agronomy

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Longevity and Parasitism Capacity of *Psyttalia concolor* (Hymenoptera: Braconidae) Fed on Sugar Solutions and Insect Honeydew

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Abstract: Sugars are important food sources required by adult parasitoid species to enhance their survival, fecundity and fitness. The beneficial capacity of *Psyttalia concolor* Szépligeti, as a biological control agent of different fruit fly pest species, is expected to increase when different sugar food sources are supplied. The objective of this study was to test the effect of seven sugars (glucose, fructose, sucrose, trehalose, melibiose, melezitose and sorbitol) on the longevity and parasitism capacity of *P. concolor*. Moreover, we evaluated the effect of two types of honeydew excreted by hemipteran pests present in olive trees, *Saissetia oleae* Olivier (Coccidae) and *Euphyllura olivina* (Costa) (Psyllidae) on the longevity of the parasitoid. Our results show a positive effect of carbohydrate (single sugars and honeydew) consumption on parasitoid survival. Female longevity increased when fed on sorbitol and melibiose, while males benefited from feeding on glucose and fructose, suggesting that hexose-nectars would benefit males. Sucrose increased the percentage of non-emerged hosts and parasitism rate while melezitose significantly decreased these percentages, compared to the other sugars offered. *P. concolor* benefited more from feeding on honeydew than on sugars, and this food item can represent an important source of energy for the parasitoid. This result indicates the importance of specific nutrients for promoting the action of *P. concolor* against pests.

Keywords: parasitoid; nutrition; carbohydrate; fecundity; survival; *Olea europaea*

Citation: Pinheiro, L.A.; Pereira, J.A.; Medina, P.; Santos, S.A.P. Longevity and Parasitism Capacity of *Psyttalia concolor* (Hymenoptera: Braconidae) Fed on Sugar Solutions and Insect Honeydew. *Agronomy* **2022**, *12*, 2401. https://doi.org/10.3390/agronomy12102401

Academic Editor: Christos Athanassiou

Received: 6 September 2022
Accepted: 29 September 2022
Published: 4 October 2022

1. Introduction

Parasitoid wasps are one important group of natural enemies of insect pests that depend on the consumption of nectar, pollen or honeydew to survive and reproduce during the adult stage [1–3]. Adult parasitoids can also complement their diet through the ingestion of host haemolymph (host feeding strategy) [4].

Nectar is mainly composed of quickly digestible carbohydrates with high-energy content. It mainly contains two hexoses (glucose and fructose) and one disaccharide (sucrose) with concentrations ranging from 7% to 70% w/w [5]. After carbohydrates, amino acids (essential, non-essential and some non-protein amino acids) are the most abundant components occurring in nectar [6]. Under laboratory conditions, parasitoids fed on floral nectar increased their longevity, fecundity (e.g., can expand the reproductive period, mature more eggs, attack more hosts) and foraging ability when compared with starved parasitoids [7–9].

Honeydew is a sugar-rich substance excreted by hemipteran insects with a specific chemical profile that depends on the honeydew producer and its host plant [10,11]. In agricultural systems, where flowering plants are frequently scarce, honeydew is probably an abundant source of carbohydrates [1].

In the field, several studies also confirmed that parasitoids benefited from feeding on nectar and honeydew [12–14]. Moreover, positive effects of sugar-rich items feeding on nutrient levels of parasitoids (sugars, glycogen and lipids) were also reported by Olson et al. [15], Lee et al. [16], Nafziger Jr. and Fadamiro [17] and Snart et al. [18]. However, nectar is not available in the same way for parasitoids, depending on factors such as (i) flower morphology (that may hamper accessibility to the nectar), (ii) quality (e.g., concentration and composition of nectar produced by the plant), (iii) quantity, (iv) distribution of plants in the foraging area and (v) detectability [19,20].

The solitary parasitoid wasp species, *Psyttalia concolor* (Szépligeti) (Hymenoptera: Braconidae) is a synovigenic koinobiont larval-pupal endoparasitoid of several Tephritidae species. Individuals of this species were collected from olives infested with olive fruit flies, *Bactrocera oleae* (Rossi) (Diptera: Tephritidae), in Tunisia, and the species was introduced in Italy as a biocontrol agent against that pest [21]. After that, it has been used in augmentative releases for the biological control of *B. oleae* in European countries (e.g., Italy, Greece, or Spain) [22] and in the USA [23]. However, very few actions resulted in good rates of parasitism (22–23%) [22,24], and the maintenance of *P. concolor* in the field was not successful. Several reasons were indicated to justify those results, such as the quality of mass-reared individuals or the abundance of pupae of the olive fruit fly in the beginning of summer [25]. The availability of sugary food items in the agroecosystem can limit parasitoid potential. Therefore, the adoption of conservation biological control strategies, through habitat manipulations and maintenance of ground covers and field margins with the adequate plant species composition, or the use of sugar sprays, as complementary measure to augmentative releases, can contribute to maintaining the species in the field and for enhancing their effectiveness [26]. In this context, the objective of this work was to study the effect of different carbohydrate food sources on longevity and parasitism capacity of *P. concolor* in order to develop strategies for optimizing its use in future conservation and augmentative biological control programs against fruit flies such as *B. oleae* or *C. capitata*. We hypothesize that sugar solutions and honeydew, supplied individually, will significantly increase longevity and parasitism capacity of *P. concolor*.

