

# The nutritional value of aphid honeydew for non-aphid parasitoids

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## Abstract

Intake of sugar-rich foods by adult parasitoids is crucial for their reproductive success. Hence, the availability of suitable foods should enhance the efficacy of parasitoids as biological control agents. In situations where nectar is not readily available, homopteran honeydew can be a key alternative food source. We studied the impact of honeydew feeding on the longevity of the larval endoparasitoids *Cotesia marginiventris*, *Campoletis sonorensis* and *Microplitis rufiventris*, all natural enemies of important lepidopteran pests. Females of these wasps lived longer when feeding on honeydew produced by the aphid *Rhopalosiphum maidis* on barley compared to control females provided with water only. However, they lived shorter than females fed with a sucrose solution. Further investigations with *C. marginiventris* showed that access to honeydew also increases the number of offspring produced, but less so than access to a sucrose solution. Moreover, it was found that females of this species need to feed several times throughout their life in order to reach optimal longevity and reproductive output. Analyses of the sugars in the honeydew produced by *R. maidis* on barley revealed that it contains mainly plant-derived sugars, but also several aphid-synthesized sugars. The sugar composition of the honeydew changed as a function of aphid colony size and time a colony had been feeding on a plant. In general, the higher the aphid infestation, the smaller the percentage of aphid-synthesized sugars in the honeydew. Experiments with honeydew sugar mimics allowed us to reject the hypothesis that the relatively poor performance of the parasitoid on a honeydew diet was due to the sugar composition. Instead, the results from additional feeding experiments with diluted honeydew showed that the nutritional value of pure honeydew is primarily restricted by its high viscosity. The possible consequences of these findings for biological pest control are discussed.

## Zusammenfassung

Die Aufnahme von zuckerreicher Nahrung durch adulte Parasitoide ist entscheidend für ihren reproduktiven Erfolg. Daher sollte die Verfügbarkeit von geeigneter Nahrung die Effizienz von Parasitoiden als Mittel der biologischen Kontrolle steigern. Wenn Nektar nicht leicht verfügbar ist, kann der Honigtau von Homopteren eine entscheidende alternative Nahrungsquelle sein. Wir untersuchten den Einfluss des Honigtauconsums auf die Lebensdauer der larvalen Endoparasitoide *Cotesia marginiventris*, *Campoletis sonorensis* und *Microplitis rufiventris*, die natürliche Feinde wichtiger Schadlepidopteren sind. Verglichen mit Kontroll-Weibchen, die nur Wasser erhielten, lebten die Weibchen dieser Wespen länger, wenn sie von der Blattlaus *Rhopalosiphum maidis* (auf Gerste) produzierten Honigtau aufgenommen hatten. Allerdings lebten sie kürzer als Weibchen, die mit einer Saccharose-Lösung gefüttert wurden.

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Untersuchungen an *C. marginiventris* zeigten, dass Zugang zu Honigtau auch die Nachkommenzahl erhöhte, wenn auch in geringerem Maße als Saccharose-Lösung. Für optimale Lebensdauer und Reproduktion mussten die Weibchen mehrmals während ihres Lebens trinken. Die Zucker-Zusammensetzung des Honigtaus variierte mit der Größe der Blattlauskolonie und der Dauer ihres Bestehens. Im Allgemeinen sank der von den Blattläusen synthetisierte Zuckeranteil im Honigtau mit der Stärke des Blattlausbefalls. Versuche mit gemischten Zucker-Lösungen (nachgeahmtem Honigtau) erlaubten uns die Hypothese zurückzuweisen, dass der relativ schlechte Lebenserfolg des Parasitoiden bei Honigtau-Diät auf die Zucker-Zusammensetzung zurückzuführen sei. Vielmehr zeigten zusätzliche Versuche mit verdünntem Honigtau, dass der Nährwert von reinem Honigtau primär durch seine hohe Viskosität eingeschränkt wird. Die möglichen Konsequenzen dieser Befunde für die Biologische Schädlingsbekämpfung werden diskutiert.

**Keywords:** Nutritional ecology; Honeydew; Carbohydrates; Parasitoids; Biological control; Aphididae; Braconidae; Ichneumonidae

## Introduction

Food is essential for adult female parasitoids to optimize their reproductive success. Feeding not only increases their longevity and fecundity (Leius, 1961; England & Evans 1997; Wäckers, 2001), but also affects flight activity (Forsse, Smith & Bourchier, 1992; Wäckers, 1994) and attraction to and/or retention in an area (Stapel, Cortesero, De Moraes, Tumlinson & Lewis, 1997). The availability of suitable food should therefore be an important consideration in attempts to optimize the effectiveness of parasitoids as biological control agents.

Adult females of some parasitoids obtain essential nutrients directly from hosts through so-called host feeding, but even these species often need non-host food sources as a source of energy (Jervis, Kidd & Heimpel, 1996). Nevertheless, parasitoid females that feed on hosts or their by-products reduce the need to shift from host searching to food foraging, whereas parasitoids that only feed on food sources that are not associated with hosts will frequently have to forage for hosts and food separately (Sirot & Bernstein, 1996; Lewis, Stapel, Cortesero & Takasu, 1998). When food is located at a distance from host sites, this switching between resources may become particularly costly, since traveling to food sites limits the amount of time available for host searching, costs energy and increases the risk of mortality (Jervis et al., 1996; Stapel et al., 1997).

