WAGENINGEN UNIVERSITY
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Effects of different sugars on longevity and fecundity of two parasitic wasps: *Lysibia nana* and *Gelis agilis* (Hymenoptera: Ichneumonidae)

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Abstract

Different sources of carbohydrates can have a positive or negative influence on the longevity of parasitoids. In this study, the effect of sugar diet on longevity and fecundity of two closely related hyperparasitoids wasps (*Lysibia nana* and *Gelis agilis*) was investigated. Both wasp species are solitary ectoparasitoids attacking pre-pupae of the primary parasitoid *Cotesia glometara*. The latter species parasitizes caterpillars of the genus *Pieris* . Wingless *G. agilis* must first host-feed for egg maturation, whereas *L. nana* only uses resources obtained from its host during larval feeding to finish egg maturation. Hyperparasitoids can have a top-down effect on primary parasitoids if they are provided with adequate food sources, which they can allocate to maintenance, locomotion or egg production. The availability of hosts can have an effect on longevity of the wasps. In this study, the two wasp species were provided with one out of five sugar solutions during their adult life. Different cohorts of wasps were either provided with sugar solutions alone or with sugars and host cocoons. The results on the performance of the effect of different sugars was compared for *L. nana* on *G. agilis*. In addition, these results were compared with longevity data reported for the primary parasitoid *C. glomerata*, which is a host for the two hyperparasitoid species. In order to find out whether wasps had a preference for a sugar type, a five-way choice experiment was done. Only in the absence of hosts, the longevity of the specialist *L. nana* was more influenced by sugar type than that of the generalist *G. agilis*. In the presence of hosts, longevity of both hyperparasitoids was similarly affected by sugar type. Fecundity of *L. nana* was marginally affected by sugar diet, whereas *G. agilis* produced very few offspring on all sugar diets. Mannose was found to be an unsuitable food source for both species. The females did not have a preference for a specific sugar and feeding duration did not vary according to the sugar on which the wasps was feeding. Consequently, effects of sugars on the performance of the wasps are probably due to the difference in the ability of the wasps to digest different sugars. When the results from Wäckers study (2001) using *C. glomerata* are compared with those determined for *L. nana* and *G. agilis* in this study, it was found that sugar quality affects the three species differently, which could have an effect on a biological control efficiency.

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Introduction

Many studies have reported that the availability and type of carbohydrates to adult parasitoids and hyperparasitoids may extend longevity and improve fecundity (Giron et al. 2002;Giron et al. 2004;Wackers 2000, 2001;Winkler et al. 2005;Winkler et al. 2009). Nutrients that can be acquired during adult life are mainly carbohydrates and proteins, whereas lipids are accumulated during larval development (Harvey et al. 2008). Fat can be converted into energy for locomotion and maintenance, but also for egg production (Ellers et al. 1998); (Rivero and Casas 1999). Proteins are used for tissue formation and the production of eggs (Jervis et al. 2008). Amino acids are gained during larval development, and in certain species, during host-feeding (Jervis et al. 2008). This study is focused on carbohydrates acquisition during adult life.

Carbohydrates are the most accessible form of energy available to insects and they provide an exogenous source of carbohydrates for all insect activities (Blum 1985). It also contributes to the oogenises in synovigenic females, meaning that females emerge from hosts with immature eggs (Jervis et al. 2008). Those eggs represent only a fraction of the total egg production. Consequently, further nutrient acquisition will allow the production of more egg and considerably influence female fertility (Jervis and Kidd 1986).

Sugars are absorbed into the insect's hemolymph as monosaccharides, such as glucose and fructose (Blum 1985). The capacity of the parasitoid to digest polysaccharides relies on the presence of specific enzymes in the insect's digestive system. These enzymes break down polysaccharides to monosaccharides. (McFarlane 1929; Nation 2008). The concentration of glucose in the hemolymph is maintained low through a costly conversion taking place in fat body cells. Here, glucose is converted into the disaccharide trehalose, to facilitate monosaccharide diffusion from the midgut (Chapman 1998a). In addition, food digestion is related to the feeding pattern of an insect and to humoral control. Insects possess only a few enzymes for the digestion of carbohydrates ((Nation 2008) and sugar quality depends on the insect's ability to digest polysaccharides (Chapman 1998b). Therefore, different sugars differ in their quality and

parasitoid wasps are able to utilize some sugars better than others. (Lehane and Billingsley 1996; Wackers 2001; Winkler 2004).

Carbohydrates can be obtained from several sources. Usually, wasps feed on floral nectar, extra floral nectaries or honeydew (Wackers 2001). Nectar upon which wasps feed on varies in sugar concentration and composition (Baker and Baker 1983). In floral nectar, glucose, fructose or sucrose can be the dominant. Other sugar types along with amino acids, vitamins and minerals are found in lower concentrations in nectar (Baker and Baker 1983). The mechanism involved in the selection of carbohydrates diet by insects is still unknown (Nation 2008). However for some hymenopteran species, such as the honeybee, recognize flowers on the amino acid content of the nectar and on the basis of pollen odor (Schoonhoven et al. 2005). There is not evidence that the parasitoid wasps use a similar mechanism.

Parasitoids larvae develop within a fixed amount of resources provided by its host. Nutrients involved in reproduction are mainly acquired during larval development of the parasitoid (Harvey et al. 2006; Jervis et al. 2008). However, adults can allocate resources either to maintenance or to reproduction depending on its physiological status (Giron et al. 2004). Lack of food sources during adulthood can result in a the allocation of larval acquired resources to maintenance purposes instead of reproduction. Thus, adult nutrition is one of the most important factors that can influence the allocation of resources to reproduction (Giron et al. 2004).

Parasitoids are used as bio-control agents of insect pests in agriculture. Provision of sugar sources in the environment may improve parasitoid performance in terms of longevity and fecundity (Wade et al. 2008a) (Wade et al. 2008b). However, these carbohydrate sources may also be consumed by hyperparasitoids, which are parasitoids that parasite another parasitoid. Sugar consumption may also have a positive impact on the hyperparasitoid reproduction capacity as well. Improving the fitness of the hyperparasitoids would result in a reduction of the number of primary parasitoids (Niyibigira 2003). Consequently, pest numbers could increase. Therefore, hyperparasitoids play an important role in the ecology of primary parasitoids and can influence greatly the outcome of primary parasitoid success and the effectiveness of biological control (Harvey et al. 2004).

