *Biol. Control* **56**, 225–229.
- Nemec, V. and Starý, P. (1990). Sugars in honeydew. *Biologia (Bratisl.)* **45**, 259–264.
- Nithiyannantham, S. and Palaniappan, L. (2013). Physicochemical studies on some disaccharides (sucrose, lactose, maltose) in aqueous media at 298.15 K. *Chem. Sci. Trans.* **2**, 35–40.
- Olson, D. M., Takasu, K. and Lewis, W. J. (2005). Food needs of adult parasitoids: behavioral adaptations and consequences. In *Plant-Provided Food for Carnivorous Insects* (ed. F. L. Wäckers, P. C. J. van Rijn and J. Bruin), pp. 137–147. Cambridge: Cambridge University Press.
- Papaj, D. R. and Vet, L. E. (1990). Odor learning and foraging success in the parasitoid, *Leptopilina heterotoma*. *J. Chem. Ecol.* **16**, 3137–3150.
- Percival, M. S. (1961). Types of nectar in angiosperms. *New Phytol.* **60**, 235–281.
- Poolman Simons, M. T. T., Suverkröpp, B. P., Vet, L. E. M. and de Moed, G. (1992). Comparison of learning in related generalist and specialist eucoilid parasitoids. *Entomol. Exp. Appl.* **64**, 117–124.

- Pulliam, H. R. (1974). On the theory of optimal diets. *Am. Nat.* **108**, 59-74.
- Romeis, J. and Wäckers, F. L. (2000). Feeding responses by female *Pieris brassicae* butterflies to carbohydrates and amino acids. *Physiol. Entomol.* **25**, 247-253.
- Röse, U. S. R., Lewis, J. and Tumlinson, J. H. (2006). Extrafloral nectar from cotton (*Gossypium hirsutum*) as a food source for parasitic wasps. *Funct. Ecol.* **20**, 67-74.
- Siekmann, G., Tenhumberg, B. and Keller, M. A. (2001). Feeding and survival in parasitic wasps: sugar concentration and timing matter. *Oikos* **95**, 425-430.
- Sih, A. and Christensen, B. (2001). Optimal diet theory: does it work, and when and why does it fail? *Anim. Behav.* **61**, 379-390.
- Stapel, J. O., Cortesero, A. M., DeMoraes, C. M., Tumlinson, J. H. and Lewis, W. J. (1997). Extrafloral nectar, honeydew and sucrose effects on searching behavior and efficiency of *Microplitis croceipes* (Hymenoptera: Braconidae) in cotton. *Environ. Entomol.* **26**, 617-623.
- Stephens, D. W. and Krebs, J. R. (1986). *Foraging Theory*. Princeton, NJ: Princeton University Press.
- Stoffolano, J. G., Jr (1973). Effect of age and diapause on the mean impulse frequency and failure to generate impulses in labellar chemoreceptor sensilla of *Phormia regina*. *J. Gerontol.* **28**, 35-39.
- Takasu, K. and Lewis, W. J. (1993). Host- and food-foraging of the parasitoid *Microplitis croceipes*: learning and physiological state effects. *Biol. Control* **3**, 70-74.
- Takasu, K. and Lewis, W. J. (1995). Importance of adult food sources to host searching of the larval parasitoid *Microplitis croceipes*. *Biol. Control* **5**, 25-30.
- Takasu, K. and Lewis, W. J. (1996). The role of learning in adult food location by the larval parasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae). *J. Insect Behav.* **9**, 265-281.
- van Alphen, J. J. M. and Vet, L. E. M. (1986). An evolutionary approach to host finding and selection. In *Insect Parasitoids* (ed. J. Waage and D. Greathead), pp. 23-61. London: Academic Press.
- Vökl, W., Woodring, J., Fischer, M., Lorenz, M. W. and Hoffmann, K. H. (1999). Ant-aphid mutualisms: the impact of honeydew production and honeydew sugar composition on ant preferences. *Oecologia* **118**, 483-491.
- Vollhardt, I. M. G., Bianchi, F. J. J. A., Wäckers, F. L., Thies, C. and Tschamtkte, T. (2010). Spatial distribution of flower vs honeydew resources in cereal fields may affect aphid parasitism. *Biological Control* **53**, 204-213.
- von Frisch, K. (1934). Über den geschmackssinn der biene. *Z. Vgl. Physiol.* **21**, 1-156.
- Wäckers, F. L. (1999). Gustatory response by the hymenopteran parasitoid *Cotesia glomerata* to a range of nectar and honeydew sugars. *J. Chem. Ecol.* **25**, 2863-2877.
- Wäckers, F. L. (2001). A comparison of nectar- and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *J. Insect Physiol.* **47**, 1077-1084.
- Wäckers, F. L. (2005). Suitability of (extra-) floral nectar, pollen, and honeydew as insect food sources. In *Plant-Provided Food for Carnivorous Insects* (ed. F. L. Wäckers, P. C. J. van Rijn and J. Bruin), pp. 17-74. Cambridge: Cambridge University Press.
- Wäckers, F. L. and van Rijn, P. C. J. (2012). Pick and mix: selecting flowering plants to meet the requirements of target biological control insects. In *Biodiversity and Insect Pests: Key Issues for Sustainable Management* (ed. G. M. Gurr, S. D. Wratten, W. E. Snyder and D. M. Y. Read), pp. 139-165. Chichester: John Wiley & Sons.
- Wäckers, F. L., Bonifay, C. and Lewis, W. J. (2002). Conditioning of appetitive behavior in the hymenopteran parasitoid *Microplitis croceipes*. *Entomol. Exp. Appl.* **103**, 135-138.
- Wäckers, F. L., Bonifay, C., Vet, L. and Lewis, W. J. (2006). Gustatory response and appetitive learning in *Microplitis croceipes* in relation to sugar type and concentration. *Animal Biology* **56**, 193-203.
- Waller, G. (1972). Evaluating responses of honeybees to sugar solutions using an artificial flower feeder. *Ann. Entomol. Soc. Am.* **65**, 857-862.
- Waller, G. D. and Bachman, W. W. (1981). Use of honey-sac load and dance characteristics of worker honeybees to determine their sugar preferences. *J. Apic. Res.* **20**, 23-27.
- Wieczorek, H. and Wolff, G. (1989). The labellar sugar receptor of *Drosophila*. *J. Comp. Physiol. A* **164**, 825-834.
- Williams, L., III and Roane, T. M. (2007). Nutritional ecology of a parasitic wasp: food source affects gustatory response, metabolic utilization, and survivorship. *J. Insect Physiol.* **53**, 1262-1275.
- Wyckhuys, K. A. G., Strange-George, J. E., Kulhanek, C. A., Wäckers, F. L. and Heimpel, G. E. (2008). Sugar feeding by the aphid parasitoid *Binodoxys communis*: how does honeydew compare with other sugar sources? *J. Insect Physiol.* **54**, 481-491.
- Wykes, G. R. (1952). The preferences of honey bees for solutions of various sugars which occur in nectar. *J. Exp. Biol.* **29**, 511-519.
- Zhou, Z., Rains, G. C. and Kulasiri, D. (2012). Development of a behavior parameter in classically conditioned parasitic wasps that detect changes in odor intensity. *Biol. Eng. Trans.* **5**, 19-31.