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## The effect of different dietary sugars and honey on longevity and fecundity in two hyperparasitoid wasps

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### ABSTRACT

In nature adult insects, such as parasitic wasps or 'parasitoids' often depend on supplemental nutritional sources, such as sugars and other carbohydrates, to maximize their life-expectancy and reproductive potential. These food resources are commonly obtained from animal secretions or plant exudates, including honeydew, fruit juices and both floral and extra-floral nectar. In addition to exogenous sources of nutrition, adult parasitoids obtain endogenous sources from their hosts through 'host-feeding' behavior, whereby blood is imbibed from the host. Resources obtained from the host contain lipids, proteins and sugars that are assumed to enhance longevity and/or fecundity. Here we conducted an experiment exploring the effects of naturally occurring sugars on longevity and fecundity in the solitary hyperparasitoids, *Lysibia nana* and *Gelis agilis*. Although both species are closely related, *L. nana* does not host-feed whereas *G. agilis* does. In a separate experiment, we compared reproduction and longevity in *G. agilis* reared on either honey, a honey-sugar 'mimic', and glucose. Reproductive success and longevity in both hyperparasitoids varied significantly when fed on different sugars. However, only mannose- and water-fed wasps performed significantly more poorly than wasps fed on four other sugar types. *G. agilis* females fed honey produced twice as many progeny as those reared on the honey-sugar mimic or on glucose, whereas female longevity was only reduced on the mimic mixture. This result shows not only that host feeding influences reproductive success in *G. agilis*, but also that non-sugar constituents in honey do. The importance of non-sugar nutrients in honey on parasitoid reproduction is discussed.

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### 1. Introduction

In nature, adult insects often depend on supplemental nutritional sources, such as sugars and other carbohydrates, as well as proteins, to maximize their life-expectancy and reproductive potential. These food resources are commonly obtained from animal or plant exudates, such as honeydew, fruit juices and both floral and extra-floral nectar. In parasitic wasps, or 'parasitoids', the benefits of sugars on fitness-related traits, such as longevity and fecundity, is well established (Jervis et al., 1993; Heimpel et al., 1997; Wäckers, 2001; Casas et al., 2003; Lee et al., 2004; Winkler et al., 2006; Wäckers et al., 2008; Desouhant et al., 2010; but see Ellers et al., 2011). It is also well known that different sugars vary in their nutritional quality for parasitoids, with some greatly extending the lifespan whilst others have little effect or may even be toxic (Jervis et al., 1993; Wäckers, 2001; Wäckers et al., 2006; Kehrlri and Bacher, 2008). In the field, therefore, it may pay parasitoids to exhibit

preferences for feeding on exudates from some species of plants or animals over others.

In addition to sugars obtained exogenously, some parasitoids also 'host-feed', imbibing blood from the host (Jervis and Kidd, 1986). Host blood is rich in proteinaceous materials, which are important in the manufacture of eggs, whereas proteins are lacking in the honeydew and nectar which are generally comprised of carbohydrates (Bernstein and Jervis, 2006). Several studies have shown that resources obtained from host-feeding may be allocated for both reproduction and/or maintenance (Heimpel and Rosenheim, 1995; Heimpel and Collier, 1996; Giron et al., 2002; Rivero and West, 2005; Kapranas and Luck, 2008; Zhang et al., 2011). Host blood is often important in the production of large, yolky 'anhydrotic' eggs (Jervis and Kidd, 1986; Bernstein and Jervis, 2006; Harvey, 2008) that are characteristically produced by many 'idiobiont' ectoparasitoids, i.e. parasitoids that attack non-feeding or non-growing host stages or arrest host development prior to oviposition (Godfray, 1994; Harvey, 2005; Jervis and Ferns, 2011). In contrast, most endoparasitoids that develop inside the host are 'koinobionts' i.e. parasitoids that allow the host

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to continue feeding and growing during parasitism (Godfray, 1994; Harvey, 2005). In koinobionts adult female wasps do not host-feed, and invest very little protein into their tiny ‘hydropic’ eggs. Instead proteins are absorbed from the host blood through a specialized extra-embryonic membrane before hatching (Jervis and Kidd, 1986; Jervis et al., 2001, 2008). Host blood has also been found to contain certain sugars, such as trehalose and sucrose that are important in reducing the metabolism of lipids (Giron et al., 2002). However, this is certainly not the rule, as the longevity of some parasitoids is not extended at all by host-feeding (Rivero and West, 2005; Harvey, 2008). Host-feeding parasitoids are generally highly synovigenic, meaning that the adult female wasps emerge with few or no ripe eggs and therefore mature most of their eggs after eclosion (Eilers et al., 2000; Jervis et al., 2001, 2008).