This study examines the effect of sugar quality on the performance of two closely related hyperparasitoid species, *Lysibia nana* Gravenhorst and *Gelis agilis* Fabricius (Hymeoptera: *Ichneumonidae*), which differ considerably in some of their life history traits. The life history and development strategies of *L. nana* and *G. agilis* have been previously compared (Harvey 2005). *L. nana* can live up to 40 days, while *G. agilis* can live up to 70 days (Harvey 2008). Although *L. nana* has a shorter life expectancy, it develops faster, lays twice as many, but smaller, eggs and has a shorter handling time than *G. agilis*. When fed on honey, *L. nana* lays up to 17 eggs per day during the fist six to eight days of its life. Then, egg production rapidly decreased to none or a few eggs per day until death. In total, a *L. nana* female can produce around a 100 eggs during its life. *G. agilis* lays one to three eggs per days until death. On average, *G. agilis* produces 40 eggs in a life-time. Both species are synovigenic, meaning that they do not have matured eggs in their ovaries at adult emergence. *L. nana* mobilizes metabolic protein for egg maturation at emergence, whereas *G. agilis* must host-feed in order to acquire the protein necessary to initiate ovigenesis (Harvey 2008). Previous experiments have shown that *L. nana*, when provided with honey and host cocoons, lives shorter than when only honey is provided. This suggests that laying egg is costly for *L. nana*. It was also shown that host-feeding and reproduction is not costly for *G. agilis*, as it do not affect longevity of this species (Harvey et al. 2008). Furthermore, *L. nana* is a more specialized secondary parasitoid species than *G. agilis* since the latter can parasite a larger number of species including *L. nana* (Harvey et al. 2009). In addition, *G. agilis* is wingless, which limits its mobility and foraging range, whereas *L. nana* can fly to locate food or hosts.

In this study, we specifically compared the effect of individual sugar diet on the longevity and the fecundity of the two hyperparasitoid wasps. Results of the hyperparasitoid experiments are compared to those reported for *Cotesia glomerata*, a primary parasitoid, which is used in this study as hosts for the two hyperparasitoids (Wäckers 2001).

2. Research aims and hypothesis

Here, longevity and lifetime reproductive success of the two hyperparasitoid species were compared when feeding on different sugars.

The questions of the research are:

1) Does the performance of the two hyperparasitoid species vary when female wasps were fed on different sugars?
Hypothesis:
It is expected that longevity and fecundity of *L. nana* and *G. agilis* vary depending on which sugar solution is provided to them. Although *G. agilis* is a closely related species, results are expected to be different compared to *L. nana*. I hypothesize that the performance of *G. agilis* is less affected by sugar quality because this species is a wingless generalist. Therefore, it must feed on what it finds in its direct surrounding.

2) Does the longevity differ in the absence of hosts?
Hypothesis:
L. nana is expected to have a longer life expectancy in the absence of hosts than with hosts (Harvey et al. 2008) for every sugar solution. It is expected that *L. nana* that in the presence of hosts, the longevity of *L. nana*, which produces a high number of offspring, would decreases due to nutrient allocation to reproduction. *G. agilis*'s longevity is expected not to be affected by host availability since additional nutrients obtained by host-feeding may also be allocated to maintenance, which could extend the longevity of the wasps (Harvey et al. 2008).

3) Does egg load after death differ according to the sugar diet and host access?
Hypothesis:
G.agilis is expected not to produce any eggs in the absence of hosts, because she has to host-feed in order to mature eggs, whereas *L. nana* is expected to produce eggs with or without hosts presence (Harvey et al. 2008). For both species, it is expected that sugar

quality would affect egg load. Yet, *L. nana* egg load after death should be lower in the presence of hosts due successful reproduction. Since sugar quality may affect egg production capacity, it is expected the egg load after death would differ according to the type of sugar offered (Heimpel et al. 1997).

4) Do hyperparasitoids have a preference for a specific sugar and do they feed longer on a specific sugar?

Hypothesis:

It is expected that wasps would not be able to discriminate between different sugar solutions and that they will feed equally long for all sugar solutions. In this case, the difference in longevity and fecundity would not be caused by a ingestion difference, but by a difference in digestion capacity.

5) Does sugar diet affect longevity of hyperparasitoids similarly as a primary parasitoid?

Hypothesis:

C. glomerata is able to use a number of sugars which were not suitable for other wasps species (Wackers 2001). Therefore, it is expected that the longevity of the primary parasitoid when feeding on different sugar diets will differ from the longevity of the hyperparasitoids.

3. Materials and Methods

3.1 Insect cultures

Both *L. nana* and *G. agilis* were supplied by the Netherlands Institute of Ecology (NIOO) in Heteren, the Netherlands. *L. nana* was originally obtained from cocoons of *Cotesia glomerata* L. (Hymeoptera: *Braconidae*) recovered from leaves of *B. napus* growing adjacent to the NIOO institute. *G. agilis* was also obtained from cocoons of *C. glomerata* placed in the garden of the institute. In the present study, *C. glomerata* cocoons were also used as hosts for the two hyperparasitoids. 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 hours. Then, parasitized cocoons were transferred to large Petri dishes 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 to 4 days before it was provided with new cocoons for oviposition. For more details on the rearing, see Harvey (2000). Adults of both species were kept in Petri dishes at 10°C, which is the optimal temperature to extend their longevity (Harvey and Witjes 2005).

The parasitoids were reared at 25 ± 2 ^{o}C with a 16:8 hour L:D light regime. *C. glomerata* were reared on *Pieris brassicae* caterpillars (for rearing details see Harvey, 2000) and were originally collected from agricultural fields in the vicinity of Wageningen university. *L. nana* and *G. agilis* cultures were reared on *C. glomerata* cocoons obtained from the general Wageningen culture. Fresh parasitoid cocoons were collected and stored in a fridge at 7°C until they were offered to the hyperparasitoid wasps.

3.2 Experimental Protocol

Longevity and fecundity

Wasps were kept individually in 9.5 cm Petri dishes. The wasps were divided into two groups. One group of wasps was kept without hosts and the other group with hosts. The latter group was used to measure fecundity and longevity in the presence of host, whereas

the former group was used to measure longevity of the wasps in the absence of hosts. Individuals in both groups were provided with one out of five different sugar solutions throughout their adult life. Twelve wasps were observed per sugar per diet (with and without hosts). Each dish was labeled with information on 1) the starting date of the experiment, 2) the wasp species, 3) the type of sugar offered to the wasp, and, for the wasps provided with hosts 4) the date at which new hosts where placed in the Petri dish. An overview of the experimental set-up is given in figures 1 and 2.

After adult emergence, single female *L. nana* were placed in a Petri dish with three males for mating purposes. After 24 hours, all males were removed. For the following six days, each female received every other day 40 fresh cocoons to parasitize. Hereafter, only 30 cocoons were offered to the wasp due to an expected decrease in fecundity. *G. agilis* received 10 cocoons every other day throughout her adult life. Unmated females were used, as *G. agilis* females do not need to mate in order to reproduce. Cocoons exposed to hyperparasitoids were kept under the same conditions as the adult wasps for the parasitoids to develop (see below). Mortality of the wasps was observed daily in order to record the longevity of the adults in days.