In nature, the primary non-host food sources available to parasitoid females are floral and extra-floral nectar, and homopteran honeydew (Wäckers & van Rijn, 2005 and references therein). Nectar availability is often limited in large monocultures, which may greatly hamper the effectiveness of parasitoids used for biological control (Winkler, Wäckers, Bukovinszky-Kiss & van Lenteren, 2006). In such situations, honeydew can be a key alternative food for parasitoids if honeydew producing Homoptera occur in the vicinity of hosts. Feeding on nearby honeydew instead of distant nectar sources should allow parasitoids to allocate more time

to searching for hosts, resulting in higher rates of parasitism.

Several laboratory studies have shown that in the presence of honeydew parasitoid females indeed live longer and achieve higher fecundity than unfed females (e.g. Hocking, 1966; England & Evans, 1997; Singh, Singh & Upadhyay, 2000). However, nectar is often a much better food source for parasitoids than honeydew (Idoine & Ferro, 1988; Wäckers, 2000; Lee, Heimpel, & Leibe, 2004; Wäckers & van Rijn, 2005). One possible explanation is that honeydew is not only composed of the main plant-derived sugars fructose, sucrose and glucose, but also contains various other compounds. These include homopteran-synthesized sugars (Mittler, 1958; Hendrix, Wei & Leggett, 1992), which may reduce the nutritional value of the honeydew (Wäckers, 2000, 2001). In fact, minimizing nutritional benefits to their natural enemies may be one of the driving forces in the evolution of honeydew sugar synthesis (Wäckers, 2000).

The composition of honeydew shows great variation both in the type of sugars present and the overall sugar concentration depending on the homopteran and plant species (Hendrix et al., 1992). Moreover, parasitoids can vary considerably with regard to the spectrum of honeydew sugars that they can utilise (Jacob & Evans, 2004; Wäckers, 2001; Winkler, Wäckers, Stingli & van Lenteren, 2005; Hausmann, Wäckers, & Dorn, 2005). This variability both from the side of the product (honeydew) and the users (parasitoids) suggests that there is an opportunity to fine-tune and manipulate the situation in crop fields to better exploit the presence of honeydew producing insects for pest control.

With the above in mind, we investigated the effect of the honeydew produced by the aphid *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae) attacking barley (*Hordeum vulgare*) on the longevity of the solitary larval endoparasitoids *Cotesia marginiventris* (Cresson) (Hymenoptera: Braconidae), *Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae) and *Microplitis rufiventris* Kokujev (Hymenoptera: Braconidae), natural enemies of important lepidopteran pests (Hegazi,

Hammad & Elminshawy, 1977; Hoballah, Degen, Bergvinson, Savidan, Tamò et al. 2004). For *C. marginiventris* we also tested, in cage experiments, how the presence of aphids affects their lifetime parasitism. As it was found that *R. maidis* honeydew was considerably less suitable than a water solution with sucrose, we analysed the honeydew for the presence of aphid-produced sugars that might explain these results. Solutions mimicking the sugar composition of *R. maidis* honeydew were then fed to *C. marginiventris* females to determine their nutritional quality. Since sugar composition did not explain the negative effect on nutritional quality, two additional experiments were conducted to determine if differences in food uptake due to differences in viscosity could explain differences in survival on honeydew and sucrose water.

## Materials and methods

### Plants

All plants were individually grown from seed in a climate chamber ( $27 \pm 2^\circ\text{C}$ , 60% r.h., 16L: 8D, and 50,000 lm/m<sup>2</sup>). Barley (variety Lyric) was used for the aphid rearing or experiments when 3–5 weeks old. Maize (variety Delprim) was used for the parasitoid performance experiments when 3–4 weeks old.

### Insects

We chose the corn leaf aphid *R. maidis* for the experiments because it excretes copious amounts of honeydew and is usually considered a minor pest (Kröber & Carl, 1991; Jauset, Munoz., & Pons, 2000; Kring & Gilstrap, 1986), so its presence in crop fields could benefit biological control agents without causing additional harm to the crop. The aphids were provided by Agroscope RAC Changins in Switzerland and reared in climate chambers ( $25^\circ\text{C}$ , 70% r.h. and 14L:10D).

*Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) eggs were received weekly from Syngenta (Stein, Switzerland) and the emerging larvae were used for parasitoid rearing or experiments. *C. marginiventris*, *M. rufiventris* and *C. sonorensis* colonies were maintained on *S. littoralis* larvae fed with artificial diet. Adults were kept in climate chambers ( $25^\circ\text{C}$ , 85% r.h. and 14L:10D) and the females used for the experiments were one day old, mated and unfed.

### Effect of honeydew on parasitoid longevity

Groups of five parasitoid females of a particular species were put in a cellophane bag ( $30 \times 15\text{ cm}$ ) covering a barley plant that was either: sprayed with a

2 M sucrose solution, infested with aphids or clean (i.e. neither treatment). Aphid infestation was obtained by placing clean barley plants together with aphid-infested plants for four to six days before an experiment started. This resulted in an estimated infestation of eight aphids/cm<sup>2</sup> at the beginning of the experiments. To provide humidity, a plastic container with water-soaked cotton wool was provided in each cellophane bag. All plants were put in a climate chamber and survival of the wasps was recorded daily.

### Effect of honeydew on *Cotesia marginiventris* performance

Groups of three *C. marginiventris* females were placed in plastic Bugdorm-2 cages ( $60 \times 60 \times 60\text{ cm}$ , MegaView Science Education Services Co. Ltd., Taiwan) with two maize plants infested with around 300 *S. littoralis* larvae and one barley plant that was either: sprayed with sucrose, infested with aphids, or clean. To investigate if *C. marginiventris* females need to feed several times through their life to optimally benefit from a food source, we added two other treatments, whereby barley plants sprayed with sucrose or infested with aphids were left in the tents only for the first two days of the experiments. The survival of the females was recorded daily and the *S. littoralis*-infested maize plants were replaced every other day. The collected larvae were reared through on artificial diet until the parasitoids formed their cocoons that were then counted.