2. Materials and Methods

2.1. Insect Rearing

P. concolor was reared on *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) in the Crop Protection Unit, ETSIAAB-UPM, following the methodology described by Jacas and Viñuela [27]. *C. capitata* individuals were obtained from a rearing culture maintained in that Unit for more than 20 generations, with occasional inclusion of new individuals collected from field. *P. concolor* individuals come from a Spanish field population. Both insects were reared at 25 ± 2 °C temperature, 75 ± 5% humidity and 16:8 h light:dark photoperiod. Parasitoids were neither exposed to food or water prior to the experiments. *C. capitata* was used as a model host to test the effect of different food sources on parasitism capacity of *P. concolor* once it is easily maintained in the laboratory.

2.2. Food Sources

Seven types of sugars were tested individually: two monosaccharides, D(+)-Glucose 1-hydrate (PanReac AppliChem, Barcelona, Spain) and D(+)-Fructose > 99% (ACROS Organics, Geel, Belgium), three disaccharides, (D+)-Sucrose > 99% (ACROS Organics, Geel, Belgium), D(+)-Trehalose dihydrate > 99% (ACROS Organics, Geel Belgium) and Melibiose > 98% (Sigma-Aldrich, Madrid, Spain), one trisaccharide, D(+)-Melezitose monohydrate 99% (ACROS Organics, Geel Belgium), and one sugar alcohol, Sorbitol > 99.5% (Sigma-Aldrich, Madrid, Spain). These sugars were selected based on the described major compounds of plant-derived-sugars (floral nectars and honeydew—see Wäckers [7]). The concentration used for all the sugar solutions was 1 M, representing the upper limit of sugars found in floral nectar and honeydew [7].

Moreover, two types of honeydew excreted by two secondary olive tree pests, the black-scale, *Saissetia oleae* (Olivier) (Hemiptera: Coccidae), and the olive psyllid, *Euphyllura olivina* (Costa) (Hemiptera: Psyllidae), on longevity of *P. concolor* were tested. *S. oleae* and *E. olivina* were reared on different 2-years olive trees, maintained, in pots, in a climate chamber at 24 ± 1 °C, 70 ± 5% relative humidity (RH) and a photoperiod of 16:8 h (L:D) at School of Agriculture, Polytechnic Institute of Bragança. Honeydew secreted ad libitum by each pest species was collected by placing a piece of Parafilm®, cut into squares (5 cm × 5 cm), under highly infested olive tree branches, for 24 h. After that period, each piece of Parafilm® containing more than 10 droplets of honeydew was offered to the parasitoids.

2.3. Longevity and Parasitism Potential of P. concolor Fed on Different "Sugars"

2.3.1. Longevity

For longevity experiments, five recently emerged (<24 h) females and five males of *P. concolor* were placed in a plexiglass cage (12 cm in diameter × 5 cm height) with a hole on the top (5.5 cm in diameter) covered with a piece of white cotton net (<1 mm^2) for ventilation. Females were identified due to the existence of an ovipositor at the end of the abdomen, structure that is absent in males. A total of six replicate cages were tested for each treatment.

Each sugar solution was offered individually in a glass vial (2 mL) with a strip (4 cm × 1 cm) of a commercial polyurethane sponge and overlaid with a strip of Parafilm®. Glass vials were glued to the base of the plexiglass cages with a piece of sticky putty and a similar vial with distilled water was also provided. Distilled water and solid diet—mixture of Brewer's yeast and powdered sugar (1:4)—were used ad libitum as negative and positive controls, respectively. Sugar solutions and solid diet were refilled on a daily basis and changed every week, while melezitose was renewed daily due to its propensity to crystallize. Parasitoid survival was assessed daily by observing each cage and removing dead individuals.

