DOI: 10.1111/aab.12959

ORIGINAL ARTICLE

Revised: 23 September 2024

WILEY

Nectar-inhabiting bacteria differently affect the longevity of co-occurring egg parasitoid species by modifying nectar chemistry

Evgenia Sarakatsani ¹	💿 📔 Jay Darryl L. E	rmio ^{1,2} 💿 Shahinoo	r Rahman ³ 💿 🛛
Patrizia Bella ¹ 💿 🛛	Alfonso Agrò ¹ 💿 🛛 🛛	Mirella Lo Pinto ¹ 💿 🕴	Ezio Peri ¹ 💿
Stefano Colazza ¹ 💿	Bart Lievens ⁴ 💿	Michael Rostás ³ 💿	Antonino Cusumano ¹ 💿

¹Department of Agricultural, Food and Forest Sciences, University of Palermo, Palermo, Italy ²Department of Pest Management, Visayas State University, Baybay City, Philippines

³Division of Agricultural Entomology, University of Göttingen, Göttingen, Germany

⁴Department of Microbial and Molecular Systems, KU Leuven, Leuven, Belgium

Correspondence

Ezio Peri, Department of Agricultural, Food and Forest Sciences, University of Palermo, 90128 Palermo, Italy. Email: ezio.peri@unipa.it

Funding information

Ministero dell'Università e della Ricerca; PRIN project 2020, Grant/Award Number: Prot. 2020T58TA3

Abstract

Flowering plants can be introduced in modern agroecosystems to support resident natural enemies in the context of Conservation Biological Control (CBC). Buckwheat (Fagopyrum esculentum) (Polygonales: Polygonaceae) has been shown to enhance the longevity of several parasitoids through the provision of high quality and easily accessible floral nectar. Yet floral nectar is ubiquitously colonized by microbes which can change nectar chemistry with consequences for parasitoids. Nonetheless, how bacteria associated with buckwheat floral nectar affect parasitoid performance is not known. In this study, adult females of Trissolcus basalis (Hymenoptera: Scelionidae) and Ooencyrtus telenomicida (Hymenoptera: Encyrtidae), two parasitoids of Nezara viridula (Hemiptera: Pentatomidae), were provided with synthetic nectar fermented by 14 bacterial isolates originating from buckwheat nectar. We recorded the effect of bacterial fermentation on female longevity and nectar chemistry. In the case of T. basalis, females consuming nectar fermented by Bacillus sp., Brevibacillus sp., Brevibacterium frigoritolerans, Saccharibacillus endophyticus, and Terribacillus saccharophilus significantly enhanced their longevity compared with females fed with non-fermented nectar. For O. telenomicida, enhanced longevity was recorded only in the case of B. frigoritolerans and Pantoea dispersa. For both parasitoids, no negative effects due to bacterial fermentation of nectar were recorded. Chemical investigations of bacteria-fermented nectars revealed an increased diversity in the composition of sugars and sugar alcohols, whereas non-fermented nectar only contained sucrose. Our findings show that nectarinhabiting bacteria are important "hidden players" in the interactions between flowers and parasitoids, an indication that a better understanding of plant-microbe-insect interactions could improve CBC programmes.

KEYWORDS

Bacillaceae, floral nectar bacteria, Nezara viridula, Ooencyrtus telenomicida, Paenibacillaceae, Trissolcus basalis

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Author(s). Annals of Applied Biology published by John Wiley & Sons Ltd on behalf of Association of Applied Biologists.