Studies examining life-history variables such as longevity and reproduction in parasitoids have rarely integrated the nutritional value of different naturally occurring sugars and host-feeding under the umbrella of a single ecophysiological framework. Instead, most studies on the nutritional value of different sugars for parasitoids have explored one demographic character, such as longevity (Wäckers, 2001; Vattala et al., 2006), or else have used non host-feeding koinobiont parasitoid species that generally invest very low *per capita* resources into each egg (Wanner et al., 2006; Winkler et al., 2006; Faria et al., 2008; Lee and Heimpel, 2008; Wu et al., 2008). In this way it is impossible to tease apart the benefits, if any, of different sugars in concert with host feeding, on reproduction and longevity in parasitoids that require both host- and non-host resources during adult life to maximize these variables.

A previous study reported that longevity in a primary gregarious endoparasitoid, *Cotesia glomerata* L. (Hymenoptera: Braconidae), differed significantly when the wasps were fed on 14 different sugars that occur naturally (Wäckers, 2001). In this study we examine the effects of several naturally occurring sugars found in e.g. honeydew and nectar, on longevity and fecundity in two closely related species of solitary hyperparasitoids, *Lysibia nana* Gravenhorst and *Gelis agilis* Fabricius (Hymenoptera: Ichneumonidae, Cryptinae) that attack the fully cocooned pre-pupae and pupae of *C. glomerata* in the field. Both species are ectoparasitoids, are wholly synovigenic and produce anhydropic eggs (Harvey, 2008). However, they also exhibit several key differences in reproductive and morphological traits. *Lysibia nana* reproduces sexually and adults are fully winged; by contrast, *G. agilis* reproduces asexually and adult females are wingless (Harvey, 2008). Another important difference between the two species is that *G. agilis* females mutilate some host cocoons with their ovipositors and feed on the blood that leaks from the wound as a pre-requisite for egg maturation; *L. nana* females, on the other hand, do not host-feed (Harvey, 2008). Furthermore, the reproductive biology and host exploitation behavior of *L. nana* is closely correlated with brood size in *C. glomerata* whereas in *G. agilis* it is not (Harvey, 2008; Harvey et al., 2011). This suggests that *L. nana* is much more specialized in attacking *C. glomerata* than *G. agilis*.

In a second experiment, we compare longevity and fecundity in the host-feeding species *G. agilis* when the wasps are provided with glucose (a high quality sugar), honey, or a ‘honey-mimic’ that consists of equivalent amounts of several sugars that are found in honey. Honey has long been used in the lab as an alternate food source to sucrose solution for insects such as parasitoids, but its actual properties in influencing parasitoid reproduction and longevity have been little explored. With respect to carbohydrates, honey consists mainly of similar proportions of fructose and glucose with smaller concentrations of maltose, sucrose, and several other complex carbohydrates (Doner, 1977). As with all nutritive sweeteners, the content of honey is dominated by sugars although it also contains trace amounts of vitamins or minerals (Martos et al., 2000;

Gheldof et al., 2002). Furthermore, honey also contains tiny amounts of several compounds thought to function as antioxidants, including chrysin, pinobanksin, vitamin C, catalase, and pinocembrin (Martos et al., 2000).

The aims of the study are twofold: first, to determine if longevity and fecundity in *L. nana* and *G. agilis* are affected when fed on different naturally occurring sugars; second, to determine if these demographic variables differ in *G. agilis* when different cohorts are reared on glucose, honey or a honey-mimic that lacks the trace minerals and compounds found in honey. We hypothesize (1) that longevity and fecundity in these two closely related hyperparasitoids will be similarly affected by qualitative differences in dietary sugars, and (2) honey provides a high-quality carbohydrate resource affecting longevity similarly as high-quality sugars offered singly or in a mixture, because sugars and honey are of high value for maintenance, whereas host-feeding is important for reproduction.