2.3.2. Parasitism Capacity

The parasitism of *C. capitata* was evaluated using similar cages to the longevity experiments, but with an additional hole (5.5 cm in diameter) on the bottom of the cage covered with a piece of white cotton net (<1 mm^2) (Figure 1A). *P. concolor* females were in contact with males for 2–3 h (following Bengochea et al. [28]) for mating and, after that, a female was placed in that cage and fed with a single sugar or solid diet (control). After seven days, 3rd instar larvae of *C. capitata* were offered to *P. concolor* females at a proportion of 20 larvae/female by placing them on the top of a glass bottle covered with a piece of Parafilm® (4 cm in diameter) (Figure 1B) for one hour (according to Bengochea et al. [28]) (Figure 1C) and this procedure was repeated during five consecutive days. Subsequently, *C. capitata* larvae were transferred into Petri dishes and placed at 25 ± 2 °C, 75 ± 5% and 16:8 h light:dark until emergence. The number of non-emerged hosts, the number of emerged hosts and the number of emerged parasitoids pupae were counted and registered to calculate the percentage of parasitism. The time elapsed from the pupal stage until emergence of *C. capitata* adults was recorded. Since previous studies showed that females need one day to adapt and learn how to parasite [29], only 4 days of parasitization were used in data analysis. A total of 25 females were tested for each treatment.

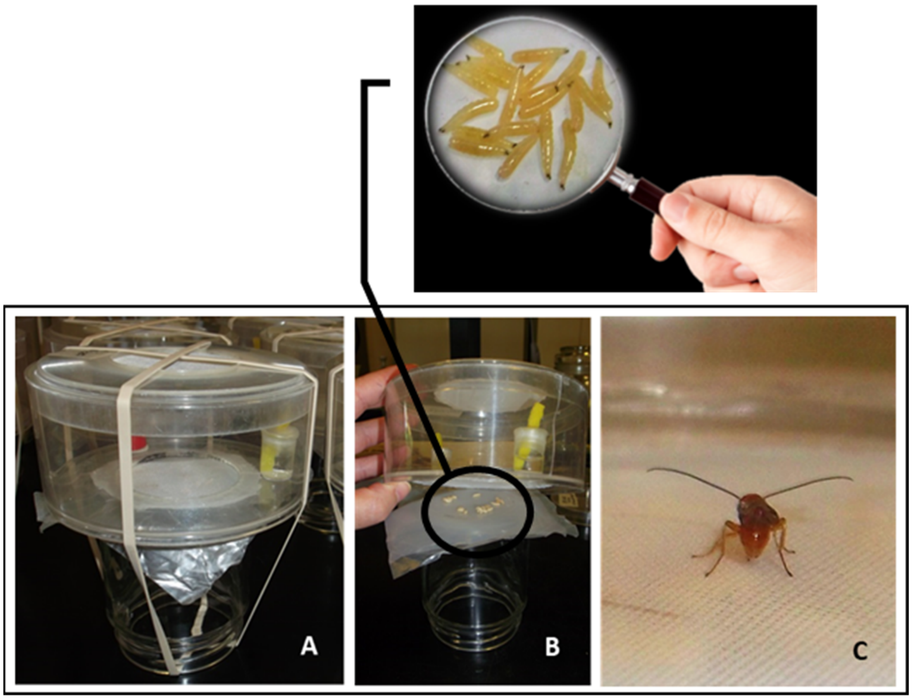

Figure 1. Parasitism tests. (**A**) General aspect of the plexiglass cage, (**B**) 3rd instar larvae of *C. capitata* offered to *P. concolor* and (**C**) detail of a *P. concolor* female parasitizing a *C. capitata* larva.

2.4. Longevity of P. concolor Fed on Honeydew

Five recently emerged (<24 h) females and males of *P. concolor* were placed in a plastic cage (diameter 7.5 cm × height 4.5 cm) with a hole in the top covered with a net for ventilation. A total of six replicate cages were tested for each treatment. Each cage was provided with (1) a Parafilm® square (5 cm × 5 cm) with honeydew from *S. oleae* or *E. olivina* + distilled water or (2) 10% of multifloral honey solution (Parque Natural de Montesinho honey) + distilled water (positive control) or (3) only distilled water (food deprived parasitoids—negative control). Water and honey were provided in 1.5 mL microcentrifuge tubes with a strip of sponge and plugged with a strip of Parafilm®. Honeydew was supplied as described in Section 2.2.

The diet offered to adults was replaced every day. The cages with parasitoids were kept in a climate chamber (25 ± 2 °C, 75 ± 5% and 16:8 h) and the survival of adults in each cage was recorded daily until all adults were dead.

2.5. Data Analysis

2.5.1. Longevity

Longevity means and standard errors were calculated separately by *P. concolor* sexes when provided with different foods. All analyses were performed with R software [30]. For survival analysis, the "survfit" function [in survival package] was used to calculate estimates of the different survival curves using the Kaplan–Meier method. The Kaplan–Meier survival curves of individual treatments were compared with the log-rank test "survdiff". A non-parametric Cox proportional hazards model ("coxph" function [in survival package]) was used to analyze the effect of the food source treatment on parasitoid survival. The assumption of proportional hazards was tested by visual inspections using the "cox.zph" function of the same package. Cox proportional hazards model fitted using "food resource", the proportional hazard assumption, was accepted, and nonlinearity was not detected. No influential observations were detected (all dfbetas <1).