1

1 | INTRODUCTION

Due to agricultural intensification, modern agricultural landscapes are generally characterized by a paucity of plant species diversity often resulting in the reduction of ecosystem services to agriculture, such as pollination and natural pest control (Cadotte et al., 2011; Wood et al., 2015). To support resident beneficial arthropods, Conservation Biological Control (CBC) offers a suite of agronomic practices such as the diversification of plant species within agroecosystems, aimed to preserve and improve the efficacy of these beneficial organisms (Hunt et al., 2021; Mele et al., 2022; Pollier et al., 2019; van Rijn & Wäckers, 2010; van Rijn & Wäckers, 2016). In fact, resident populations of predators and parasitoids that attack economically important insect pests often require additional resources to maintain their populations within and around agricultural fields. One common strategy of CBC is the establishment of flowering plants, which provide shelter (Griffiths et al., 2008; Wyss, 1995) and food resources such as pollen, floral and extrafloral nectar (Lu et al., 2014: Wang et al., 2022) and plant guttation (Urbaneja-Bernat et al., 2020) to a wide range of predators and parasitoids. Interestingly, nectar and pollen have recently been identified as the main drivers of CBC success (Gurr et al., 2024). Nectar in particular is a high-quality and easily accessible sugar-rich solution indispensable for covering the energetic and nutritional requirements of natural enemies such as adult parasitoids (Russell, 2015).

Although floral nectar is initially sterile, it rapidly becomes colonized by microorganisms, which are dispersed by flower-visiting insects, air or rain (Colazza et al., 2023; Lenaerts et al., 2016). Commonly, nectar is inhabited by a limited number of phylogenetically related species (Herrera et al., 2010; Pozo et al., 2015), including ascomycetous yeasts such as *Metschnikowia*, as well as bacteria from the phyla Actinomycetota, Bacillota, and Pseudomonadota (Álvarez-Pérez et al., 2012; Pozo et al., 2015). Microbial fermentation can strongly affect the chemical properties of nectar, such as the composition and concentration of sugars and amino acids (Herrera et al., 2008; Lenaerts et al., 2017), as well as the pH (Lenaerts et al., 2017; Vannette et al., 2013). At the same time, microbes can change the concentrations of secondary metabolites (Vannette et al., 2013; Vannette & Fukami, 2016), hydrogen peroxide (Martin et al., 2022) and ethanol (Ehlers & Olesen, 1997; Rering et al., 2018).

These microbe-induced changes in nectar chemical properties can alter its nutritional value, and in turn modify the nectar reward of a given nectariferous flowering plant to foraging parasitoids. Little is known about the effects of nectar-associated microbes on parasitoids, but it is becoming increasingly evident that microbes can impact the performance of insect parasitoids (Cusumano & Lievens, 2023). Consequently, the role of microbes as "hidden players" in the interactions between flowering plants and parasitoids should not be ignored when implementing CBC programmes.

A few species among the vast majority of flowering plant taxa have dominated the literature with respect to their effects on parasitoid performance (Russell, 2015). One important flowering resource widely used in CBC is buckwheat *Fagopyrum esculentum* Moench due to its demonstrated benefits to parasitoids (Araj & Wratten, 2015; Foti et al., 2017, 2019; McIntosh et al., 2020; Rahat et al., 2005). In the floral nectar of buckwheat, several bacteria have been found that can affect nectar odours and, consequently, enhance the attractiveness of the nectar to parasitoid species such as *Trissolcus basalis* (Wollaston) (Cusumano et al., 2023). This species is an egg parasitoid that has been successfully used for biological control of the southern green stink bug, *Nezara viridula* L. (Caltagirone, 1981; Conti et al., 2021), a serious polyphagous pest of many annual crops. The eggs of this stink bug species can also be parasitized by other egg parasitoids, such as *Ooencyrtus telenomicida* (Vassiliev), which co-occurs with *T. basalis* in Sicilian agroecosystems (Peri et al., 2011).

These two parasitoid species have distinct nutritional requirements given that *O. telenomicida* performs host-feeding while *T. basalis* does not (Cusumano et al., 2012, 2022); thus, it is anticipated that nectar provisioning will exert varying effects on their lifehistory traits, such as longevity (Jervis et al., 2008). Parasitoid efficacy can often be limited by adult female longevity and it is known that extending the lifespan of adult parasitoids not only affects the timing and frequency of encounters between males and females, but also increases the probability of encountering appropriate host stages (Rosenheim, 2011). Lifespan extension is particularly important for egg parasitoids since host eggs are often inconspicuous and suitable for parasitism for a limited amount of time (Conti & Colazza, 2012; Vinson, 1998).