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 1999). Three droplets of 10 µL of a specific sugar solution were pipeted in each Petri dish. Sugars were renewed every day. For the control treatment, three droplets of 10 µL of water were pipeted in the Petri dishes. Wet cotton wool was placed in each Petri dish to insure that the parasitoid would not dry out. In each dish with cocoons, the newly emerged wasps, both parasitoid and hyperparasitoid, were counted. The number of offspring was counted as in the protocol by Harvey (2006). (1) Both hyperparasitoid species, (2) *C. glomerata* and (3) dead hosts were scored in relation to the type of sugar offered to the female wasp. A host was considered to be 'dead' when neither a hyperparasitoid or a parasitoid were not able to emerged successfully (Harvey, Vet et al. 2006).
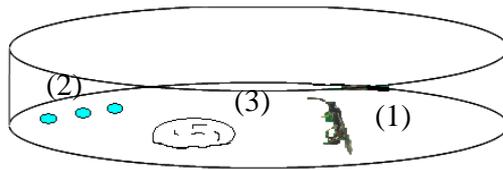
Fig. 1. Experimental set-up of wasp treatment without cocoons. (1) female wasp individually kept in a Petri dish, (2) sugar solution which was changed every day (concentration of 1M), (3) wet cotton ball providing water to wasps.
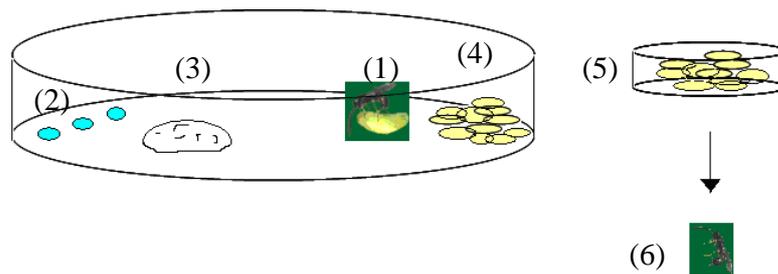

Fig. 2. Experimental set-up of wasp treatment with cocoons. (1) Female wasp individually kept in a Petri dish, (2) sugar solution which was changed every day (concentration of 1M), (3) wet cotton ball providing water to wasps, (4) every other day, 40 cocoons were given to *L. nana* and 10 cocoons were given to *G. agilis*, (5) Cocoons exposed to hyperparasitoids were kept under the same conditions as the adult wasps for the parasitoids to develop, (6) emerged offspring from both parasitoid and hyperparasitoid were counted.

After hyperparasitoid death, mature eggs remaining in the ovaries were counted by dissecting the adult in a drop of water on a glass slide using two pairs of forceps and a cecum, as done by Harvey et al. (2008).

The sugars selected for this study were based on a pilot experiment, where *L. nana* was provided with 11 different sugar solutions (Harvey and Wäcker, unpublished results). Maltose, sucrose, fructose, and glucose, similar in quality, prolonged longevity by a factor between 14.2 and 35.7 compared to water. Five of these sugars were selected to be offered to wasps in the present study: two monosaccharides, glucose and mannose, and three disaccharides, trehalose, melibioze and maltose. All these sugars can be found in floral nectar. Glucose, maltose and trehalose can also be found in honeydew (Wackers 2001).

When the results from the study by Wäckers (2001) (Fig. 3.) and the pilot experiment by Wäckers and Harvey (unpublished results) are compared, it was found that *C. glomerata* lived relatively longer than *L. nana* when feeding on mannose. The

opposite was found when wasps were feeding on maltose, making maltose and mannose interesting to test. Trehalose plays an important role in sugar absorption as it can directly diffuse into the insect midgut and serve as a gradient to absorb other sugars (Lehane and Billingsley 1996). Therefore, this sugar was also included in the experiments. Finally, melibiose was chosen since it is intermediate sugar quality. s
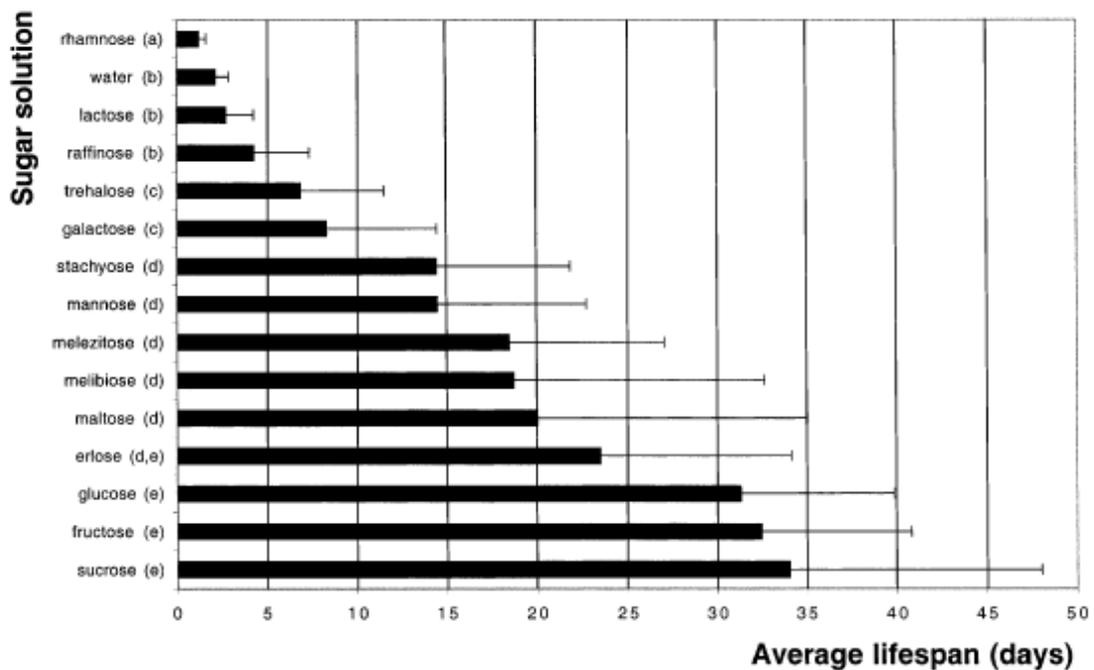

Fig. 3. Mean longevity of female *C. glomerata* when feeding on solutions of individual sugars. Different letters indicate significant differences (Fisher PLSD). Error bars represent standard deviations. (Wackers 2001).