2.5.2. Parasitism Capacity

The effect of sugars on percentage of non-emerged hosts and parasitism rate was analyzed using a Kruskal–Wallis test using the function Kruskal test from the Agricolae package. Data analyses were performed with R software.

3. Results

3.1. Longevity and Parasitism Capacity of Psyttalia concolor Fed on Different Sugars

3.1.1. Longevity

Survival curves obtained by Kaplan–Meier estimates for females (A) and males (B) of *P. concolor* fed on different food resources are shown in Figure 2. The Cox proportional hazards model showed that death hazards were significantly different among diet treatments for females ($\chi 2 = 412$, df = 8, $p < 0.001$) and males ($\chi 2 = 409$, df = 8, $p < 0.001$). All food resources significantly reduced the risk of dead females and males, when compared with only water ($p < 0.05$). For females, sorbitol and melibiose significantly reduced the risk of death when compared with all the sugars and solid diets ($p < 0.01$). Melibiose significantly reduced the risk of death in 99% when compared with only water ($p < 0.05$). For solid diet, the results indicate that this treatment had a smaller contribution to the difference in the daily hazard of death when compared with other food sources by a factor of exp(beta) $\approx$ 1%, which is not a significant contribution. Glucose significantly reduced the risk of death of females in 31% ($p < 0.05$) when compared with sucrose (Figure 2A), while for males, glucose reduced the risk of death in 2% ($p < 0.001$) when compared with sucrose and in 1% when compared with melibiose ($p < 0.01$). For males, fructose significantly reduced the risk of death when compared with trehalose (18% reduction, $p < 0.01$) and melezitose (17% reduction, $p < 0.01$) (Figure 2B).

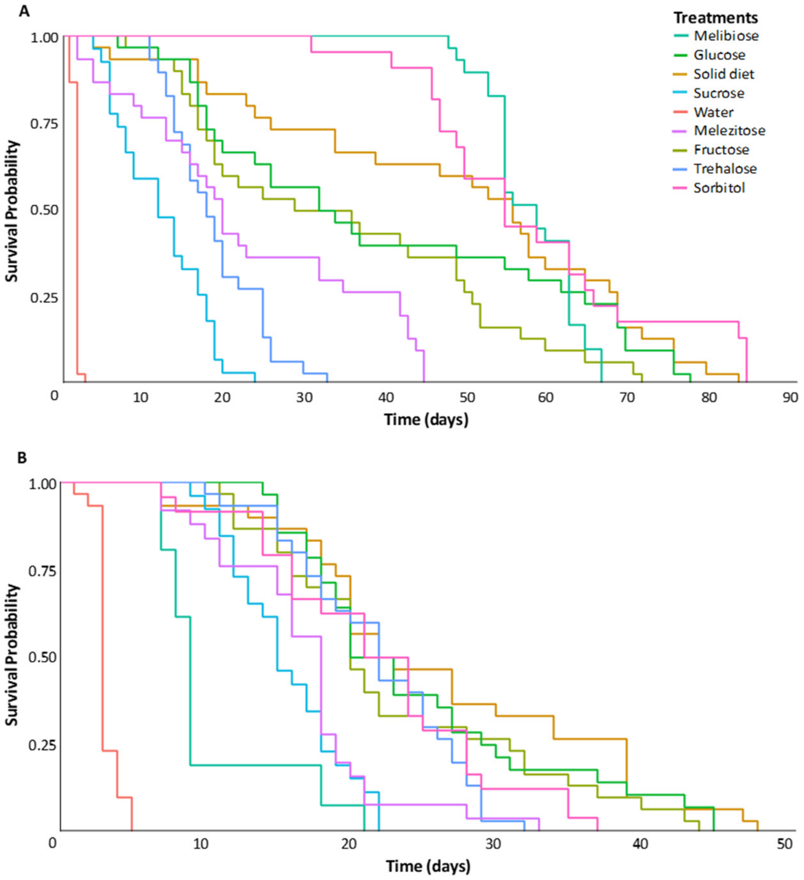

Figure 2. Survival curves obtained by Kaplan–Meier estimates for *Psyttalia concolor* females (**A**) and males (**B**) fed on single sugars.

The highest longevity obtained for females fed on sugars solution was observed on sorbitol (58.91 ± 3.26 days), melibiose (58.59 ± 1.04 days), solid diet (positive control, 48.87 ± 4.25 days) and glucose (40.13 ± 4.29 days) (Figure 3A). Males fed with diet (positive control, 26.33 ± 2.03 days), glucose (24.82 ± 1.77 days) and fructose (23.23 ± 1.72 days) presented the highest longevity (Figure 3B).