## 2. Materials and methods

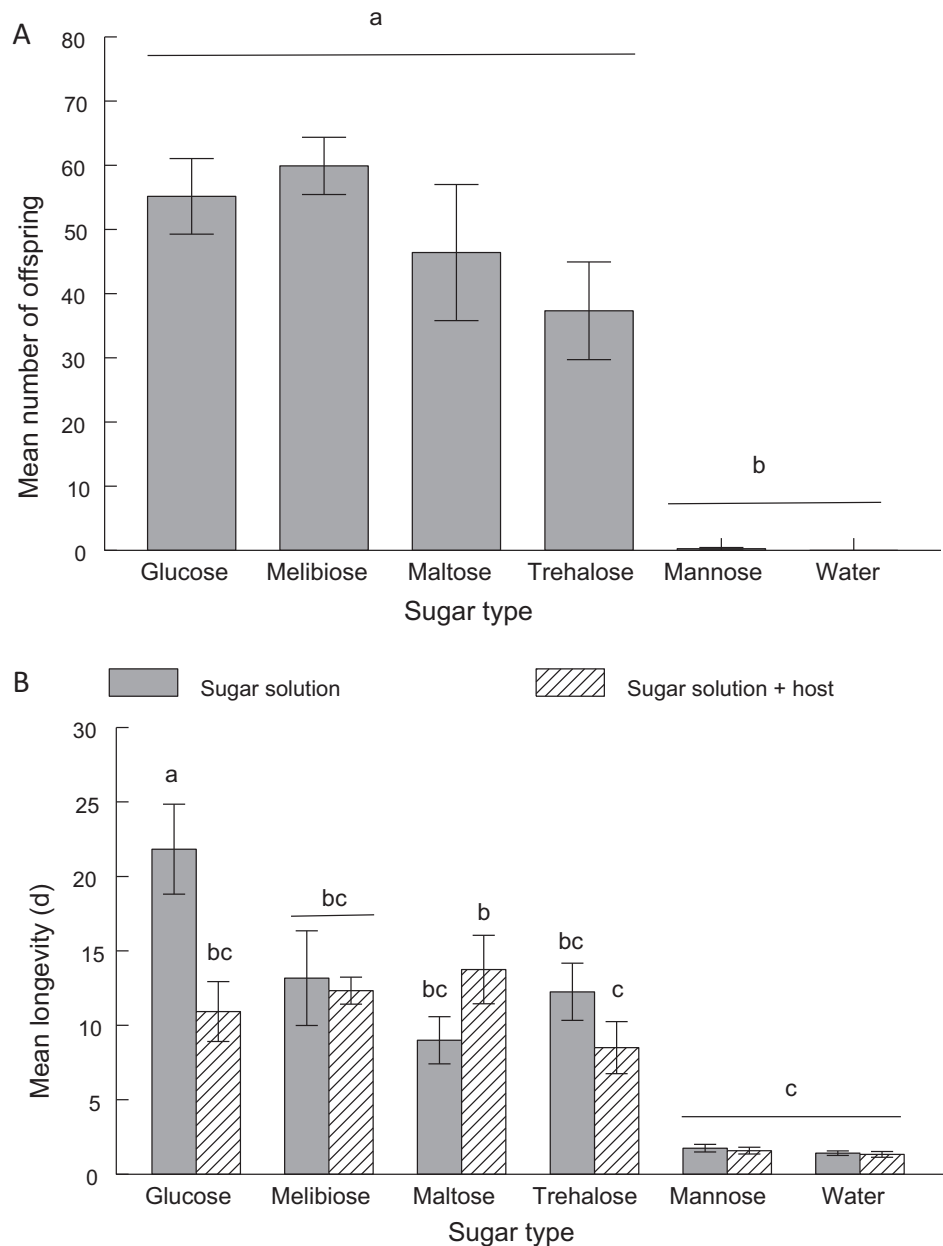
### 2.1. Insect cultures

*Lysibia nana* and *G. agilis* have been reared for several years at the Netherlands Institute of Ecology (NIOO) in Heteren, the Netherlands. Both hyperparasitoids were originally obtained from cocoons of *C. glomerata* recovered from leaves of black mustard plants (*Brassica nigra*) growing adjacent to the NIOO institute. Both species were reared according to the protocol described in Harvey (2008). Cultures of *L. nana* were generated from 200 to 300 *C. glomerata* cocoons placed in rearing cages with 50 adult *L. nana* wasps for 24 h. Then, parasitized cocoons were transferred to large Petri dishes (18 cm) until adult emergence. The rearing protocol for *G. agilis* was similar, except that *G. agilis* had access to a small number of cocoons for host-feeding purposes 3–4 days before it was provided with new cocoons for oviposition. The parasitoids were reared at  $25 \pm 2^\circ\text{C}$  with a 16:8 h L:D light:dark regime. *Cotesia glomerata* was reared on *Pieris brassicae* L. (Lepidoptera: Pieridae) caterpillars and was originally collected from agricultural fields in the vicinity of Wageningen University.

### 2.2. Experimental protocol

#### 2.2.1. Experiment 1: longevity and fecundity in *L. nana* and *G. agilis* reared on different sugars

The aim of this experiment was to determine if different, naturally occurring sugars affected longevity and fecundity in two hyperparasitoids of *C. glomerata*. A subset of the 14 sugars used in an earlier study by Wäckers (2001) were selected on the basis of a wide spectrum of effects on longevity in the primary parasitoid, *C. glomerata*, the host of *L. nana* and *G. agilis* in this study. Five of these sugars were selected to be offered to wasps in the present study: two monosaccharides, glucose and mannose (molar masses 180.2 g/mol), and three disaccharides, trehalose, melibiose and maltose (molar masses 342.3 g/mol). All of these sugars are found in floral nectar, whereas glucose, maltose and trehalose are also found in honeydew exudates from aphids (Wäckers, 2001). The concentration used for all sugar solutions was 1 M. This concentration represents the upper limit of sugars found in floral nectar and honeydew (Wäckers, 2001). Three droplets of 10  $\mu\text{L}$  of a specific sugar solution were placed in each Petri dish using a pipette. These sugars were refreshed daily. For the control treatment, three droplets of 10  $\mu\text{L}$  of water were also placed in the Petri dishes using a pipette. Cotton wool moistened with water was placed in each Petri dish to ensure that the droplets would not evaporate.