Although buckwheat has been widely demonstrated to increase parasitoid longevity, the effect of bacteria associated with buckwheat on nectar chemistry and parasitoid performance remains unexplored. To this end, synthetic nectar solutions were prepared and then undergone fermentation, by nectar-inhabiting bacteria isolated from buckwheat flowers. Subsequently, we conducted bioassays and chemical investigations with the aim to shed light on the effects of bacterial fermentation of the nectar on the performance of *T. basalis* and *O. telenomicida*. In particular, a no-choice feeding bioassay was carried out to examine the microbe-mediated effects on the longevity of adult parasitoid species. Also, a chemical analysis was performed to investigate changes in sugar/sugar-alcohols resulting from microbial metabolic activity in the attempt to link parasitoid performance with nectar chemistry.

2 | MATERIALS AND METHODS

2.1 | Parasitoid rearing

The colonies of *T. basalis* and *O. telenomicida* originated from parasitized sentinel *N. viridula* egg masses that had previously been placed in tomato fields in Palermo, Italy, where infestations of *N. viridula* had been recorded. Both parasitoids were kept in 16-mL glass tubes (70– 100 wasps/tube) in a climate chamber ($24 \pm 2^{\circ}$ C, $80 \pm 5\%$ RH, 16 h:8 h L:D) of the Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo. The colonies were provided with 50/50% honey-water solutions and, for their reproduction, mated females were exposed to *N. viridula* eggs, obtained from the stink bug rearing in the department. Parasitoid females provided with only water were used in the experiments when they were about 24 h old.

2.2 | Synthetic nectar solutions

In this study, the following bacteria were used which were originally isolated from buckwheat nectar by Cusumano et al. (2023): phylum Bacillota - Bacillus sp. (Bacillus sp1) SAAF 22.2.6, Bacillus sp. (Bacillus sp2) SAAF 22.2.27, Brevibacillus sp. SAAF 22.4.13, Brevibacterium frigoritolerans SAAF 22.2.4, Saccharibacillus sp. SAAF 22.4.25, Staphylococcus epidermidis SAAF 22.3.11 and Staphylococcus hominis SAAF 22.3.10. Terribacillus saccharophilus SAAF 22.2.3: phylum Pseudomonadota-Pantoea agglomerans SAAF 22.4.2, Pantoea dispersa SAAF 22.3.3, Pantoea sp. (Pantoea sp1) SAAF 22.4.5, Pantoea sp. (Pantoea sp2) SAAF 22.4.17; phylum Actinomycetota-Cellulosimicrobium sp. SAAF 22.3.25, Curtobacterium sp. SAAF 22.4.18. The bacterial isolates were plated on trypticase soy agar (TSA) (Oxoid; Basingstoke, UK) and incubated for 2-3 days at 25°C. Then, 10 mL aliquots of trypticase soy broth (TSB) (Oxoid; Basingstoke, UK) were inoculated with a single bacterial colony from the TSA plates and kept on a rotary shaker of 150 rpm at 25°C for 1 day.

We prepared the synthetic nectar by mixing a filter-sterilized sucrose solution at a concentration of 50% w/v (Carlo Erba Reagents, Val-de-Reuil, France) with 3.16 mM casamino acid (OmniPur, Merck KGaA, Darmstadt, Germany), as described by Vannette and Fukami (2014). Next, the synthetic nectar was inoculated with individual bacterial isolates following the protocol described by Lenaerts et al. (2017).