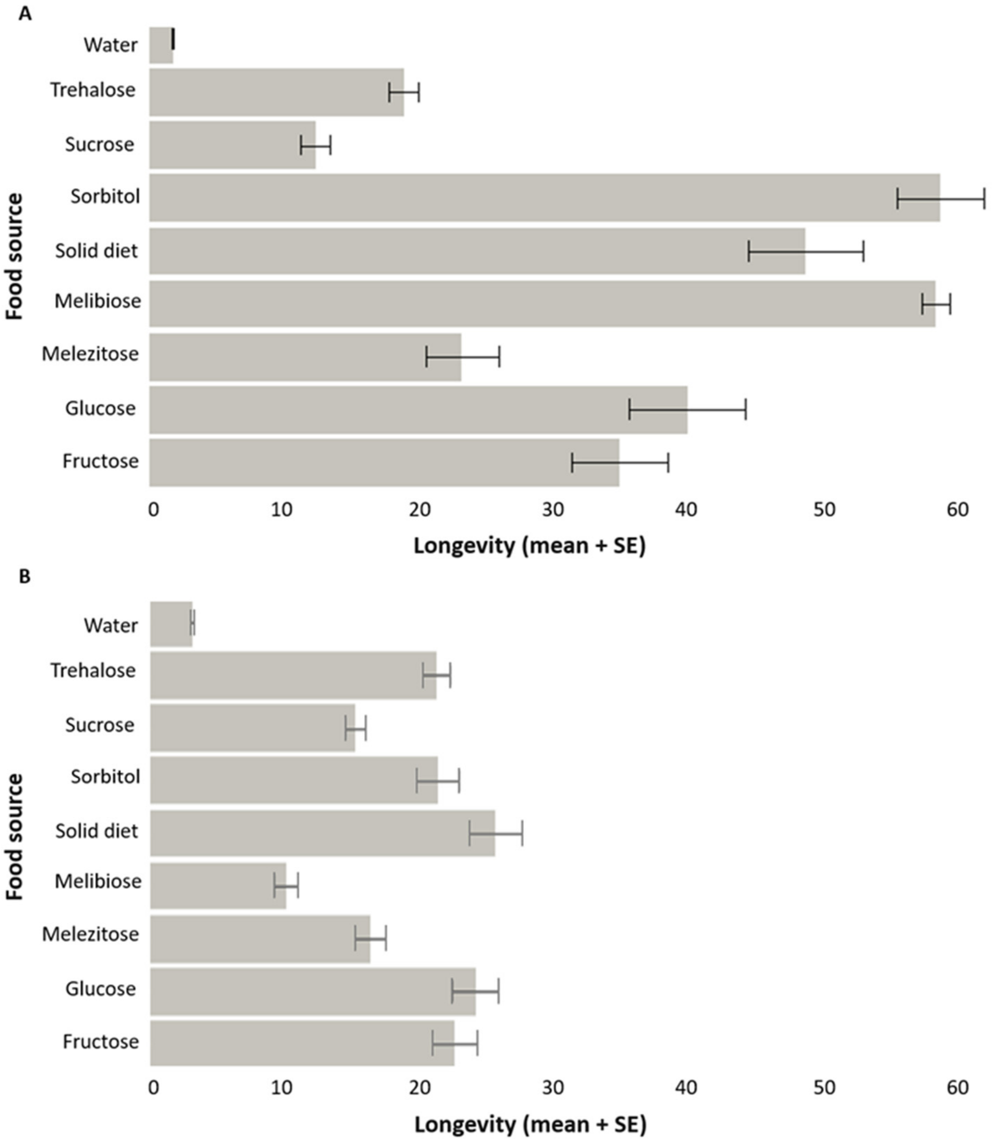

Figure 3. Longevity (mean + SE) (days) for *Psyttalia concolor* females (**A**) and males (**B**) fed on different solutions of single sugars.

3.1.2. Parasitism Capacity

The effects of each food source on parasitism capacity parameters are summarized in Table 1. The mean percentage of non-emerged hosts was higher than 83% for all food sources, except for parasitoids fed on melezitose (48.90% ± 6.53, mean ± standard error). Sucrose originated the highest percentage of non-emerged hosts (94.95 %), but these results were only significantly different from results obtained for melezitose (Table 1). The percentage of parasitism varied from 48.36% ± 6.51 (mean ± SE), obtained for *P. concolor* fed on melezitose, to 74.45 ± 2.52, obtained for *P. concolor* fed on the solid diet (positive control) and statistically significant differences were found between these two types of food (Table 1).

Table 1. Percentage of non-emerged hosts and parasitism rate (mean + SE) in the different treatments with single sugars and solid diet.