**Fig. 1.** Effects of different sugars and water on mean fecundity (A) and longevity (B) in *Lysibia nana*. Line bars represent standard error of the mean. Bars with different letters are significantly different (Tukey–Kramer tests,  $P < 0.05$ ). Sample size for each treatment = 12.

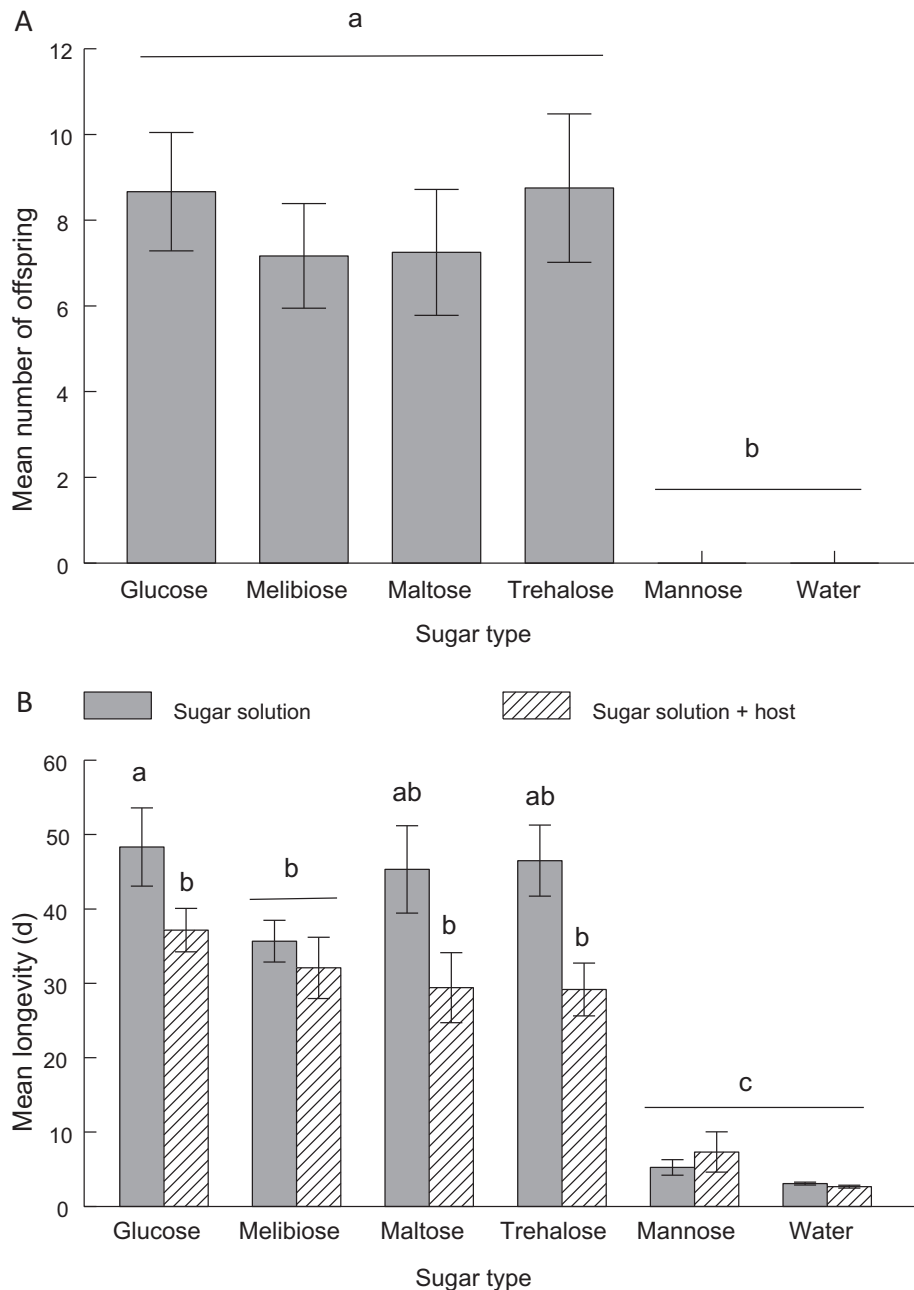
Wasps were kept individually in 9.5 cm Petri dishes and were divided into two groups. One group of wasps was kept without hosts whereas the other group was provided continually with 20 (*L. nana*) or 10 (*G. agilis*) host cocoons (<24 h old) that were replaced every 48 h. Cocoon numbers were based on a previous study that measured reproduction in both species reared only on honey (Harvey, 2008). Individuals in both groups were provided with one of five different sugar solutions throughout their adult life. Twelve wasps were observed per sugar per diet (with and without hosts). After adult emergence, individual female *L. nana* wasps were placed in a Petri dish with three males for mating purposes. After 24 h, all males were removed, and the wasps were presented with cocoons as described above. After exposure to parasitoids the cocoons were removed and placed in marked vials. The number of emerging adult hyperparasitoids was thereafter recorded. Hyperparasitoid mortality was monitored daily in order to ascertain the longevity of the adult wasps in days. Longevity was

also recorded in wasps that had no access to hosts throughout their adult lives.