To inoculate the nectar, 2 ml from the TSB cultures were centrifuged at 10,000 rpm for 5 min. Next, bacterial pellets were washed with sterile saline solution (0.9% NaCl) and resuspended in sterile saline solution to adjust the cell concentration to an optical density of 1 (OD 600) (about 10⁸ cfu/ml). From the bacterial suspensions obtained, 100 µl was used to inoculate 10 ml of synthetic nectar in 20 ml Falcon tubes. In addition, synthetic nectar which was not inoculated with any bacterial isolate was used as control. Nectar fermentation took place for 5 days, during which both inoculated and non-inoculated synthetic nectar were incubated in static conditions at 25°C. The 5-day incubation period was selected since it represents the anthesis duration for several nectariferous flowering species (Lenaerts et al., 2016; Peay et al., 2012). At that time, bacterial densities ranged between 1×10^4 and $5.6\times 10^8\,cfu/ml$ which is comparable with those observed in the field for other plant species (Fridman et al., 2012). After the fermentation, all nectar solutions were filtered (pore size 0.2 µm, Exacta + Optech Labcenter SpA, Italy) to prepare cell-free solutions. The absence of microbial contamination was confirmed by plating a subsample of each nectar solution on TSA. Lastly, aliquots of the nectar were prepared in glass amber vials of 2 ml and subsequently stored at -80°C (Peay et al., 2012) until use for bioassays and chemical analyses.

2.3 | Bacterial effects on parasitoid longevity

To assess the effect of bacterial fermentation on parasitoid longevity, a survival bioassay was carried out following the methodology described by Ermio et al. (2024). Briefly, females of T. basalis and O. telenomicida were isolated individually in a glass vial (volume 5 ml) which was closed with a cotton lid. At the side of the glass vial, for each treatment, a droplet of the test nectar was ad libitum deposited on a piece of parafilm[©] and replaced every 2 days using a sterile pin. All vials were kept in a climate chamber at 24 ± 2°C, 80 ± 5% RH, 16 h:8 h L:D. Parasitoids were inspected daily to record the total number of days each female stayed alive (longevity days). For both T. basalis and O. telenomicida, 15 adult females were used per each treatment, and 15 treatments (i.e., 14 bacterial isolates and the nonfermented synthetic nectar as control) were carried out, leading to a total of 225 tested females for each parasitoid species. Natural nectar was not included in the treatments mainly because our aim was to investigate the effects of individual bacterial isolates on nectar chemistry and parasitoid performance whereas natural nectar samples likely harbour microbial communities-which may consist of mixtures of bacteria and fungi-and which may differ in space, climatic conditions and time (Russell & McFrederick, 2022).

2.4 | Bacterial effects on nectar chemistry

To carry out sugar analyses, 200 µl of nectar samples from both nonfermented and fermented treatments, were aliquoted into 1.5 ml Eppendorf tubes, lyophilized, and subsequently weighed. For methoxyamination. 10 µl of methoxyamination reagent (prepared by dissolving 20 mg/ml of methoxyamine hydrochloride in pure pyridine) was added per milligram of nectar sample. The mixtures were vortexed until fully dissolved, subjected to 3 min of sonication, and then placed on a shaker for 90 min. For internal standardization, 20 µl of adonitol (20 ng/µl in methanol) was added to gas chromatography screw-capped glass vials containing a glass insert (200 µl). The internal standard was dried using a speed vac (RVC 2-25 CD plus, Germany) at 30°C for 300 min. Subsequently, an aliquot of 20 µl of the sample with methoxyamination reagent was transferred to a glass vial containing adonitol. Finally, 20 µl of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA) was added and the mixture was vortexed for 10 s. Samples were analysed using a gas chromatograph-mass spectrometer (Agilent Technologies, GC: 7890B, MSD: 5977B) fitted with a Restek Rtx-5 w/Integra-Guard column (30 m \times 0.25 mm ID \times 0.25 μ m). An autosampler (Agilent Technologies, PAL RSI 85) was used to inject 1 µl of the sample with a split ratio of 0.1:1. The inlet temperature was 205°C and the pressure was 17.2 psi. The oven temperature was held at 70°C for 2 min and then increased gradually to a final temperature of 325°C, held for 10 min. Helium (1 ml min⁻¹) was used as the carrier gas. Due to the very high concentration of sucrose in the samples, the mass spectrometer was switched off at the retention time where sucrose appeared to avoid damage to the instrument. n-Alkane standards (C8-C40) were analysed for determining retention indices. Metabolite derivatives (sugars and

license

sugar alcohols) were identified by comparing their mass spectra and retention indices with data bank entries in the Golm Metabolome Database (GMD), and Fein BinBase database with the help of MS-DIAL (ver. 4.8) software. Metabolites were quantified relative to the peak area of the internal standard. Furthermore, the pH in the different nectars was determined with a pH electrode (WTW Inolab, Weilheim, Germany).