Choice experiment

Insects belonging to the Hymenoptera family, eat until their stomach is full (Chapman and Boer 1995). The termination of a meal is partly determined when the stomach is stretched and partly determined by hormonal changes in the digestive system. After a meal, the wasp becomes inactive (Chapman and Boer 1995). To determine if *L. nana* and *G. agilis* can discriminate among different sugars, the five sugar solutions, each at a concentration of 1 M, were presented to a newly emerged female wasp in a Petri dish. The time of her meal and sugar type ingested were scored. A short drinking time (<10 s) can represent a rejection of the sugar. A rejection event followed by a acceptance event,

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i.e. a longer drinking time of another sugar type, could mean that wasps can discriminate among sugar diets based on taste. The experiment was ended when the wasp became inactive. Wasps that did not drink from any sugar within 30 minutes were excluded from the statistical analysis. A total of 50 wasps per species were tested. An overview of the experimental set-up is given in figure 4.
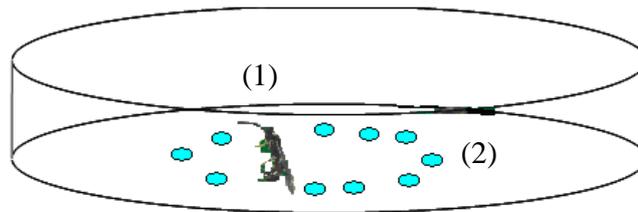

Fig. 4.Experimental set-up of choice experiment. (1) Individual female wasp kept in a Petri dish where the first (2) sugar, randomly disposed in a circle, upon which it fed on was scored as well as the feeding-time.

3.3 Statistics analysis

As the fecundity data, measured as lifetime number of offspring, did not follow a normal distribution, non-parametric statistics were used to analyze the data. Fecundity of wasps feeding on different sugar diets was analyzed using a Kruskal-Wallis test followed by multiple pair-wise Mann-Withney U tests. Both test were run using SPSS. To analyze the effect of sugar diet and host availability on hyperparasitoid longevity, a two-way ANOVA was performed. The effect of sugar diet on the number of matured eggs after the female had died was analyzed using one-way ANOVA. The fate of the host cocoons after hyperparastioid exposure was analyzed using ANOVA (sugar type as main factor). All ANOVA tests were performed using GenStat 12th edition followed by multiple comparisons using LSD. The sugar choice experiments were analyzed into two parts: 1) the sugar choice was analyzed using a χ^2 test, using Excel (H_0: Wasps have no preference for any of the sugars), 2) the duration of feeding on the different sugar was analyzed using one-way ANOVA with sugar as main factor. The relationship between longevity and fecundity was compared using regression analysis.

4. Results

4.1 Lysibia nana: longevity and lifetime reproductive success

For *L. nana,* the interaction between sugar solution and host availability on the longevity was significant (ANOVA, $F_{5,139}$: 4.13, P= 0.002,), when wasps fed on glucose without hosts they lived the longest compared to all other treatments (Fig.5a.). Host availability, affected longevity only when wasps were feeding on glucose (Fig.5a.).
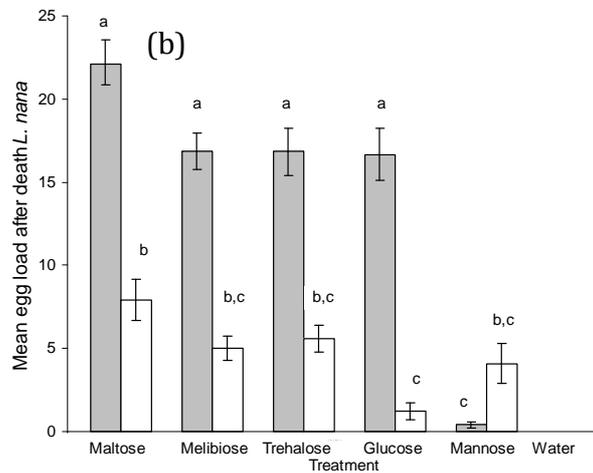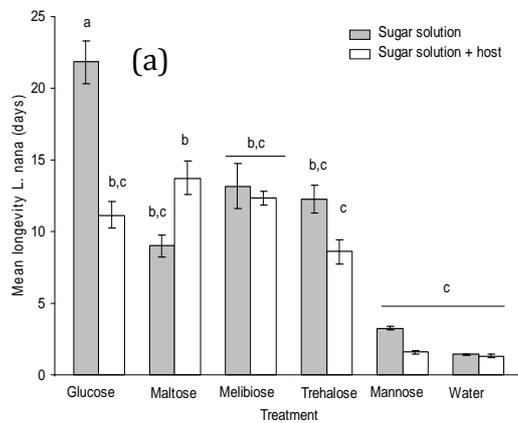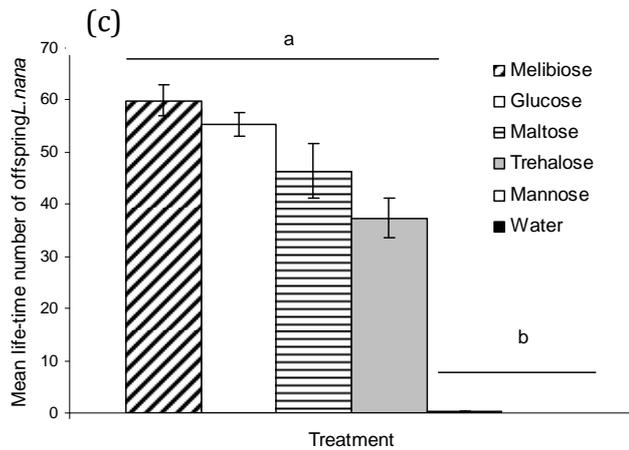
Fig. 5. *L. nana* **performance according to sugar type and host availability. (a) Mean longevity (in days) in relation to sugar treatment (b) mean egg load after death, (c) mean life-time number of offspring . Line bars represent standard error of the mean (n=12). Bars with the same letter are not significantly different (LSD with α=0.05). (a) and (b), Mann-Whitney U tests (c) with α=0.05.**

The interaction between sugar solution and host availability was significant on the egg load after death (ANOVA, $F_{5,139}$= 7.22, P < 0.001). The females provided with hosts had a lower number of eggs in their ovaries after death on all sugar types, except mannose, than the females that had not access to hosts. The egg load of the wasps fed on mannose was not affected by host availability (Fig.5b).

L. nana fecundity was significantly higher on all sugar diets compared to the water treatment (Mann-Whitney U test, P < 0.001), except for the mannose treatment (Mann-Whitney U test, P= 0.514) (Fig. 5c). Wasps fed on melibiose produced the highest number of offspring. However, the number of offspring did not differ significantly from the glucose, maltose, and trehalose treatment, respectively (Mann-Whitney U-test, P =0.9, P=0.56, P=0.05, respectively).