### Honeydew collection and analysis

Individual barley plants were infested with aphids in a clip-cage ( $1.5 \times 1.5\text{ cm}$ ). In order to evaluate the effect of infestation rate and time since infestation on honeydew composition, three initial aphid densities were used (10, 100 and 500 aphids of mixed ages). The first honeydew collection was made three days after infestation, and subsequently the honeydew was collected at intervals of one week from the infestation date, for three consecutive weeks.

For the first and second collections the clip-cages that were used for the initial infestation were replaced with a new clip-cage that was left attached to the plant for 24 h. These collection cages were then placed in a 100% r.h. environment for 24 h and a micro-capillary was used to collect 1  $\mu\text{L}$  of honeydew, which was diluted in 50  $\mu\text{L}$  of 70% ethanol. For the subsequent collections, the clip-cages were removed, the plants were placed in new cellophane bags and Petri dishes were placed at the bottom of the plants for 24 h. The honeydew was collected from the Petri dishes using the same method described for the collection from clip-cages. The sugars

in the honeydew were analysed using the method described by Steppuhn & Wäckers (2004).

### Longevity of *C. marginiventris* on honeydew mimics

HPLC analysis showed that the honeydew is mainly composed of the plant sugars glucose, fructose and sucrose and the aphid-synthesized sugars maltose, erlose and trehalose. To investigate if the observed reduction in survival of parasitoids on the honeydew might be due to the aphid produced sugars, we measured the longevity of *C. marginiventris* when feeding on three different 1 M sugar mixtures that mimic honeydew saccharide composition. These were: (1) glucose (33.3%), fructose (33.3%) and sucrose (33.3%); (2) glucose (25%), fructose (25%), sucrose (25%), maltose (12.5%) and erlose (12.5%); and (3) glucose (25%), fructose (25%), sucrose (25%), maltose (10%), erlose (10%) and thehalose (5%). Longevity on these solutions was compared with the longevity of wasps fed on a 1 M sucrose solution, wasps fed on *R. maidis* honeydew, and unfed wasps.

Groups of three 12–24 h old females that had been food-deprived were placed in plastic cups (height 2.5 cm, diameter 4.5 cm). Small droplets of one of the sugar solutions were distributed on the lid of the cup using a total of 8  $\mu$ L of solution per cup. To collect honeydew, plastic lids were placed under aphid infested barley plants for 24 h, which was sufficient to cover the lids with honeydew. Humidity was kept high by placing a wet dental roll in each cup. The lids with sugar solutions were replaced twice per week and those with honeydew every other day. The cups with parasitoids were kept in a climate chamber (25 °C, 85% r.h. and 14L:10D) and the number of wasps alive was recorded daily.

### Relationship between honeydew intake and survival

During the aphid-produced sugars did not explain the relatively poor performance of honeydew-fed parasitoids, an experiment was added to test for a possible difference in uptake of honeydew and sucrose solution during feeding. For this we measured the intake of *R. maidis* honeydew or of a 2 M sucrose solution by *C. marginiventris* during a single feeding bout and subsequently determined the effect of this consumed sugar quantity on parasitoid survival.

*C. marginiventris* females were used when 24–30 h old. To make sure that the food intake was solely motivated by sugar need, parasitoids had been provided with ad libitum water prior to the experiments. The food sources were presented to the wasps as a 1  $\mu$ L droplet on a microscope slide (7.6  $\times$  2.6 cm) and were left in a 100% r.h. environment during the tests. The time spent feeding was recorded and quantity of food consumed was

determined by weighing the individual females on a precision scale (Mettler MX5;  $\pm$ 1  $\mu$ g) before and immediately after exposure to honeydew or a sucrose solution. Subsequently, each individual was placed in a glass tube (1.2  $\times$  7.5 cm) and its survival determined. Humidity was provided by a wet strip of filter paper in the glass tubes.

The correlation between intake and survival showed that the better survival on a sucrose solution was only due to a greater intake of this food source. This might indicate that honeydew's high viscosity makes it a difficult food source to feed on. To investigate this hypothesis we conducted an additional experiment, which measured the percentage weight gain and subsequent survival of *C. marginiventris* when feeding on pure honeydew, honeydew diluted with 10% of water, honeydew diluted with 30% of water or a 2 M sucrose solution. The methodology used was the same as for the previous experiment.

### Statistical analysis

Effects of different diets on survival in the first experiment were compared using survival analyses. Differences between survival curves were analysed with a log-rank test using S-Plus 6.2. Differences in parasitism rate (numbers of cocoons formed) by *C. marginiventris* females kept in cages with different food sources were tested by ANOVA and the means compared using the Tukey's test with SPSS 12.0.

The percentage of weight gained by *C. marginiventris* after one feeding bout on honeydew and sucrose solution was compared with a *t*-test, and subsequent longevity after feeding on these two food sources was compared using a Mann-Whitney test. The correlation between honeydew or sucrose solution intake and subsequent survival was analysed in a GLM of longevity with terms for food type, intake (% weight gain during one feeding bout) and their interaction. The time spent feeding on honeydew and sucrose was compared using a Rank Sum test. All these analysis were performed using SPSS 12.0.

The percentage weight gained by *C. marginiventris* after one feeding bout on undiluted or diluted honeydew or sucrose solution was compared by ANOVA and the means compared using the Tukey's test. The longevity of unfed *C. marginiventris* or those that had one feeding bout on undiluted or diluted honeydew or on a sucrose solution was compared with Kruskal Wallis ANOVA by ranks and the differences between the means were compared using Tukey's test. The degree of correlation between the intake of undiluted or diluted honeydew and of sucrose solution and the subsequent parasitoid survival was determined by linear regression analysis. All analyses were performed using SPSS 12.0.