2.5 Statistical analysis

Longevity data were analysed using Generalized Linear Models (GLMs), fitting a gamma error distribution and a reciprocal link function (Crawley, 2007). For each parasitoid species we fitted two different GLMs: in a first GLM we tested whether parasitoid longevity was significantly different when wasps were fed with nectars fermented by the three different phyla of bacterial isolates. To do so, we pooled together the results of all isolates within a given phylum (eight Bacillota isolates, four Pseudomonadota isolates and two .Actinomycetota isolates). We hypothesized that isolates belonging to the same phylum would elicit similar effects in terms of parasitoid longevity given the phylogenetic relatedness of the strains. Thus, we expected to observe differences among the three phyla (Bacillota, Pseudomonadota and Actinomycetota). In a second GLM, we tested whether parasitoid longevity was significantly different between the 14 fermented nectars and the control, non-fermented synthetic nectar. For both GLMs, Fisher's LSD test was used for post hoc comparisons of means. Significance of the fixed terms in the model was determined directly with Ftests (Crawley, 2007). Model fit was assessed with diagnostic plots. All statistical analyses of longevity data were performed with R software version R 4.1.3 (R Core Team, 2022).

Projection to latent structures discriminant analysis (PLS-DA) was used to analyse peak areas of the sugar/sugar-alcohols detected after bacterial fermentation of the nectar media. In these analyses we excluded the control as it only contained sucrose. We carried out three separated PLS-DA, one where we compared all Bacillota isolates, one for the Pseudomonadota isolates and one for the Actinomycetota isolates. Peak areas were first log-transformed, mean-centred and subsequently scaled to unit variance before they were subjected to the analysis using MetaboAnalyst (Xia et al., 2009). The results of the analysis were visualized in score plots, which reveal the sample structure according to model components, and loading plots, which display the contribution of the variables to these components. The variable importance in the projection (VIP values), which indicates the relative ranking of the compounds contributing the most in explaining statistical differences in the PLS-DA, was also calculated (Wold, 2001).

3 RESULTS

3.1 Bacterial effects on parasitoid longevity

In the case of T. basalis, the effect of nectar fermentation among the three phyla (Bacillota, Pseudomonadota and Actinomycetota)

was statistically significant (GLM: F = 17.634, df = 2, p <.001) (Figure 1a). Post-hoc comparisons revealed that females provided with nectar fermented by Bacillota and Actinomycetota lived significantly longer than those provided with nectar fermented by Pseudomonadota (p < .001), whereas no significant difference was found between Bacillota and Actinomycetota (Figure 1a). The effect of individual isolates on the longevity of the parasitoids was statistically significant (GLM: F = 3.515, df = 14, p < .001) (Figures 1b and S1). When compared with the control nectar, differences were observed (p < .05), with positive effects on the longevity of the parasitoids for the following isolates: Bacillus sp. SAAF 22.2.6 increased the longevity by 42.30%, Brevibacillus sp. SAAF 22.4.13 by 39.61%, B. frigoritolerans SAAF 22.2.4 by 37.5%, S. endophyticus SAAF 22.4.25 by 42.08% and T. saccharophilus SAAF 22.2.3 by 40.17%. Regardless of the bacteria species involved, longevity of T. basalis was never significantly decreased compared with T. basalis fed with control nectar

In the case of O. telenomicida, there was no statistically significant effect of nectar fermentation among the three phyla (GLM: F = 0.1735, df = 2, p = .8408) (Figure 2a). However, the effect of individual isolates on the longevity of females was statistically significant (GLM: F = 4.554, df = 14, p <.001). When compared with the control nectar, differences were observed (p < .05) for B. frigoritolerans SAAF 22.2.4 and P. dispersa SAAF 22.3.3 (Figures 2b and S2), which increased female longevity by 26.88% and 24.88%, respectively. As for T. basalis, longevity of O. telenomicida was never significantly decreased by any of the bacteria species involved, when compared with females fed with control nectar.