Food Source	Non-Emerged Hosts (%) *	Parasitism Rate (%) *
Solid diet	89.85 ± 2.25^{a}	74.45 ± 2.52^{a}
Sugars		
Monosaccharides		
Fructose	92.05 ± 1.61^{a}	63.34 ± 1.51^{ab}
Glucose	90.60 ± 1.57^{a}	62.99 ± 1.51^{ab}
Disaccharides		
Melibiose	83.30 ± 3.77^{a}	52.16 ± 3.35^{bc}
Sucrose	94.95 ± 1.22^{a}	64.60 ± 1.63^{ab}
Trehalose	85.10 ± 3.44^{a}	62.58 ± 2.59^{ab}
Trisaccharides		
Melezitose	48.90 ± 6.53^{b}	48.36 ± 6.51^{c}
Sugar alcohol		
Sorbitol	85.20 ± 2.10^{a}	62.42 ± 1.63^{ab}
Kruskal–Wallis chi-squared	57.404	42.496
Df	7	7
p-value	<0.001	<0.001

* Data followed by the same letter on the same column are not significantly different ($p \geq 0.05$).

3.2. Longevity of *P. concolor* Fed on Honeydew

The survival curves obtained by Kaplan–Meier estimates for females (A) and males (B) of *P. concolor* fed on different honeydew solutions are shown in Figure 4. The Cox proportional hazards model PHM showed that female and male death hazards were significantly different among diet treatments ($\chi 2 = 158$, df = 3, $p < 0.001$; $\chi 2 = 127$, df = 3, $p < 0.001$, respectively). Females fed on *E. olivina* reduced the risk of death by 5% when compared with honey ($p < 0.001$) (Figure 4A).

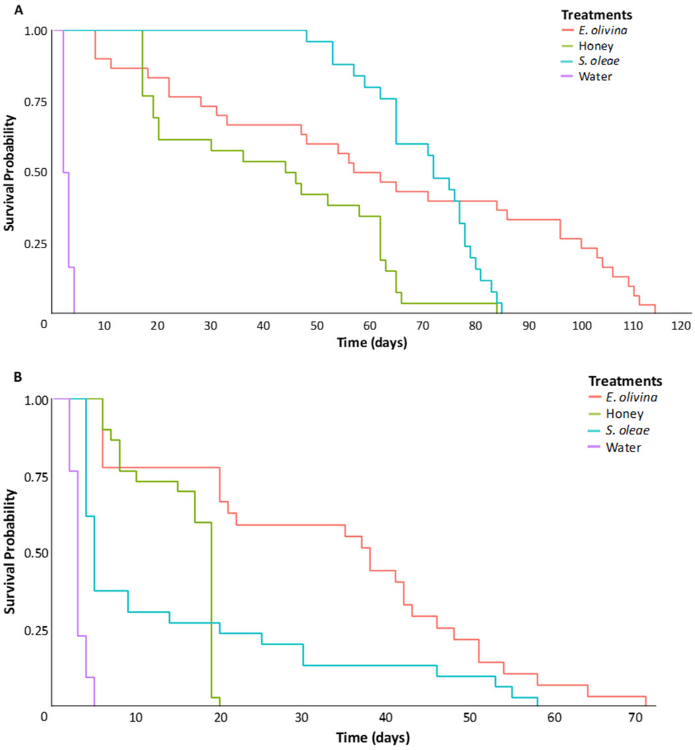

Figure 4. Survival curves obtained by Kaplan–Meier estimates for *Psyttalia concolor* females (**A**) and males (**B**) fed on different honeydews.

The mean longevity for *P. concolor* females fed on *S. oleae* honeydew was 70.40 ± 2.12 days and on *E. olivina* honeydew was 62.27 ± 6.77 days, reaching higher values than those fed on honey and water only (Figure 5A). For *P. concolor* males, the highest survival was observed on individuals fed on *E. olivina* (33.26 ± 3.78 days) (Figure 5B) and this food item reduced the risk of death by 65% when compared to females fed on water only ($p < 0.01$) (Figure 4B).