## Results

### Effect of honeydew on parasitoid longevity

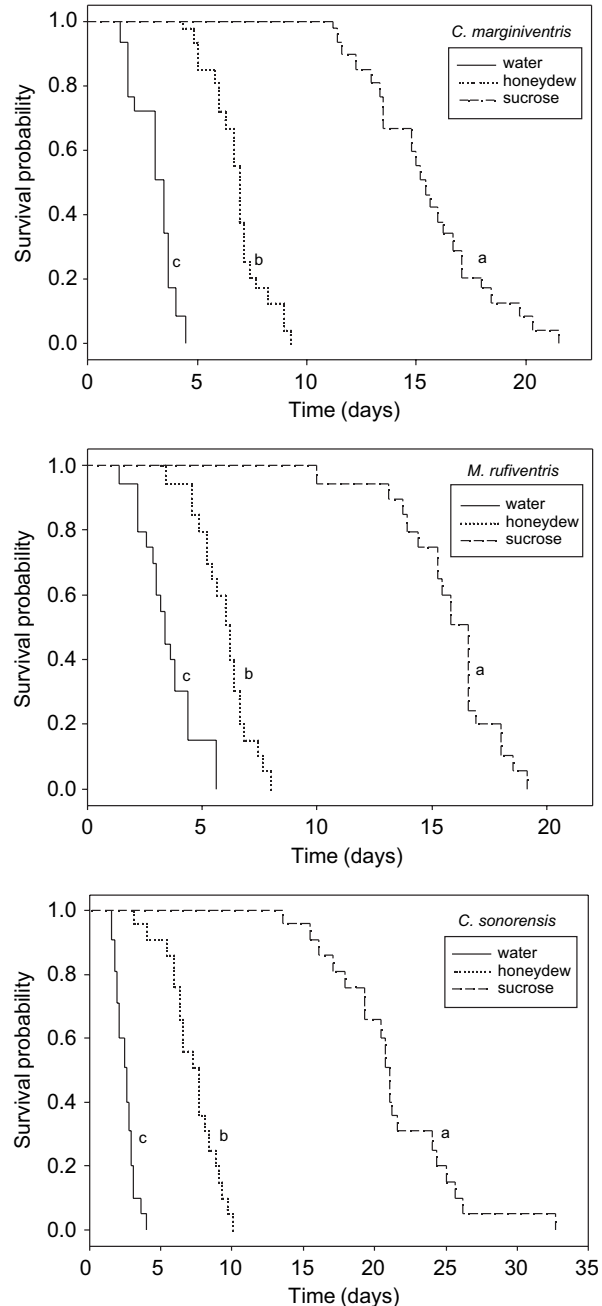
Diet significantly affected the survival of *C. marginiventris*, *M. rufiventris* and *C. sonorensis* ( $n = 24$ ,  $\chi^2 = 125$ ,  $df = 2$ ,  $p < 0.001$  for *Cotesia*;  $n = 20$ ,  $\chi^2 = 83.1$ ,  $df = 2$ ,  $p < 0.001$  for *Microplitis*; and  $n = 24$ ,  $\chi^2 = 104$ ,  $df = 2$ ,  $p < 0.001$  for *Campoletis*). For all three species, honeydew increased longevity, but survival was considerably lower than for the wasps that had fed on the sucrose solution (Fig. 1).

### Effect of honeydew on *C. marginiventris* performance

Results for longevity in the cage experiments were similar to those obtained in the previous experiment and this was reflected in the level of parasitism. Survival ( $n = 8$ ,  $\chi^2 = 38.1$ ,  $df = 4$ ,  $p < 0.01$ ) and offspring production ( $F_{4,35} = 24.86$ ,  $p < 0.001$ ) differed significantly among the food sources. *C. marginiventris* females lived longer and produced more offspring when continuously feeding on honeydew than when unfed or fed with honeydew or sucrose for two days only. However, relative to females that had been continuously fed with sucrose solution their lifespan and offspring production were reduced (Figs. 2 and 3). Parasitoids whose feeding on honeydew or sucrose had been restricted to the first two days only showed a slightly increased longevity, which was not translated in an increased offspring production.

### Honeydew analysis

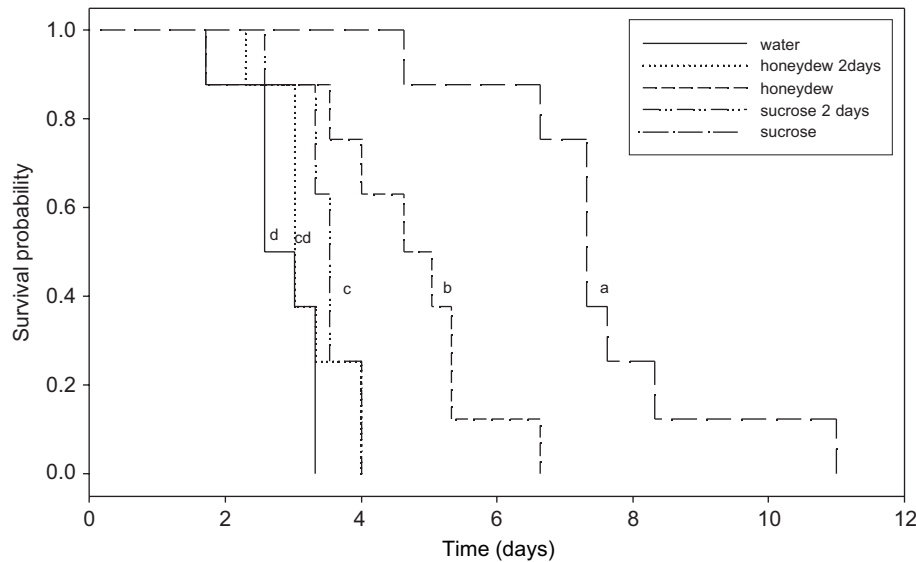
The composition of the honeydew produced by *R. maidis* feeding on maize plants showed some changes as a function of initial infestation rate and time after infestation (Fig. 4). The honeydew was mainly composed of the phloem sugar sucrose and its hexose components, fructose and glucose, which made up between 64 and 94% of the sugars. Maltose and erlose were the most important aphid-synthesized sugars in the honeydew, trace amounts of melezitose were found in all collections and trace amounts of raffinose and melibiose were detected during the 2nd collection of the initial 500 aphid infestation. One observed trend was a decrease in the percentage of aphid-synthesized sugars with increasing aphid density (e.g. in the first collection there was 21.9% of aphid-synthesized sugars for 10 aphids, 17.4% for 100 aphids, 4.5% for 500 aphids) and time after infestation. Over time, there was also a shift from fructose to glucose excretion.