#### 2.2.2. Experiment 2: the effects of glucose, honey, and a honey 'mimic' on longevity and fecundity in *G. agilis*

The aim of this experiment was to determine if host-feeding alone is essential for maximal reproduction in *G. agilis*, or that differences in the nutritional quality of a high quality sugar (glucose), honey (which is known to contain both carbohydrates and amino acids) and a honey sugar mimic (lacking amino acids) affects reproductive output in this hyperparasitoid.

This experiment was carried out in exactly the same way as experiment 1, except that different cohorts of *G. agilis* females were provided *ad libitum* from eclosion with 10 host cocoons (refreshed every 48 h) when provided with one of the three diets (honey, honey-mimic, glucose). Glucose was chosen because it was the sugar on which *G. agilis* achieved optimal performance (longevity, fecun-



**Fig. 2.** Effects of different sugars and water on the mean fecundity (A) and longevity (B) in *Gelis agilis*. Line bars represent standard error of the mean. Bars with different letters are significantly different (Tukey–Kramer tests,  $P < 0.05$ ). Sample size for each treatment = 12.

dity) in experiment 1. The honey-mimic was prepared as follows: fructose: 17.5 g, glucose: 17.5 g, maltose: 7 g, sucrose: 1.5 g, dissolved in 100 ml water. These variable concentrations of sugars accurately reflect the relative sugar composition of honey at half their concentrations (the mixture was diluted for the sugars to dissolve), but also lack the trace elements that are also found in honey (e.g. proteins, amino acids, vitamins).

Here, we also measured cumulative fecundity, which is the total number of offspring produced by all parasitoids within each treatment added together daily.

### 2.3. Statistical analysis

For both hyperparasitoid species, data for fecundity and longevity in experiment 1 followed a normal distribution and were, therefore, analyzed using one way Anova's for fecundity and general

linear models for longevity, the latter with sugar type and host accessibility as factors. When assumptions of equal variances were violated, Anova was conducted using an unequal variance structure (Little Rubin, 2002). Multiple comparisons were made using Tukey–Kramer tests. For experiment 2, data were analyzed using one-way Anova's, again with Tukey–Kramer tests for multiple comparisons. All analyses were performed using the statistical package SAS 9.2.

## 3. Results

### 3.1. Experiment 1

In *L. nana* there was a significant effect of treatment (sugars and water) on lifetime reproductive success ( $F_{5,65} = 18.76$ ,  $P < 0.001$ ). However, this effect was generated primarily by the mannose

and water treatments, where very few progeny were produced (Fig. 1A). On the other 4 sugar types, glucose, trehalose, melibiose and maltose, fecundity did not differ significantly, even though slightly more progeny were produced for wasps fed on melibiose (Fig. 1A). Longevity in *L. nana* also varied significantly with treatment ( $F_{5,42.7} = 40.3$ ,  $P < 0.001$ ), but marginally not with the presence or absence of cocoons ( $F_{1,63} = 3.01$ ,  $P = 0.09$ ), although there was a significant interactive effect between these variables on longevity ( $F_{5,42.7} = 2.61$ ,  $P = 0.04$ ). Wasps without host access lived longest when fed on glucose and shorter in the other host access/feeding treatments, particularly water and mannose, where all wasps survived <5 days (Fig. 1B).

In *G. agilis* there was also a significant effect of treatment (sugars and water) on lifetime reproductive success ( $F_{5,66} = 12.21$ ,  $P < 0.001$ ). Moreover, as with *L. nana*, this effect was generated primarily by the mannose and water treatments where no progeny were produced at all (Fig. 2A). Fecundity in wasps reared on the other 4 other sugar types did not differ significantly and averaged around 7–8 per female (Fig. 2A). Longevity in *G. agilis* also varied significantly with treatment ( $F_{5,39.3} = 103.9$ ,  $P < 0.001$ ) as well as with the presence or absence of cocoons ( $F_{1,79.7} = 13.29$ ,  $P = 0.005$ ). Furthermore, there was a significant interactive effect between these variables on longevity ( $F_{5,39.3} = 3.11$ ,  $P = 0.02$ ). Wasps without host access lived longest when fed on glucose, maltose and trehalose and shorter in the other host access/feeding treatments, particularly water and mannose (Fig. 2B).

### 3.2. Experiment 2

#### 3.2.1. *G. agilis*: longevity and lifetime reproductive success

*G. agilis* wasps must host-feed in order to produce eggs during their lifetime. Females host-feed by repeatedly inserting and removing the ovipositor from host cocoons, eventually imbibing host blood through their mouthparts at the aperture of the wound site (Fig. 3).