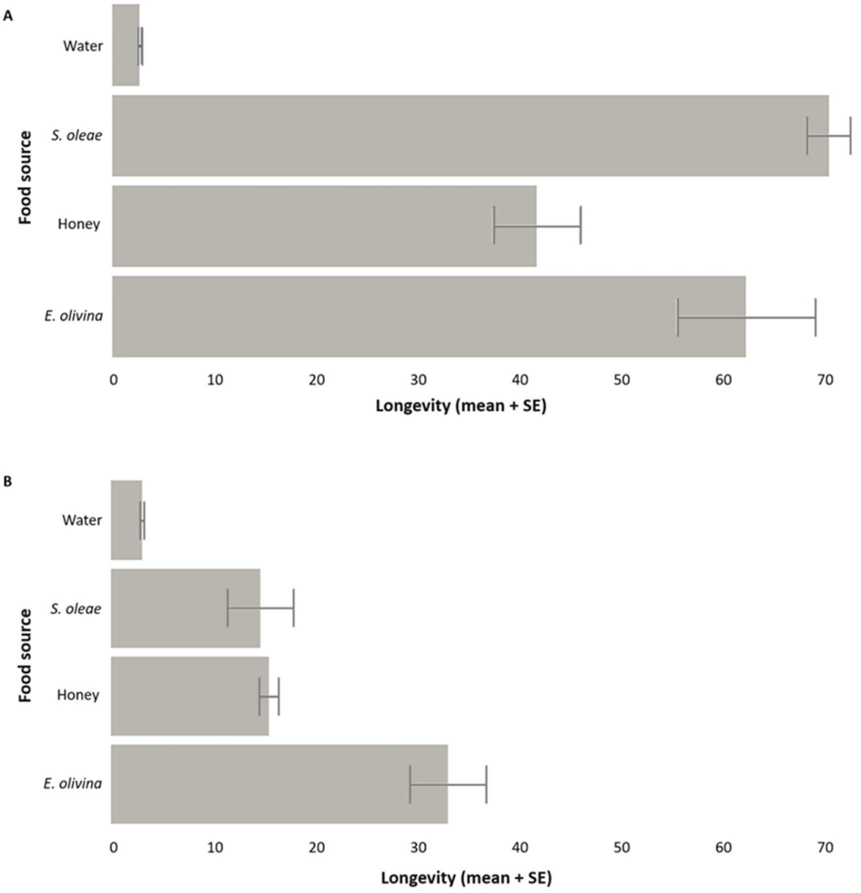

Figure 5. Longevity (mean + SE) (days) for *Psyttalia concolor* females (**A**) and males (**B**) fed on different honeydews.

4. Discussion

The sugar compounds and honeydew food sources tested in this work showed positive effects both on female and male lifespans; although, in general, the benefit for females was higher than for males when the same sugar was supplied. Similarly, Williams III et al. [31] found that females of *Psyttalia lounsburyi* (Silvestri) (Hymenoptera: Braconidae), another parasitoid of *B. oleae*, presented higher longevity than males. The higher longevity observed for females is likely due to higher nutritional requirements than males in order to provide energy for host forage and oviposition [32]. Females of *P. concolor* lived more when fed on sugars typically present in Mediterranean nectars (and only sporadically found in honeydew) [33], i.e., sorbitol and melibiose. In addition to its nutritional value, Burke and Waddell [34] showed that sorbitol is a sugar that provides memory persistence, an important ability to the reinforcement of appetitive memory, even though it is not considered a sweet sugar. Moreover, the accumulation of sorbitol in parasitoids is also associated with a tolerance to low temperatures [35], which benefits the presence of these insects in agroecosystems when winter inflicts severe cooling or freezing conditions. These results indicate that flowers with nectar containing sorbitol and melibiose, such as *Asparagus acutifolius* L. (Asparagaceae) and *Convolvulus arvensis* L. (Convolvulaceae), could be tested in future studies in order to evaluate their potential to promote parasitoid population maintenance in agroecosystems, following a conservation biocontrol strategy.

P. concolor females fed on sucrose showed lower longevity when compared to the other tested sugars and the positive control. In contrast, other studies evidenced the positive effect of sucrose-feeding in other braconids, such as *Cotesia glomerata* (L.) [13], *Chelonus elaeaphilus* Silvestri [9] and *P. lounsburyi* [31]. This finding showed that longevity responses to sugars vary among species, as well as their ability to digest and synthesize sugars [36–38].

In our study, only recently emerged wasps were tested but longevity can change in individuals with previous sexual interactions, as demonstrated by Benelli et al. [39]. These authors showed that female and male longevity was strongly reduced, compared with the virgin females, when male–female interactions were considerable. Thus, in future experiments, the mating status of wasps can be an additional factor to test.

In synovigenic species, such as *P. concolor*, the percentage of attacked hosts is an important factor for biological control efficiency. In our study, melezitose resulted in significantly lower percentages of non-emerged hosts and parasitism rates than the other sugars tested. This effect can be ascribed to the fact that melezitose is a sugar that quickly crystallizes, making the sugar uptake difficult for the parasitoid [40].

In general, the parasitism rate observed in *P. concolor* fed on sugars averaged 60–65%, which indicates a risk of egg limitation in the field by this braconid, as similarly observed in other synovigenic parasitoids [41]. The nutrients necessary for egg production are partially acquired by proteins and lipids, while carbohydrates enhance the lifespan fecundity of parasitoids [3]. It is important to consider that parasitoids will need other elementary nutrients such as vitamins, mineral salts, steroids, nucleic acids for normal development and reproduction.