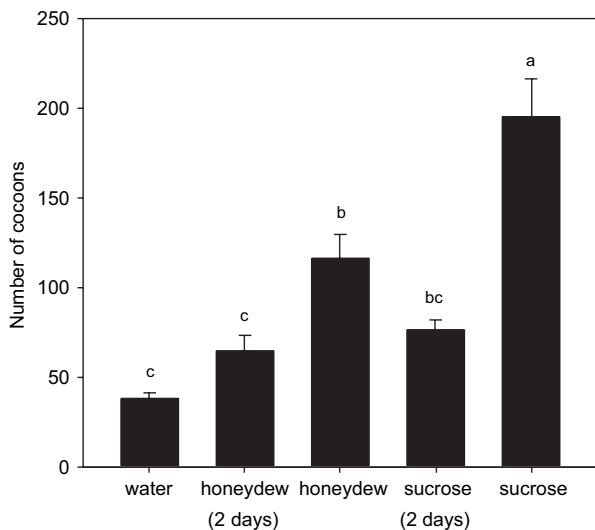
**Fig. 1.** Survival curves showing the survival probability of *C. marginiventris*, *M. rufiventris* and *C. sonorensis* when provided with water, honeydew or sucrose solution. Different letters indicate significant differences between curves ( $p < 0.001$ ).

### Longevity of *C. marginiventris* on honeydew mimics

There were considerable differences among the food treatments with respect to their effect on parasitoid survival ( $n = 20$ ,  $\chi^2 = 119$ ,  $df = 5$ ,  $p < 0.001$ ) (Fig. 5), but the survival probabilities of *C. marginiventris* feeding on the different sugar solutions (honeydew mimics) did not differ significantly ( $n = 20$ ,  $\chi^2 = 1.1$ ,  $df = 3$ ,  $p = 0.77$ ). Survival after feeding on any of the



**Fig. 2.** Survival curves showing the survival probability of *C. marginiventris*, when provided water, honeydew or sucrose solution continuously or for the first 2 days of their adult lives. Different letters indicate significant differences between curves ( $p < 0.01$ ).



**Fig. 3.** Total number of offspring (number of cocoons) produced by *C. marginiventris* when provided water, honeydew or sucrose solution continuously or for the first 2 days of their adult life (average  $\pm$  SE). Different letters indicate significant difference between treatments ( $p < 0.05$ ).

sugar solutions was significantly higher in comparison to honeydew ( $n = 20$ ,  $\chi^2 = 74.6$ ,  $df = 4$ ,  $p < 0.001$ ). Honeydew feeding, in turn, enhanced survival relative to feeding on water only ( $n = 20$ ,  $\chi^2 = 20$ ,  $df = 1$ ,  $p < 0.001$ ).