3.2 Bacterial effects on nectar chemistry

Analysis of sugars and sugar alcohols revealed that bacterial fermentation resulted in the de novo production of 14 metabolites compared with the non-fermented synthetic nectar, which consisted only of sucrose. Overall, all these compounds were detected in all nectars inoculated with the bacterial isolates, but in different proportions (Table 1). A comparison by PLS-DA among the eight isolates of bacteria within the Bacillota phylum resulted in a significant model (permutation test, p < .001) (Figure 3a). The first five compounds, ranked in descending order of VIP values, are: erythrose, sophorose, galactinol, fructose and 1-kestose. Within the Pseudomonadota phylum, a comparison by PLS-DA among the four bacterial isolates also resulted in a significant model (permutation test, p < .001) and the following compounds have the highest VIP values: fructose, tagatose, L-iditol, glucose and galactose (Figure 3b). Within the Actinomycetota phylum, a comparison by PLS-DA among the two isolates of bacteria resulted in a significant model (permutation test, p < .001) where the following five compounds have the highest VIP values: tagatose, raffinose, trehalose, galactose and fructose (Figure 3c). The pH of the bacteriafermented nectar ranged from 5.21 (±0.03) in the case of Bacillus sp2 SAAF 22.2.27 to 6.62 (±0.03) in the case of Brevibacillus sp. SAAF 22.4.13 (Table 1).



FIGURE 1 Boxplot illustrating the distribution of longevity (days) for the adult female egg parasitoid *Trissolcus basalis* fed on synthetic nectar fermented by the different bacterial isolates. The synthetic nectar was fermented by: for Bacillota–*Bacillus* sp1 SAAF 22.2.6, *Bacillus* sp2 SAAF 22.2.27, *Brevibacillus* sp. SAAF 22.4.13, *Brevibacterium frigoritolerans* SAAF 22.2.4, *Saccharibacillus endophyticus* SAAF 22.4.25, *Staphylococcus epidermidis* SAAF 22.3.11, *Staphylococcus hominis* SAAF 22.3.10, and *Terribacillus saccharophilus* SAAF 22.2.3; for Pseudomonadota–*Pantoea agglomerans* SAAF 22.4.2, *Pantoea dispersa* SAAF 22.3.3, *Pantoea* sp1 SAAF 22.4.5, and *Pantoea* sp2 SAAF 22.4.17; for Actinomycetota– *Cellulosimicrobium* sp. SAAF 22.3.25 and *Curtobacterium* sp. SAAF 22.4.18. (a) Comparison among the 3 phyla (different letters indicate significant differences, GLM, $p \le .05$). (b) Comparison among the 14 bacterial isolates and the non-fermented synthetic nectar (asterisks indicate significant differences, GLM, $p \le .05$). Pink = Bacillota, Olive green = Pseudomonadota, Green = Actinomycetota; Blue = Non-fermented synthetic nectar. All biological replicates (n = 15) indicate cell-free nectars.

4 | DISCUSSION

This study demonstrates that bacterial fermentation of nectar affects the longevity of two egg parasitoids of stink bugs. To our knowledge, this is the first study that assessed how parasitoid longevity can be affected by nectar-inhabiting bacteria in buckwheat, a flowering plant which is widely used in Conservation Biological Control (CBC) programmes.

We found that the longevity of *T. basalis* females feeding on certain fermented nectars was significantly prolonged by approximately 40% compared to non-fermented synthetic nectar. In particular, nectar fermented by the bacterial isolates *Bacillus* sp. SAAF 22.2.6, *Brevibacillus* sp. SAAF 22.4.13, *B. frigoritolerans* SAAF 22.2.4, *S. endophyticus* SAAF 22.4.25 and *T. saccharophilus* SAAF 22.2.3 had positive effects. This observed longevity is also higher compared to the mean longevity of females exposed to excised inflorescences of buckwheat (Rahat et al., 2005). Furthermore, we did not record any negative effect due to fermentation by nectar-inhabiting bacteria on the longevity of *T. basalis*. It is noteworthy that all bacteria that enhanced longevity of *T. basalis* belong to the phylum Bacillota, suggesting a specific effect elicited by these bacterial isolates on the positive fitness-related effects of *T. basalis*.