Several sugars occurring in floral nectars and hemipteran honeydew, such as glucose, fructose, trehalose and sorbitol, gave similar results for male longevity. These results indicate that males of *P. concolor* appeared to be more generalist feeders than females, suggesting that hexoses-dominant nectars would benefit this gender. These sugars can be offered/preserved in agroecosystems through wild flowering plant with hexose-dominant nectars such as *Raphanus raphanistrum* (Brassicaceae) and *Malva neglecta* (Malvaceae) or hexose-rich nectars, such as *Daucus carota*, *Conopodium majus* and *Foeniculum vulgare* (Apiaceae) and *A. acutifolius* and *Anarrhinum bellidifolium* (Scrophulariaceae) [9]. Plants should benefit the natural enemy but not pests or serve as hosts of pathogens such as *Xylella fastidiosa*.

The longevity of males fed on melibiose reached an average of 10 days, while on glucose and fructose, the longevity was more than doubled. This is consistent with a study on *C. glomerata* dealing with a quantification of food intake, which suggested that, contrarily to melibiose, glucose and fructose were immediately converted into energy, fulfilling the nutritional needs for that parasitoid [42].

Notably, research conducted to assess the parasitoid longevity when feeding on honeydew has inconsistent results since some studies indicated that honeydew did not increase parasitoid survival, while others showed that honeydew effectively increased it. There is a high variation of types of honeydew showing different carbohydrate contents, which provides different longevities on parasitoids [43]. Moreover, a lower longevity may be attributed to the difficulty in feeding due to high viscosity and to the tendency of some honeydew sugars to rapidly crystallize [44]. In our study, honeydew enhanced the survival of females and males of *P. concolor*, presenting higher values than single sugars. Honeydew from *S. oleae* and *E. olivina* was a good food source for females, presenting higher longevity than honey solution (positive control). However, for males, *E. olivina* exhibited the best longevity performance, with *S. oleae* and honey showing similar results. In contrast, Villa et al. [45] found that *E. olivina* and *S. oleae* honeydew reduced the death hazard of *Elasmus flabellatus* (Fonscolombe) (Hymenoptera: Eulophidae) females comparatively to the other tested flowers but not to honey solution (positive control). Results obtained by Wang et al. [46] indicated that *S. oleae* honeydew also increased the longevity of parasitoids *Psyttalia humilis* (Silvestri) (Hymenoptera: Braconidae) and *Scutellista caerulea* (Fonsc.) (Hymenoptera: Pteromalidae) with a single meal.

In the field, when flowers produce little, if any, nectar, *E. olivina* and *S. oleae* honeydew can be the primary exogenous carbohydrate source available [1]. However, these food sources are usually present for a few months and often at low densities [31]. On the other hand, one of the advantages of these food sources is related to the plant community inhabiting the agroecosystem as a whole, since honeydew producers may be associated to the crop itself, the weeds close to this culture or even the surrounding vegetation [1]. Thus, the presence of honeydew when nectar is rare extends the survival of *P. concolor* (even as a single meal) and potentially enhances the parasitism of the *B. oleae* population.

In a conservation biological control program, a manipulation technic used as an alternative to flowering plant management involves the application of solutions rich in carbohydrates and/or proteins to crops, to act as artificial food supplements, also known as artificial food sprays or artificial honeydews [14,47,48]. This strategy acquires primary importance in crops where vegetation has been removed through farming practices such as tillage and herbicide application, leading to a reduction in floral resources [49], or when a low density of plants during the Mediterranean summer months can eventually provide limited sugar resources (due to low quantity and quality of resources and detectability by the insects) [50–52].

It is worth noticing that experiments with caged individuals are not necessarily representative of the basic needs of insects in the field, but they can be used as a preliminary indication of the importance of certain food sources for the lifespan of parasitoids [7]. Under field conditions, the nutritional requirements of *P. concolor* are likely higher than in the laboratory due to their foraging behavior and parasitizing hosts. Our findings suggest that a nutritious source of carbohydrates (sugar or honeydew) is important for *P. concolor* longevity and reproduction, especially for females. Thus, it is important to supply food through selected flowering plants or artificial food devices under field conditions. Considering the benefits of sugar sources to parasitoids, it is important to not only develop an effective field delivery system, such as feeding containers, smears on wooden stakes, and foliage sprays, but also to dedicate oneself to the formulation of methodologies that ensure the conservation and availability of these sugars in the field for a longer period of time.

Author Contributions: L.A.P. performed the laboratory experiments and analyzed the data, S.A.P.S., J.A.P. and P.M. conceived and designed the experiment and funding acquisition, P.M. provided assistance during the laboratory experiments and all the authors contributed to writing the paper. All authors have read and agreed to the published version of the manuscript.

Funding: The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support by national funds FCT/MCTES to CIMO (UIDB/00690/2020 and UIDP/00690/2020) to the Associate Laboratory SusTEC (LA/P/0007/2020), and to the project OLIVESIM—Managing ecosystem services in olive groves using advanced landscape agent-based models PTDC/ASP-PLA/30003/2017.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank Luis Quirós (ETSIAAB-UPM) for helping to maintain and rear both *Psyttalia concolor* and their host, *Ceratitis capitata*.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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