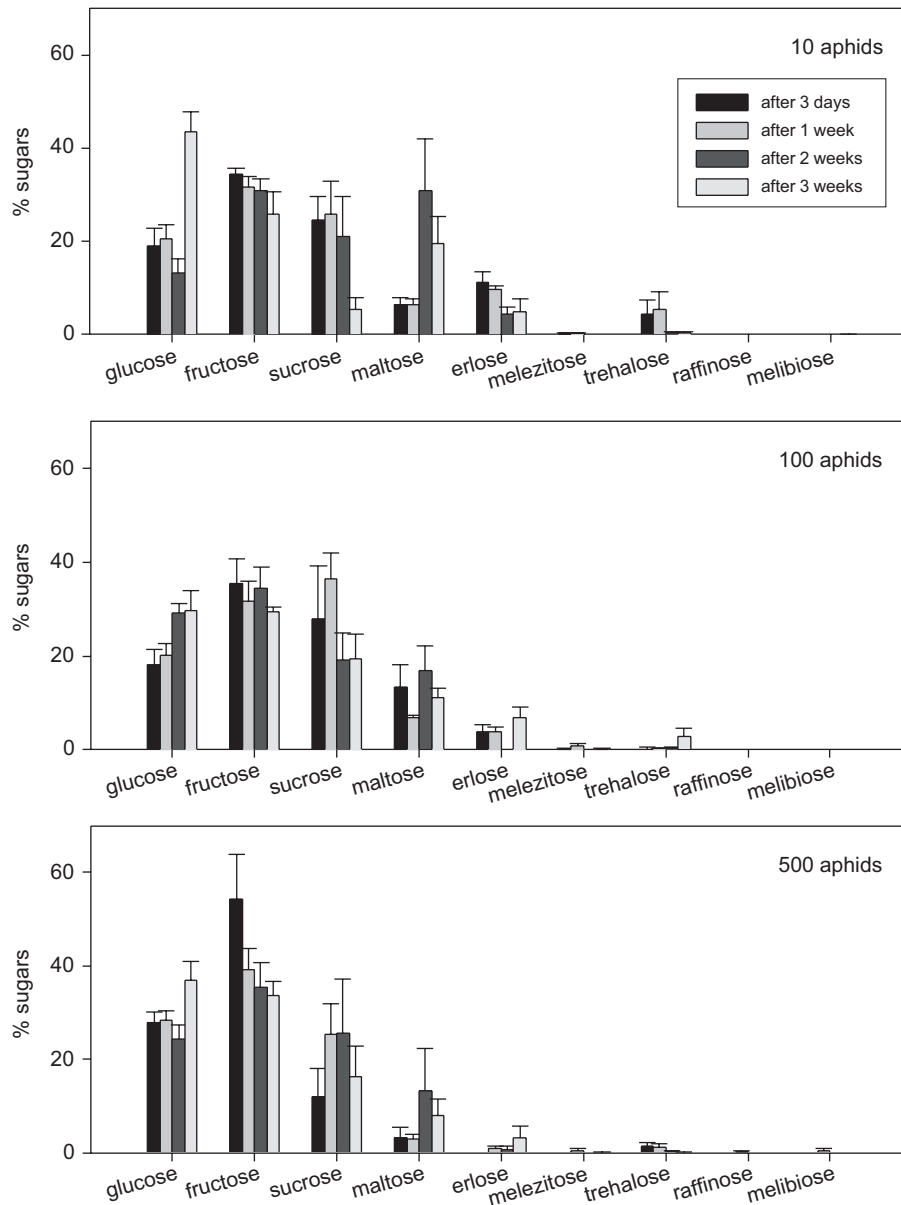
### Relationship between honeydew intake and survival

During the single feeding bout, parasitoids consumed less honeydew than sucrose (means of 11.70% on

honeydew and 22.59% on sucrose;  $t = -6.62$ ,  $df = 38$ ,  $p < 0.001$ ). Again, the resulting average longevity of wasps fed on honeydew was shorter than that of wasps fed on sucrose ( $T_{20} = 223.5$ ,  $p < 0.001$ ). GLM showed that the observed difference in survival in this experiment was solely due to food intake, and if this is taken into account then there was no effect of food source (Table 1, Fig 6).

Moreover, there was a significant difference ( $T = 570.5$ ,  $p < 0.001$ ) in the amount of time *C. marginiventris* spent feeding on the two food sources (means of 6.9 min on honeydew and 14.35 min on sucrose). The long feeding time but small intake of honeydew implies that the wasps have difficulty ingesting this food source.

The results from the experiment with diluted honeydew confirmed the above conclusions. There was a positive relationship between intake and longevity of females that fed on undiluted or diluted honeydew or on a sucrose solution ( $R^2 = 0.554$  for undiluted honeydew;  $R^2 = 0.612$  for 90% honeydew;  $R^2 = 0.5913$  for 70% honeydew;  $R^2 = 0.494$  for sucrose solution;  $p < 0.001$  for all treatments). There was a significant difference ( $F_{3,68} = 37.82$ ,  $p < 0.001$ ) in weight gain among the *C. marginiventris* females that had fed on different solutions (means of 10.77% for undiluted honeydew; 22.31% for 90% honeydew; 32.86% for 70% honeydew; 37.47% for sucrose solution). These differences in food intake were reflected in the longevity data ( $H = 55$ ,  $df = 4$ ,  $p < 0.001$ ) (Fig. 7). Females that fed on undiluted honeydew lived longer than unfed females, but shorter than females that fed on diluted honeydew or on sucrose.

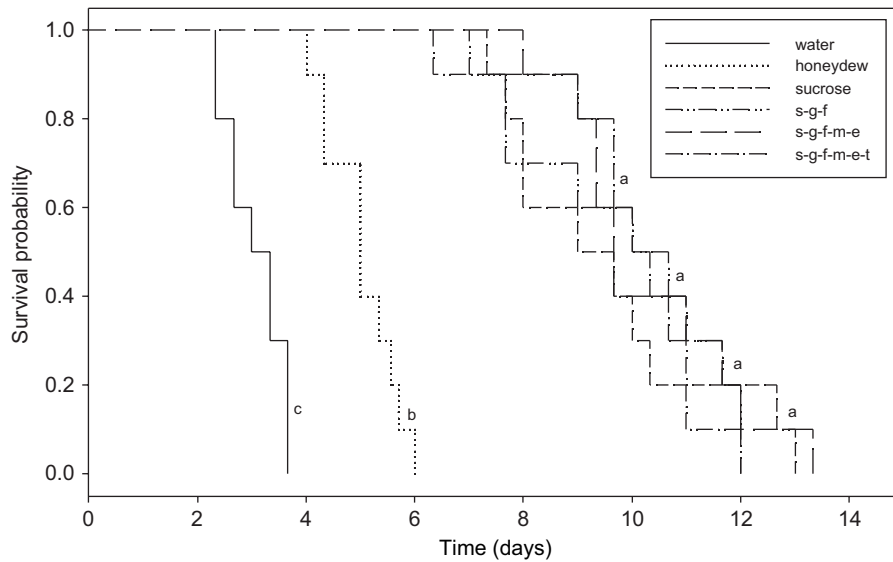


**Fig. 4.** Sugar composition of the honeydew produced over time by different infestation rates of *R. maidis* feeding on barley plants (average  $\pm$  SE). See Materials and Methods for details.