In figure 6, the mean life-time number of offspring produced by *L. nana* females are presented according to the female's age. The majority of the eggs were laid before she was 10 days old.
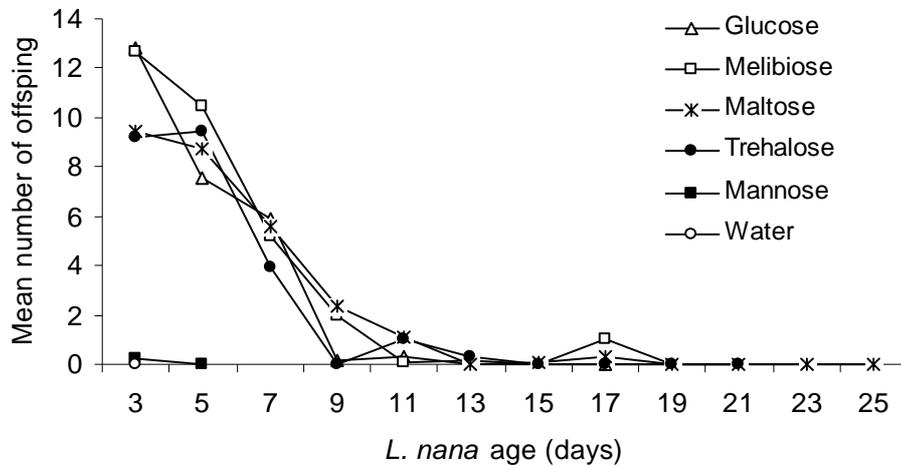
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Fig. 6. **Mean number of offspring in relation to the age of *L. nana* females.**

4. 2 Gelis agilis: longevity and lifetime reproductive success

The interaction between sugar type and host availability on the longevity of *G. agilis* was significant (ANOVA, $F_{5,139}=2.51$, P= 0.03). Deprived from hosts, wasps feeding on glucose, trehalose, maltose and melibiose lived longer than wasps that had been feeding on mannose (Fig.6.). The lifespan of wasps was significantly longer in the absence of host only when the females had been provided with a glucose diet. The availability of hosts did not significantly affect longevity when the other sugar types were offered to the wasps. *G. agilis* fed on mannose did not live longer than wasps provided with water only.
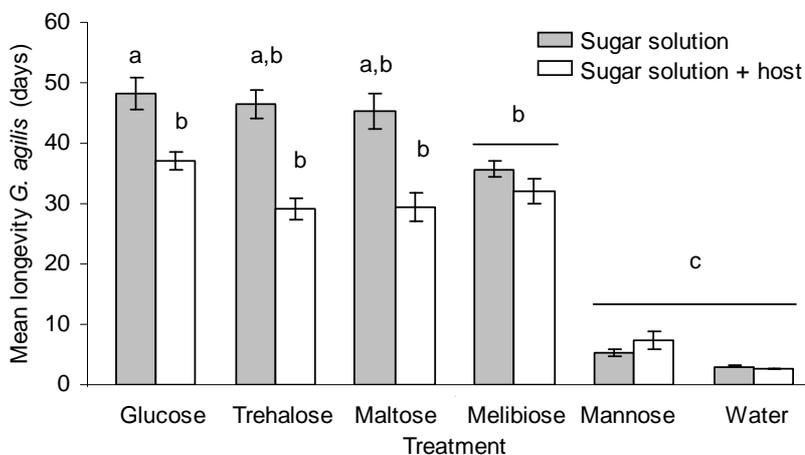

Fig. 6.Mean longevity of *G. agilis*. Females (in days) in relation to sugar diet. Line bars represent standard error of the mean (n= 12). Bars with the same letter are not significantly different (LSD with *α*=0.05).

Table 1. Mean life-time number of offspring of *G. agilis* per sugar time.

	Mean number of offspring	Standard error of mean
Glucose	1.83	0.911
Melibiose	1.75	0.854
Trehalose	0.58	0.260
Maltose	1.08	0.645
Mannose	0	0
Water	0	0

P = 0.027, Kruskal-Wallis

Sugar type affected *G. agilis* fecundity (Kruskal-Wallis, P = 0.03,). However, the mean total number of offspring produced per wasps was extremely low, less than two offspring compared to 40 offspring when fed with honey (Harvey 2008)(Table 1.). In figure 7 the fate of the cocoons (%) that had been exposed to hyperparasitoid females is presented according to the offered sugar diet. The percentage of *C. glomerata* emerging from the cocoons significantly higher on the mannose and water treatment than in the other sugar treatments (ANOVA, $F_{5,139=}$ 16.23 P < 0.001) (Fig.7.). Treatments resulting in a high percentage of dead cocoons, all sugars except mannose, coincided with a low percentage of cocoons developing into *C. glomerata*, since the number of cocoons producing *G. agilis* was negligible. (ANOVA, $F_{5,139}$:15.37 P < 0.001) (Fig.7.).
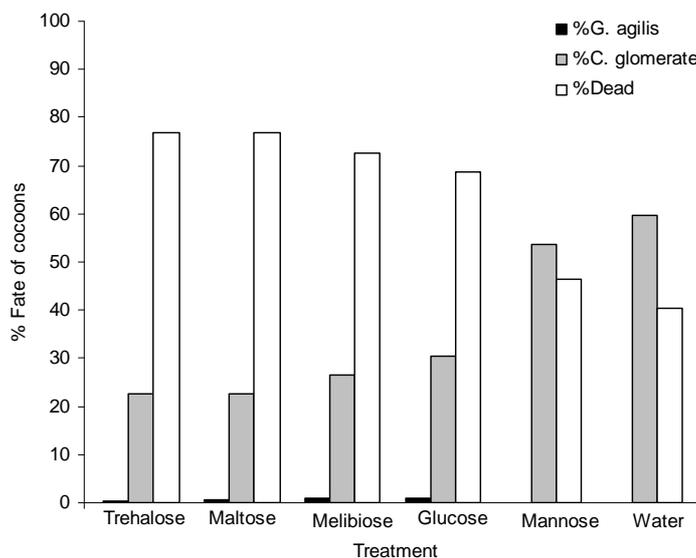

Fig. 7. Fate distribution of *C. glomerata* cocoons according to sugar treatment. The fate of the cocoons was recorded as percentage of *C. glomerata* emerging from cocoons, percentage of dead cocoons and percentage of *G. agilis* offspring.
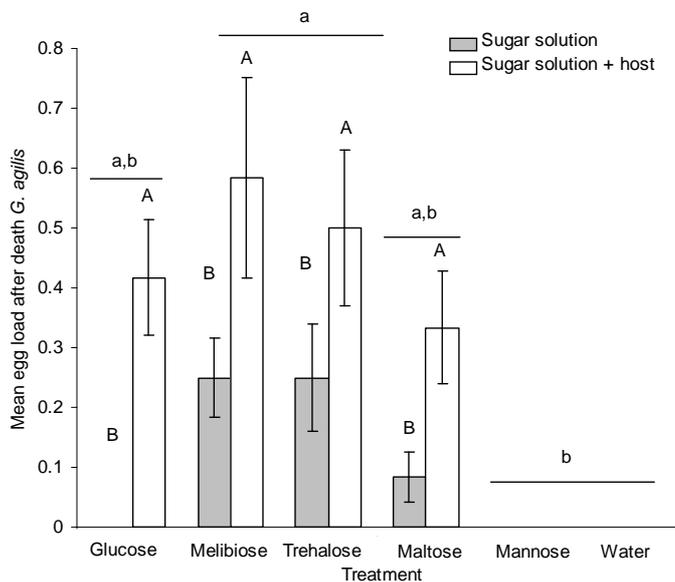
Fig. 8. Mean egg load after death of *G. agilis* in relation to sugar type, host availability (n= 12). Line bars represent standard error of the mean. Bars with the same letter are not significantly different(LSD with α=0.05). Small letters, multiple comparisons egg load according to sugar treatment, capital letter comparisons egg load according to host availability. Bars with the