The number of progeny produced by *G. agilis* females varied significantly with feeding treatment ( $F_{2,27} = 15.18$ ,  $P < 0.001$ ). Wasps provided continually with hosts and honey produced approximately twice as many progeny as wasps provided with hosts and either glucose or the honey-mimicking sugar (Fig. 4A). Furthermore, longevity in *G. agilis* varied significantly with feeding treatment ( $F_{2,27} = 15.18$ ,  $P < 0.001$ ). Adult longevity was similar in wasps fed on glucose and honey but about two weeks less in wasps fed on the honey-mimic (Fig. 4A). An overview of the cumulative

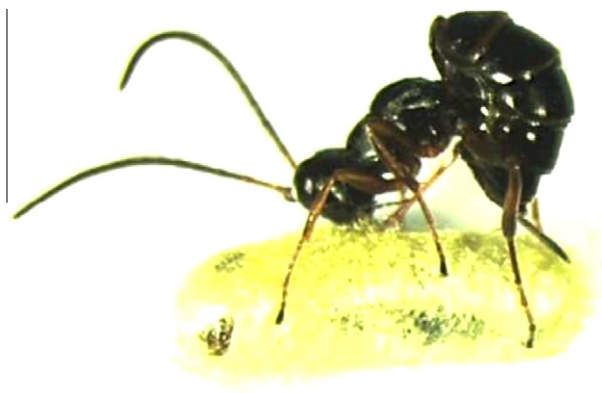
fecundity in *G. agilis* revealed that the wasps produced similar numbers of progeny over about the first 6–8 days, but then reproductive output in wasps fed on glucose and the honey-mimic declined much more rapidly than individuals fed on honey (Fig. 4B).

## 4. Discussion

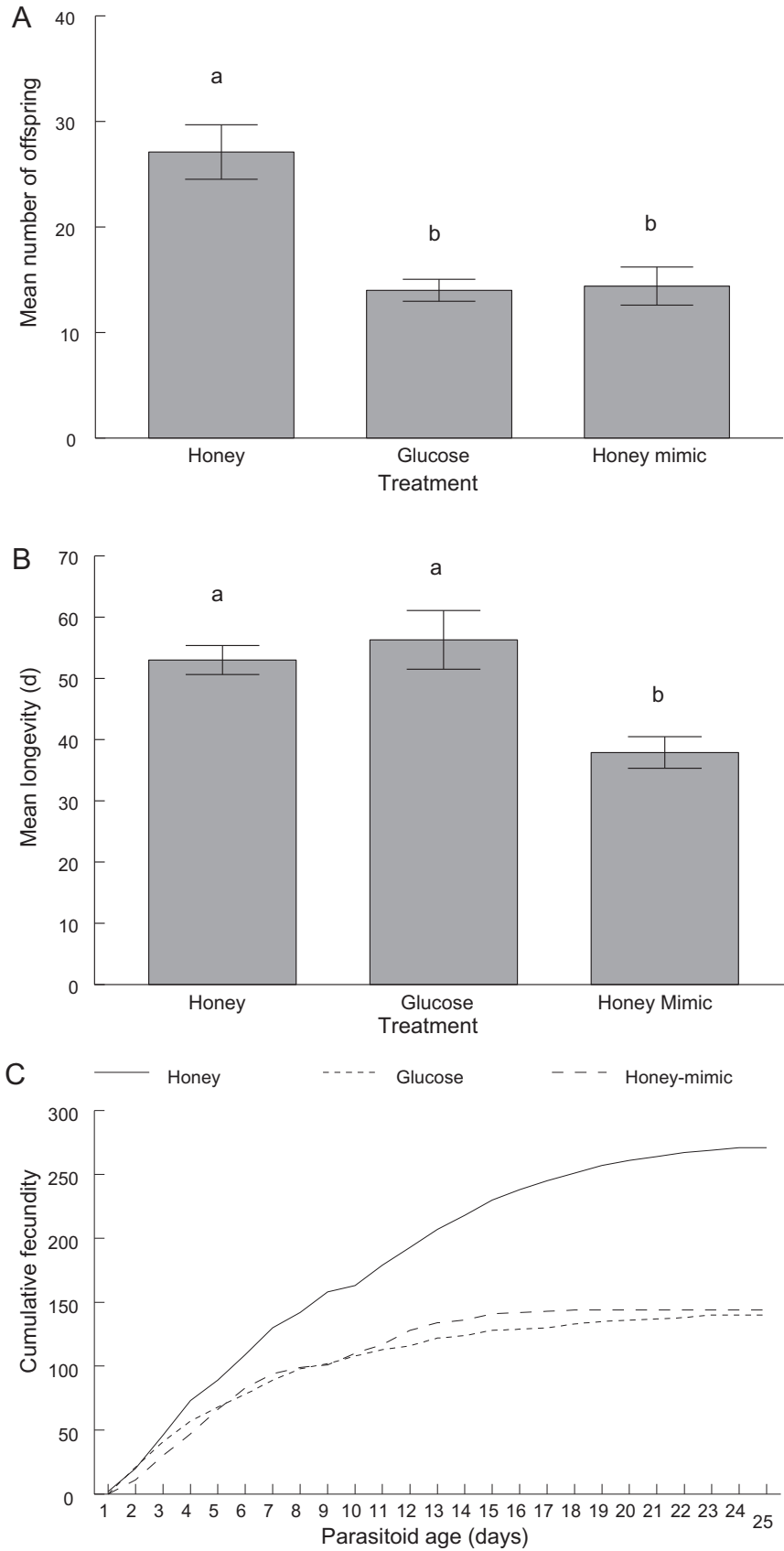
The results of experiment 1 revealed that the different sugars had similar effects on the longevity and fecundity in the specialist hyperparasitoid *L. nana* and in the generalist, *G. agilis*. There were significant inter-specific differences in these fitness correlates, as has been shown in previous studies when wasps were fed honey (Harvey, 2008). However, within each species similar numbers of offspring were produced and wasps had similar life-spans on four of the five sugars tested. There was a significant reduction in longevity and offspring production when the wasps were reared on diets of water only or mannose. A previous study (Wäckers, 2001) reported much more variation in the quality of the same sugars on longevity in the primary parasitoid *C. glomerata*, which serves as an important, and perhaps the main, host in the field for *L. nana*. In contrast with both hyperparasitoids, *C. glomerata* survived longer on mannose and much shorter on trehalose. The latter sugar is of particular interest here because trehalose is an important blood sugar in insects (Giron et al., 2002) and *G. agilis* host-feeds at least several times during adult life. Moreover, both hyperparasitoids produce large, yolky anhydropic eggs that are much larger than the tiny, mostly yolkless hydropic eggs that are produced by *C. glomerata* and many other koinobiont endoparasitoids (Jervis and Kidd, 1986; Harvey, 2008; Jervis et al., 2008). Our results suggest that trehalose may constitute an important sugar for host-feeding parasitoids or parasitoids that invest high amounts of *per capita* resources into a comparatively small number of large eggs. *C. glomerata* females can produce up to 2000 eggs in their lifetime (Le Masurier, 1991), whereas *G. agilis* has a realized fecundity of < 40, even under optimal conditions (Harvey, 2008; this study).