## Discussion

Studies assessing parasitoid longevity when feeding on honeydew have yielded inconsistent results. Some studies found that parasitoid survival on honeydew is as low as when wasps are given no food, others show that honeydew increases parasitoid survival but not as much as nectar or honey, whereas some found that honeydew has the same effect on parasitoid survival as nectar, honey or sucrose solutions (reviewed by Wäckers & van Rijn, 2005). In our study, honeydew feeding increased survival in all three parasitoid species tested, but the positive effect of feeding on a sucrose solution was considerably higher (Fig. 2).

The additional experiments with *C. marginiventris* females show that the prolonged survival as a result of feeding was also reflected in an increase in offspring production and that they need to feed more than once in order to optimize longevity and reproductive output (Fig. 3). When wasps were only given access to food during the first two days of their lives, survival and reproductive success of honeydew fed wasps was not or barely better than that of unfed wasps. Similar effects of feeding frequency on survival have been found for other parasitoids (Fadamiro & Heimpel, 2001; Siekmann, Tenhumberg & Keller, 2001; Wäckers, 2001). However, all these studies were performed in the lab in the absence of hosts. Under more realistic conditions, with the wasps



**Fig. 5.** Survival curves showing the survival probability of *C. marginiventris* when provided (1) water; (2) honeydew; (3) sucrose solution; (4) sucrose, glucose and fructose; (5) sucrose, glucose, fructose, maltose and erlose; or (6) sucrose, glucose, fructose, maltose, erlose and trehalose. See Materials and Methods for details regarding the sugar proportions. Different letters indicate significant differences between curves ( $p < 0.001$ ).

**Table 1.** GLM for the effect of food type, percentage of weight gained after one feeding bout and their interaction on the longevity of *C. marginiventris*

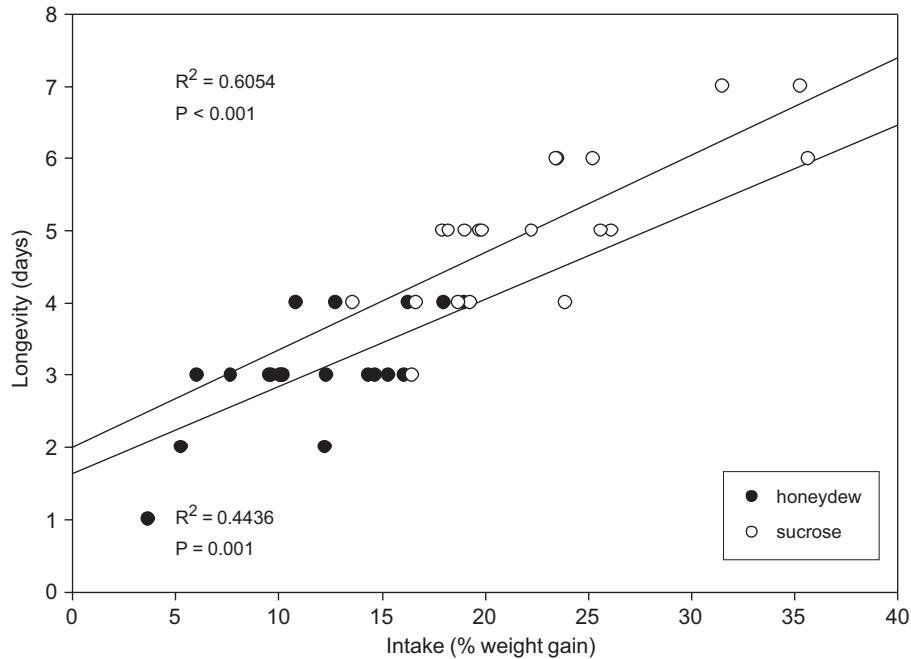
Source	Type III Sum of Squares	df	Mean Square	F	P
Intercept	10.59	1	10.59	26.57	0.000
Food	0.10	1	0.10	0.25	0.616
Intake	14.75	1	14.75	37.00	0.000
Food * intake	0.05	1	0.05	0.12	0.730
Error	14.36	36	0.40		
Total	728.00	40			

exhibiting their foraging behaviour and parasitizing hosts, nutritional requirements can be expected to be higher (Hoferer, Wäckers & Dorn, 2000; Casas et al., 2005). Sisterson & Averill (2002) estimate that in the wild parasitoid females spend 25% of their time searching for food. Indeed, the impact of food on parasitoid offspring production is far more pronounced under (semi-)field as compared to laboratory conditions (Winkler et al., 2006).

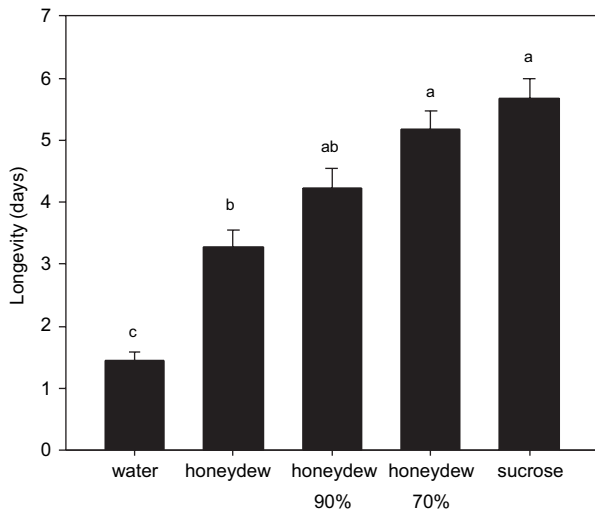
The relatively poor performance of wasps that were provided honeydew was initially thought to be due to sugar composition, as aphids often excrete substantial amounts of sugars that they synthesize themselves as well as plant-derived sugars. These aphid-produced sugars have a lower nutritional value than glucose, fructose and sucrose, the sugars present in most flower nectars (Wäckers, 2001). Our analyses of honeydew produced by *R. maidis* revealed that glucose, fructose and sucrose are the dominant sugars, but it also

contained low levels of the aphid-produced sugars, mainly maltose, erlose, and trehalose. The results from the longevity tests with honeydew mimics (Fig. 5) show that at these levels the aphid-synthesized sugars did not affect the performance of *C. marginiventris* females.