The effect of the interaction between sugar type and host availability on the egg load of *G. agilis* was not significant (ANOVA $F_{5,139}$ = 0.58, P = 0.72) (Fig.8.). Sugar diet had a significant effect on the egg load after death (ANOVA $F_{5,139}$ = 2.44, P= 0.04) (Fig.8.). *G. agilis* feeding on all sugar types, except on mannose, had significantly more matured eggs after death than the water treatment. *G. agilis* had significantly more matured egg in its ovaries when wasps had access to hosts (ANOVA, $F_{1,139}$=5.04, P = 0.03) (Fig.8.).

4.3 Relationships between longevity and fecundity for L. nana and G. agilis

L. nana females that lived longer produced more offspring than those that had a shorter lifespan (y= 3.7263x + 2.6388, R^2 = 0.67) (Fig.10.). This relationship was not found for *G. agilis* since in most cases, the females did not produced sany offspring (y = 0.062x-0.5501, R^2 = 0.2632) (Fig.10.).
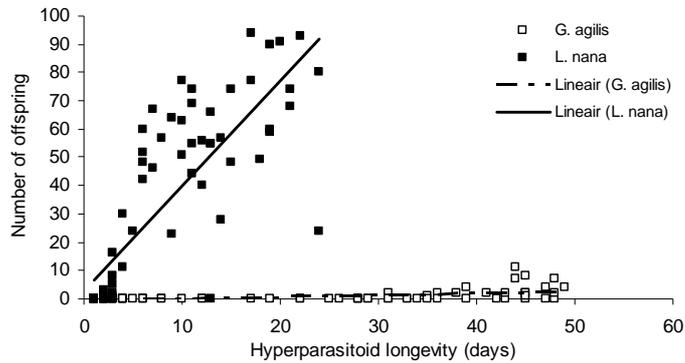
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Fig. 10. Relationship between longevity (days) and the number of offspring for *L. nana* and *G. agilis*. *L. nana* (closed squares, solid line): y= 3.7263x + 2.6388, R^2 = 0.6705; *G. agilis* (open squares, dashed line): y = 0.062x-0.5501, R^2 = 0.2632. Sample sizes are: 72 *L. nana* and 72 *G. agilis*.

4.4 Choice experiment

Both species did not have any preference for a specific sugar type (χ^2: 4.00, df: 4, P = 0.41 for *L. nana* and χ^2: 7.47, df: 4, P = 0.11 for *G. agilis*) (Table 2.). Feeding durations on the five sugar solutions, for both *L. nana* and *G. agilis*, were not significantly different (ANOVA, $F_{4,46}$ = 1.03, P = 0.40 and $F_{4,42}$ = 0.87, P = 0.80 respectively,) (Table 2.). Four *G. agilis* females (8%) did not feed during the experiment and, therefore, were excluded from the statistical analysis.

Table 2. Sugar preference and feeding duration for *L. nana* and *G. agilis*.

Sugar/species	*L. nana* (n= 50)		*G. agilis* (n=50)	
	Number of wasp choosing sugar	Mean feeding time (s)	Number of wasp choosing sugar	Mean feeding time (s)
Maltose	13	181 ± 39.9	4	92 ± 21.1
Melibiose	12	148 ± 24.9	12	148 ± 31.8
Trehalose	11	117 ± 17.3	10	125 ± 17.2
Glucose	9	148 ± 20.0	6	119 ± 10.8
Mannose	5	98 ± 30.5	14	106 ± 8.0
Non response	0		4	

Sugar choice, χ^2: 4.00 , P= 0.406 for *L. nana*, χ^2: 7.48, P= 0.11 for *G. agilis*. Feeding time, ANOVA, provide F statistic P= 0.40 for *L. nana*, P= 0.80 for *G. agilis*, ±Standard error of mean.

5. Discussion

5.1 Does the performance of the two hyperparasitoid species vary with sugar diet?

It was hypothesized that the performance of the two hyperparasitoid species would vary according to the sugar diet offered, where *G. agilis*, being a generalist, would be less affected by sugar type than the specialist *L. nana*. Indeed, in the absence of hosts, sugar diets could be divided into three groups with respect to sugar quality for *L. nana*, whereas for *G. agilis* only two groups could be distinguished. In the presence of hosts, two groups of sugars could be made both for *L. nana* and *G. agilis*, meaning that in the presence of hosts *L. nana* is affected by sugar quality similarly as *G. agilis*. Therefore, the effect of sugar quality on longevity disappears when the hyperparasitoids have access to hosts. For both species the mannose diet resulted in a longevity not significantly longer than the water treatment. Thus, in the absence of hosts only, sugar type affect more the specialist *L. nana* than the generalist *G. agilis*, whereas in presence the longevity of both species was equally affected.

Sugar diet had also little effect on fecundity of both species. The mean total number of offspring for both species did not differ significantly for all sugar treatments, except when the wasps were provided with mannose, which resulted in a very low offspring production for *L. nana* and no offspring for *G. agilis*. This can be explained by two factors. First, the limited effect of sugar diet on parasitoid fecundity could be related to the fact that wasps were kept under laboratory conditions. Wasps were confined to a small Petri dish, which reduces the carbohydrates requirements considerably compared to natural conditions. Sugars offered to wasps differed in quality since longevity was affected. Yet, a lower quality sugar may provide enough energy to fulfill locomotion requirements of the wasps under laboratory conditions. Thus wasps kept in Petri dishes may not have to divert proteins and lipids reserves, gained from the larval development, to fulfill energy need for locomotion. Consequently, reproduction relies more on host quality during larval development than on sugar diet during adult life. Reproduction capacity may be more affected by food source quality under natural conditions (Wackers

et al. 2007). Second, there was considerable variation among individual reproduction rates of *L. nana*. Although the means life-time number of offspring were not significantly different, a trend could be observed that wasps feeding on melibiose produced more offspring than wasps feeding on trehalose. Although sugar quality had little effect on fecundity, a trend was observed. In general, *L. nana* had stopped laying eggs days before she died, i.e. she produced most of her offspring within the first 10 days of her life. For *L. nana* females, there was a positive relationship between reproduction and longevity, where wasps that lived longer produced more wasps. This contradicts the hypothesis that costs related to reproduction result in a shorter lifespan. Sugar type has an effect on longevity of *L. nana* in the presence of hosts as well as on fecundity . Therefore, testing a larger number of wasps could have resulted in significant effects of sugar quality on fecundity. Due to the fact that *G. agilis* did not reproduce, this relationship could not be established for this species.