Positive effects due to bacterial fermentation of the nectar media were also found for *O. telenomicida* females, although enhanced longevity was recorded only in the case of *B. frigoritolerans* SAAF 22.2.4 and *P. dispersa* SAAF 22.3.3—by 26.88% and 24.88% respectively—when compared with non-fermented synthetic nectar. These isolates belong to different phyla (Bacillota and Pseudomonadota, respectively), indicating that non-related bacteria can enhance longevity in this parasitoid species.

The egg parasitoid species studied in this study have distinct nutritional requirements given that *O. telenomicida* performs host-feeding while *T. basalis* does not; thus, it is not surprising to observe divergent effects from different fermented nectars, although the fermentation by *B. frigoritolerans* SAAF 22.2.4 enhanced longevity in both stink bug egg parasitoids. To the best of our knowledge, the effects of nectar-inhabiting bacteria on parasitoids have only been studied in the aphid parasitoid *Aphidius ervi* (Haliday) where positive, negative or neutral effects have been reported (Lenaerts et al., 2017). Fermentation by *Lactococcus* sp. increased the longevity of A. *ervi*, whereas *Asaia* had a detrimental effect on the longevity of the aphid parasitoid (Lenaerts et al., 2017). The negative effects of the latter bacterium, which belongs to the group of acetic acid bacteria, could

5

6



FIGURE 2 Boxplot illustrating the distribution of longevity (days) for the adult female egg parasitoid *Ooencyrtus telenomicida* fed on synthetic nectar fermented by the different bacterial isolates. The synthetic nectar was fermented by: for Bacillota–*Bacillus* sp1 SAAF 22.2.6, *Bacillus* sp2 SAAF 22.2.7, *Brevibacillus* sp. SAAF 22.4.13, *Brevibacterium frigoritolerans* SAAF 22.2.4, *Saccharibacillus endophyticus* SAAF 22.4.25, *Staphylococcus epidermidis* SAAF 22.3.11, *Staphylococcus hominis* SAAF 22.3.10, and *Terribacillus saccharophilus* SAAF 22.2.3; for Pseudomonadota–*Pantoea agglomerans* SAAF 22.4.2, *Pantoea dispersa* SAAF 22.3.3, *Pantoea* sp1 SAAF 22.4.5, and *Pantoea* sp2 SAAF 22.4.17; for Actinomycetota–*Cellulosimicrobium* sp. SAAF 22.3.25 and *Curtobacterium* sp. SAAF 22.4.18. (a) Comparison among the 3 phyla (different letters indicate significant differences, GLM, $p \le .05$). (b) Comparison among the 14 bacterial isolates and the non-fermented synthetic nectar (asterisks indicate significant differences, GLM, $p \le .05$). Pink = Bacillota, Olive green = Pseudomonadota, Green = Actinomycetota; Blue = Non-fermented synthetic nectar. Each treatment comprised of 15 replicates.

be due to the dramatic decrease in the pH which reduced the quality of the nectar for the parasitoid. In contrast, bacteria belonging to Bacillota are not known to cause such drastic reductions of pH in the fermented media (Schleifer, 2009), and our results confirm this finding. Other nectar-inhabiting microbes, such as yeasts, have been reported to affect attraction and performance of parasitoids, such as the aphid parasitoid *A. ervi* (Sobhy et al., 2018) and the egg parasitoids *T. basalis* and *O. telenomicida* (Ermio et al., 2024).