The composition of the honeydew changed over time and as a function of aphid infestation (Fig. 4). Sap feeders are thought to synthesize oligosaccharides primarily to reduce the osmotic pressure of the phloem sap (Fisher, Wright & Mittler, 1984; Wäckers, 2000). If the level and duration of aphid infestation affects the osmotic pressure of phloem sap, the aphids may adapt their sugar synthesis accordingly. Other factors such as host plant and homopteran species (Hendrix et al., 1992; Völkl, Woodring, Fischer, Lorenz & Hoffmann, 1999), homopteran age (Henneberry, Jech, Hendrix, & Steele, 1999), and ant attendance (Yao & Akimoto 2001) were shown to affect the sugar composition of the honeydew.



**Fig. 6.** Regression analysis comparing the intake of honeydew or sucrose solution (in % of body weight gained) during a single meal and subsequent survival of *C. marginiventris* females.



**Fig. 7.** Longevity of *Cotesia marginiventris* that were provided water only or that had one feeding bout on pure honeydew, on honeydew diluted with 10% of water (honeydew 90%), honeydew diluted with 30% of water (honeydew 70%) or on a sucrose solution. Different letters indicate significant differences between treatments ( $p < 0.001$ ).

This study appears to be the first to show changes in honeydew composition due to aphid infestation rate and duration. Given the results with the honeydew mimics, it is unlikely that the observed differences have important consequences for the nutritional value of honeydew for parasitoids.

The poorer performance on honeydew is best explained by differences in food intake, which, on average, was considerably less for honeydew than for a sucrose solution, and in cases where they were equal wasp survival was very similar (Fig. 6). When wasps were given diluted honeydew their food intake and subsequent survival increased significantly and was similar to that of wasps that had fed on a sucrose solution. This, together with the finding that wasps spent more time feeding on honeydew, allows us to reject the hypothesis that the lower intake of honeydew was due to a lack of feeding stimulation, but rather implies that the high viscosity of the honeydew impairs its intake by the parasitoids. The tendency of some honeydew sugars (raffinose and melezitose) to rapidly crystallize is likely to contribute to this high viscosity (Wäckers, 2000). In most of our experiments, the wasps were fed under conditions of high humidity. Under field conditions intake may be even more constrained by viscosity (Corbet, Willmer, Beatment, Unwin & Prys-Jones, 1979; Olson & Wäckers, 2007).

### Consequences for biological control

The results confirm that parasitoids only reach their full potential as biological control agents if they have regular access to suitable food sources. There are various examples where higher levels of parasitism were achieved in the field when wasps were provided with food (e.g. Evans & Swallow, 1993; Baggen & Gurr,

1998; Heimpel & Jervis, 2005) and the failure of introduced parasitoids to establish has sometimes been attributed to the lack of food sources for the adults (Hocking, 1966; Stiling, 1993).

It should be taken into account that food sources in the field are not only exploited by natural enemies, but may also benefit pests (Baggen & Gurr, 1998; Romeis, Städler & Wäckers, 2005). In this context, the use of selected food sources that primarily or exclusively benefit the natural enemies is highly desirable. Parasitoids may accept and benefit from a broader range of sugars compared to lepidopteran pests (Wäckers, 1999, 2001; Romeis & Wäckers, 2002; Winkler et al., 2005), implying that in some situations, moderate aphid populations in the field could contribute to successful biological control.

The fact that the wasps spent much longer feeding on honeydew yet ingested a smaller volume than wasps feeding on the sucrose solution implies that there is an increased risk of predation associated with honeydew feeding (Wäckers & van Rijn, 2005). Limited food intake per feeding event will increase the frequency of food foraging bouts (Stapel et al., 1997) and will make the wasps more vulnerable to predators (Heimpel, Rosenheim & Mangel, 1997; Völkl & Kroupa, 1997).

Despite the limitations to the suitability of honeydew as food for parasitoids, it is frequently consumed. Field caught parasitoids commonly contain honeydew sugars (Wäckers & Steppuhn, 2003) and Casas, Driessen, Mandon, Wielaard, Desouhant, van Alphen et al. (2003) showed that wasps can forage within a habitat for days in a situation where homopteran honeydew seems to be the only food source available. This suggests that this food source can be exploited in the field to enhance the efficacy of biological control agents. A better understanding of factors that determine the quality of honeydew as food may allow us to develop methods to manipulate these factors.

## Acknowledgements

We would like to thank the past and present members of the E-vol lab at the University of Neuchâtel for support and input in all stages of the work. This article was greatly improved by comments from Marco D'Alessandro, Matthias Held and Russ Naisbit. Special thanks to: Marie-Eve Wyniger and Roel Wagenaar (NIOO-KNAW Heteren) for technical assistance; Yves Borcard and several students for the parasitoid rearing; and Syngenta (Stein, Switzerland) for the supply of *Spodoptera littoralis*. This study was funded by the Swiss National Centre of Competence in Research "Plant

Survival", a research program of the Swiss National Science Foundation.

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