Proteins and lipids accumulated during larval development of the parasitoid are more important for egg production than ingestion of carbohydrates during adult life (Jervis et al. 2008). Consequently, host quality is more important for egg production of parasitoid females than the quality of carbohydrate sources during adult life. The smaller and older the cocoons are at the time of parasitism, the lower the fecundity and longevity of the emerging *L. nana* wasp are (Harvey et al. 2006). This phenomenon is probably due to the fact that after a *C. glomerata* older than 60 h has started to harden and are no longer palatable, making the host no longer suitable to digest for the hyperparasitoids (Harvey et al. 2006).This relationship between host age and time of parasitism has not been tested using *G. agilis*. However, other studies have shown that a the parasitism of older hosts resulted in a lower survival rate and a lower development time (Sandlan 1982; Carpenter et al. 1994; Ueno 2004). It could be argued that host quality could be a factor for the exceptionally low reproduction rate of *G. agilis*. Yet, if hosts were too old, a very low reproduction rate would have been also observed for *L. nana*, since female wasps of both species were obtained form the same rearing facility. To confirm this hypothesis, experiments on hosts age effect on survival and development time of *G. agilis* are needed.

In the experiments with *G. agilis*, the percentage of dead cocoons was high on all

sugar treatments, except on mannose. Females feeding on mannose and the water control did not live long enough to reproduce. High cocoon mortality could be due to an increase in the number of hosts that were used for host-feeding, which kills the host. This may suggest that *G. agilis* may have used hosts more for host-feeding than for oviposition, as was also found for the parasitoid *Euplemus vuilletti* (Casas et al. 2005). Alternatively, *G. agilis* eggs may not have successfully developed. Thus, cocoon mortality could have been due to host-feeding or due to the fact that eggs of *G. agilis* failed to live.

Honey and nectar, when compared to a single sugar diet, are more complete food sources; honey and nectar contain several sugar types, proteins, amino acids, vitamins and minerals (Baker and Baker 1983). An unbalanced diet can results in an increased food intake by the insect until the requirements of the lacking nutrient is obtained (Chapman 1998b). Consequently, *G. agilis* females may have increased the number of hosts they fed upon, which may explain the high proportion of dead hosts. *G. agilis* lived long enough to be able to reproduce, but failed in doing so. The single sugar diets may fulfill requirements for maintenance and locomotion but not for reproduction. Therefore, the extremely low reproduction rate of *G. agilis* could be due to an unbalanced diet and *G. agilis* may need specific minor nutrients found in nectar for egg production.

The fecundity and life expectancy of *L. nana* and *G. agilis* were strongly reduced when compared to results obtained by Harvey (2008) where the same wasps species were fed on honey. A lower parasitoid performance on single sugar diets may be due to the inability to produce specific enzymes needed to digest different sugars (Nation 2008). The opposite was found for the wasp *Bathyplectes curculionis*. In fact, this wasp lived longer on a glucose diet than on honey (Jacob and Evans 2004). Jacob argued that a more complex diet may require a longer feeding time to gain the same benefits as is obtained from a single sugar diet. Further studies would be needed to determined accurately the enzyme production for those two species.

For both species, mannose-fed wasps did not live longer nor did they produce more offspring than water-fed wasps. *G. agilis* feeding on mannose did not produce any offspring and did not have any eggs in her ovaries after death. Longevity was also found to be as short as on the water treatment when, females of both species were feeding on mannose. This suggests that mannose is not a suitable carbohydrate for reproduction and

longevity. Glucose-fed wasps of both species performed well. Glucose and mannose molecules are very similar, yet their effect on *L. nana* and *G. agilis* performances is remarkable. Mannose differs from glucose by the inversion of the C-2 chiral center. This simple change apparently leads to a drastically different bioactivity of the two molecules (SciFinder 2010) (Fig.11.). This difference has an important impact on digestion efficiency (Chapman 1998a). Both mannose and glucose are absorbed in the mid-gut. Yet, the conversion from mannose to trehalose, which allows its absorption in the mid-gut, occurs at a slower rate and is energetically more costly for mannose than for glucose (Chapman 1998a). Moreover, mannose is found to be toxic for some species, e.g. the honeybee (Fuente 1986). High levels of mannose in lime tree during drought is toxic to honeybees (Crane 1977). This toxicity could be due to the fact that the honeybee do not produce enough of the enzyme mannosephosphate isomerase, which catalyzes the conversion of mannose into fructose. However, in the same study, *Drosophila melanogaster* and *Ceratitis capitata*, both diptera, were able to digest mannose (Fuente 1986). Further experiments are needed to determine if mannose is toxic to *L. nana* and *G. agilis* and at which concentration mannose has a significant effect on hyperparasitoid fitness.
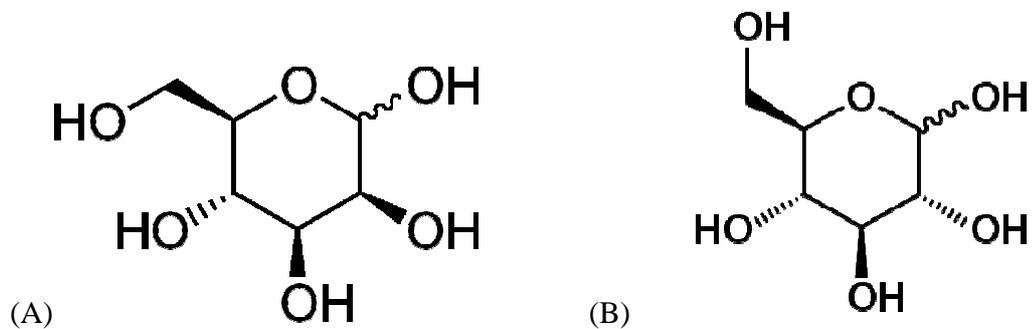

Fig. 11. Molecular structure of (A) D-mannose and (B) D-glucose.