The utilization efficiency of different sugars varies between parasitic and non-parasitic Hymenoptera (Wäckers, 2001). For instance, other studies have also reported high mortality of mannose-fed parasitoids, such as *Bracon brevicornis* (Ponnamma and Kurian, 1983) and other Hymenoptera, including honey bees, which die within only a few hours of feeding on this sugar (Sols et al., 1960; De la Fuente et al., 1986). Previous work has shown that some koinobiont parasitoids, such as *Diadegma semiclausum*, can only transport monosaccharides (e.g. glucose and mannose) across the gut wall, although this has not been widely tested across different phylogenetic groups (Wäckers et al., 2006). Other koinobionts, such as *Cotesia glomerata*, apparently possess saliva in the gut lumen that break down non-monosaccharide sugars (e.g. trehalose, melibiose and maltose, which are disaccharides; Wäckers, 2001).

One important common trait that the hyperparasitoids studied here, as well as *B. brevicornis* and honey bees exhibit, is the production of yolky eggs that depends either on exogenous resources to mature eggs, such as proteins from host-feeding, or on redirection of capital resources obtained during larval development and carried over to the adult stage (Jervis et al., 2001). Furthermore, like *G. agilis*, the primary parasitoid *B. brevicornis*, along with other species in the same genus, must first host-feed in order to mature eggs (Benson, 1973; Jervis and Kidd, 1986). Several other studies have also reported that melibiose, which is a suitable sugar for *C. glomerata* and both of its hyperparasitoids, is also toxic to bees (Barker and Lehner, 1974). The combined evidence of various studies suggests that the suitability of different sugars for parasitoid wasps and other hymenopteran insects may be closely connected with



**Fig. 3.** Photograph showing an adult female of the hyperparasitoid *Gelis agilis* during a bout of host-feeding on a cocoon of its primary parasitoid host, *Cotesia glomerata*. The female initially inserts her ovipositor into the host and then expands the wound by masticating the host with it, finally drinking host blood that seeps from the wound site. Resources obtained from host-feeding are assumed to be used for reproduction in *G. agilis*.



**Fig. 4.** Effects of honey, glucose and a honey-mimicking sugar on fecundity (A) longevity (B), and cumulative fecundity (C) in *Gelis agilis*. Wasps were provided with cocoons of *Cotesia glomerata* in all treatments. Line bars in (a) and (b) represent standard error of the mean. Bars with different letters are significantly different (Tukey–Kramer tests,  $P < 0.05$ ). Sample size for each treatment = 10.

certain aspects of the reproductive biology, such as the level of resource investment in eggs, a point which has thus far been ignored.

Several studies have reported that the resources accrued from host-feeding in parasitoids are rich in proteins that are primarily utilized for reproduction, whereas resources obtained from non-host materials (e.g. sugars from plant exudates or aphid secretions, as well as honey) are primarily utilized for maintenance (Wäckers, 2001; Rivero and West, 2005; Irvin et al., 2007). Whereas honey has been widely used to maintain insect cultures in lieu of other sugars, we found that non-sugar components in honey dramatically enhanced reproductive output in *G. agilis*, even though the longevity of wasps fed on honey and glucose did not differ. Wasps fed on glucose, as well as on a honey-mimicking solution that contained approximately the same sugars in similar relative concentrations (but none of the trace elements) found in honey, produced only half of the progeny compared to wasps fed on honey. Furthermore, wasps fed on honey and glucose lived up to two weeks on average longer than those fed on the honey-mimic. Our results suggest that the effects of host-feeding and honey in the diet of *G. agilis* are additive and, more importantly, reveal that host-feeding alone does not allow the parasitoid to achieve optimal egg production and fecundity.

Honey has been shown to contain at least 181 substances and is a highly saturated solution made up of sugars, proteins, enzymes, amino acids, minerals and vitamins (Alvarez-Suarez et al., 2009). It also contains vitamins B1, B2, C and nicotinic acid and is known to possess antimicrobial, antioxidant and hygroscopic characteristics (Echingo and Takenaka, 1974; Martos et al., 2000; Gheldof et al., 2002). More than 95% of honey is made up of carbohydrates, whereas proteins and amino acids make up only approximately 0.5% (see Alvarez-Suarez et al., 2009, for a review). The most dominant protein found in honey produced by two honeybees within the genus *Apis* is royalactin or Major Royal Jelly Protein 1 (MRJP1) (Won et al., 2008). One of the many functions of this protein involves the stimulation of reproductive maturation in honeybees (Drapeau et al., 2006). However, the sole action of MRJP1 in honeybees was found to be responsible for queen differentiation in which juvenile hormone titers increased and *vitellogenin* genes (precursors of *yolk protein*) were expressed at a higher rate. These responses led to increased egg maturation rates and a similar response to a diet containing royalactin, in which an increase in fecundity was also observed in the fruit fly *Drosophila melanogaster* (Kamakura, 2011). Therefore, the presence of this protein in honey-fed wasps could have accounted for the increased fecundity of *G. agilis* observed in this study.

It should also be noted that the effects of honey in amplifying egg production as shown here is probably manifested in the vast majority of insect species, where the adult females must invest sufficient resources into each egg for the completion of embryogenesis. Endoparasitoids are unique amongst insects in that their eggs are immersed in a protein-rich solution (the host blood). The chorion of hydropic eggs in most endoparasitoids is comprised of two separate layers, the serosa and the amnion, in which the developing wasp embryo is enclosed (Beckage and de Buron, 1994; Le Ralec, 1995). This 'porous' layer in the egg chorion enables nutrients in the host blood to be absorbed by the developing wasp embryo; the embryo is thus able to obtain the vast majority of essential nutrients from this solution for the completion of successful embryogenesis (Sabri et al., 2011). This adaptation is lacking in parasitoids that produce yolky, anhydropic eggs, such as *G. agilis*, where maternal protein investment is crucial. Many studies have shown that the hydropic eggs of koinobionts are tiny at oviposition but swell to many times their initial size by the time of hatching, whereas anhydropic eggs remain about the same size (Godfray, 1994). Given that koinobionts invest very little nutrients into their eggs, we argue that honey-fed endoparasitoids will not produce

more offspring than wasps fed on high quality sugars like glucose and fructose, although this remains to be empirically tested.

In summary, our results reveal that mannose, a sugar that a previous study found to be utilized by a primary parasitoid, *C. glomerata*, is unsuitable for two of its hyperparasitoids, *L. nana* and *G. agilis*. Conversely, both hyperparasitoids performed well on trehalose, which is of low quality for *C. glomerata*. These results are particularly interesting, given that *L. nana* is probably quite specialized on *C. glomerata* in nature and that both species may thus share a strong recent co-evolutionary history. We argue that there may be factors associated with phylogeny or reproductive biology that account for the different patterns of sugar utilization by koinobiont and idiobiont parasitoids. Furthermore, it has been shown that non-sugar components in honey enhance egg production in a parasitoid species. Future studies should aim to explore resource utilization efficiencies in adult parasitoids reared on different diets focusing on establishing possible links with certain life history and reproductive strategies in these fascinating insects. In this way it might be possible to enhance methods of mass-rearing these insects for biological control, as well as unraveling evolutionary constraints that are trait-mediated.

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