5.2 Does longevity of two hyperparaitoids differ in the presence or absence of hosts?

The hypothesis was that host availability would not affect the longevity of *G. agilis*, whereas *L. nana* would be negatively affected. Indeed, *L. nana* lived significantly longer on a glucose diet in the absence of hosts than when hosts were present. On the other sugar diets, host availability had no effect on longevity of *L. nana*. The access to hosts did not significantly reduce longevity of *G. agilis*, except when the females were feeding on glucose. However, a trend of a shorter life span could be observed when hosts were available to *G. agilis*. As it was hypothesized by Harvey (2008) for *L .nana*, the difference in life expectancy could be explained by the fact that egg production is costly and, consequently, could result in a shorter life span. For *G. agilis* it can be argue that individual sugar diet is not enough to fulfill its nutritional need, resulting in a shorter longevity in the presence of hosts.

5.3 Does egg load after death differ depending on sugar diet and host access?

It was expected that *G. agilis* would not produce any eggs in the absence of hosts, because she has to host-feed in order to finish egg maturation. *L. nana* was expected to produce eggs both in the absence and presence of hosts. *G. agilis* females had more eggs in their ovaries when hosts were available to them. Harvey (2008) found that female *G. agilis* wasps do not produce eggs unless hosts are supplied to them. In my experiment, five wasps did produce eggs in the absence of hosts. Still, *G. agilis* had more eggs in her ovaries when the female were provided with hosts. This may suggest that G. agilis would also mobilizes nutrients reserves for egg maturation. Egg loads of *L. nana* were found to be higher in the absence of hosts than in presence of hosts for each sugar treatment, except on mannose, as they probably laid their eggs.

It was also expected that the egg load would differ according to the quality of the sugar diet. As sugar type did not affect fecundity of the females, it is logical to find similar egg loads irrespective of sugar diet. The comparison of egg loads in the absence

of hosts showed that the egg loads were similar irrespective of sugar diet with the exception of the mannose diet.

5.4 Do hyperparasitoids have a preference for a specific sugar and do they feed longer on a specific sugar?

My hypotheses that both wasp species would not have a preference for a specific sugar solution and would not feed longer on a particular sugar were confirmed. *L. nana* and *G. agilis* fed on the first sugar they found. After feeding on the first sugar, they stayed near that sugar for a while. Then, they either started feeding again on the same sugar or they would search for a different food source. Some wasps, of both species, just stopped moving after feeding. The experiment was then ended.

Indeed, it has been found that honeybees are able to choose a flower on the basis of the amino acids content (Schoonhoven et al. 2005). In addition, honeybees can differentiate among plant species based on the volatiles emitted by the flowers (Schoonhoven et al. 2005). In my experiments, wasps did not have any volatiles cues neither were amino acids added to the diet. Therefore, the wasps were not able to distinguish between sugars based on its content. These results re-enforce the hypothesis that the quality of a sugar diet is determined by the waps's ability to digest the carbohydrate and not by the amount of carbohydrates ingested by the wasp.

5.5 Do sugar diets affect the performance of the hyperparasitoids similarly as the primary parasitoid?

It was expected that *C. glomerata* would be able to utilize more sugar types than the hyperparasitoids. In order to compare the longevity of the three wasp species, the performance on each of the sugars was compared to the performance of the same wasp species on glucose. Results presented in this thesis were compared to those reported by Wäckers (2001) (Fig.3.). Glucose was found to result in the longest longevity for the three species and, therefore, the relative longevity was set to 100% for each of the three species when feeding on this sugar. Figure 12 gives a general overview of the effect of sugar quality relative to glucose for each of the three species with respect to longevity. It

can be observed that parasitoids and hyperparasitoids perform differently on the various sugars. This can have an impact on the interaction of these species. Depending on sugar type available, parasitoids may be promoted or vise versa (Wackers 2001; Giron et al. 2004). As a consequence, carbohydrates availability could affect bio-control efficiency. An experiment involving the interaction between the herbivore, the parasitoid and its hyperparasitoids, were the performances of each species would be observed could give more information on the effect of individual sugars in a biological control context.
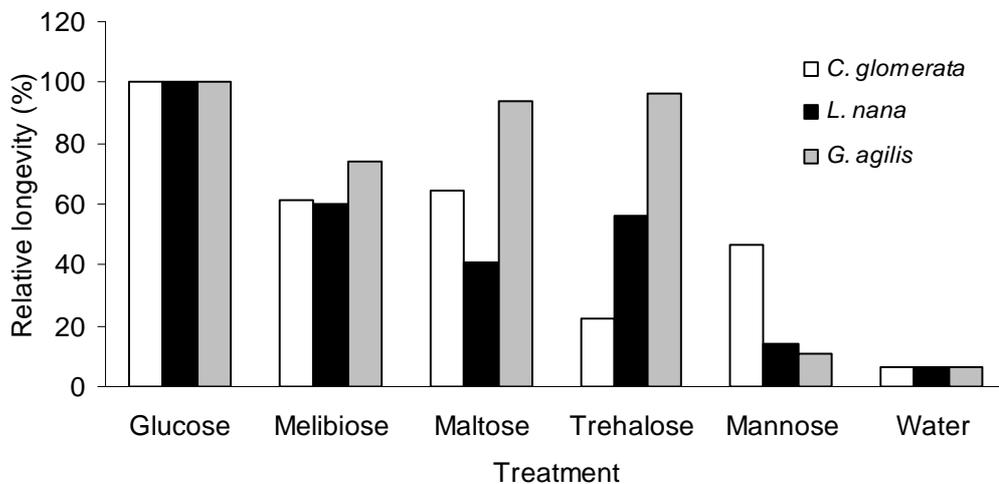

Fig.12. Relative longevity comparison between parasitoid *C. glomerata*, and hyperparasitoids *L. nana* and *G. agilis* when fed on different sugar solution.

6. Conclusion

Results of this investigation reveal that when different sugar diets are offered to *L. nana* and *G. agilis*, parasitoid performance was significantly affected by sugar quality. I have shown that sugar diet influences longevity of *L. nana* more than *G. agilis* in the absence of hosts. Glucose, one of the most prevalent sugars in flower nectar, had the most positive effect on the longevity and fecundity of the hyperparasitoid wasps, whereas mannose, which may be used by the flower as an antimicrobial agent in nectar, had the most negative effect. These results suggests that mannose may be toxic for *L. nana* and *G. agilis*. The latter species produced very few offspring irrespective of sugar diet. More experiments involving sugar mixtures could clarify the nutrient needs for reproduction of *G. agilis*. Moreover, an experimental set-up where the females would be able to fly, reflecting more natural conditions, would probably allow for larger differences between performance values according to sugar diet. Further research focusing on the changes of fat reserve during adulthood wasps would allow for a better understanding of resource allocation. In addition, a genomic approach to determine the production of different enzymes for carbohydrate digestion would give more information about the effect of sugar quality on the hyperparasitoid's physiology. Finally, as carbohydrates quality influences longevity of the parasitoid *C. glomerata* and the hyperparasitoids *L. nana* and *G. agilis* differently, a tritrophic experimental design would reveal the effect of individual carbohydrates on parasitoid interactions in a biological control context.

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