From a chemical perspective, we found that bacterial fermentation greatly influenced the sugar profile of the nectar. The nonfermented synthetic nectar contained only sucrose, whereas a total of 14 sugars and sugar alcohols were identified in the fermented products of all bacteria, although in different proportions. While previous studies focusing on nectar fermentation by microbes have mainly examined the dynamics between sucrose, glucose, and fructose (Lenaerts et al., 2017; Sobhy et al., 2018), we show here that bacterial fermentation of nectar results in a huge variety of sugars and sugar alcohols, providing a more complete picture of the effects of bacterial fermentation from a chemical perspective. It is reasonable to assume that the positive effects of the six bacterial isolates (five Bacillota and one Pseudomonadota) on the longevity of *T. basalis* and *O. telenomicida* are due to the diversity and abundance of these sugars and sugar alcohols, which may complement the nutritional needs of the parasitoids. Nevertheless, only a single strain of each bacterial species was tested in this study, so the effect of intra-specific microbial diversity on parasitoids' longevity should be explored in future studies. Also, the role of bacterial cells themselves on parasitoid performance should be investigated given that nectar, in nature, is ingested together with microbial cells (Cusumano & Lievens, 2023). In fact, microorganisms known to inhabit floral nectar, such as *Metschnikowia* yeasts, have been identified in internal organs of insect parasitoids under field conditions (Srinatha et al., 2015).

According to our PLS-DA model comparing the nectars fermented by Bacillota, the compounds most likely to correlate with parasitoid performance (based on the highest VIP values) were erythrose, sophorose and galactinol, fructose and 1-kestose. In addition, a PLS-DA model among the four nectars fermented by Pseudomonadota showed that the highest VIP values were found for fructose, tagatose, L-iditol, glucose and galactose. Hexoses (glucose and fructose) are usually encountered in high concentrations in the floral nectar of exposed nectaries (McIntosh et al., 2020). For instance, fructose accounts for 50% of the total sugars in buckwheat nectar (Lee & Heimpel, 2003). Although parasitoid species may vary in their ability to utilize different nectar sugars, glucose and fructose are known to

Automatory in the part of the	Phylum														
Herandandandandandandandandandandand13.6 (-104) 37.($+0.71$)53.0($+1.06)$ 52.3($+1.06)$ 52.3($+1.06)$ 23.6($+5.71$)36.6($+5.71$)55.0($+2.76$)50.5($+1.26)$ 50.5($+$	Bacillota									Pseudomonado	ota			Actinomycetota	
13 13<	Ba.sp1 Ba.sp2 Br.sp	Ba.sp2 Br.sp	Br.sp		Br.fr	Sa.en	St.ep	St.ho	Te.sa	Pa.ag	Pa.di	Pa.sp1	Pa.sp2	Ce.sp	Cu.sp
25064549 6.34 (±1.15) 4.57 (±1.85) 1.018 (±2.83) 1.50 (±0.03) 2.64 (±0.74) 3.61 (±1.87) 6.31 (±1.87) <td>0.28 (±0.17) 1.11 (±0.95) 2.53 (±0</td> <td>1.11 (±0.95) 2.53 (±0</td> <td>2.53 (±0</td> <td>(99</td> <td>1.36 (±0.44)</td> <td>17.61 (±1.85)</td> <td>3.37 (±0.71)</td> <td>15.20 (±4.06)</td> <td>6.21 (±2.65)</td> <td>18.23 (±1.05)</td> <td>22.38 (±6.54)</td> <td>7.90 (±2.51)</td> <td>34.68 (±6.37)</td> <td>5.50 (±2.49)</td> <td>5.05 (±2.19)</td>	0.28 (±0.17) 1.11 (±0.95) 2.53 (±0	1.11 (±0.95) 2.53 (±0	2.53 (±0	(99	1.36 (±0.44)	17.61 (±1.85)	3.37 (±0.71)	15.20 (±4.06)	6.21 (±2.65)	18.23 (±1.05)	22.38 (±6.54)	7.90 (±2.51)	34.68 (±6.37)	5.50 (±2.49)	5.05 (±2.19)
95 (±2.6) 205 (±0.3) 205 (±0.3) 3279 (±10.2) 427 (±13.2) 239 (±13.2) 14432 (±13.2) 14432 (±13.2) 14432 (±13.2) 14432 (±13.2) 14432 (±13.2) 14432 (±13.2) 14432 (±13.2) 14432 (±13.2) 14432 (±13.2) 14432 (±13.2) 14441 (±12.2)	2.44 (±1.24) 40.57 (±7.85) 5.17 (±2.5	40.57 (±7.85) 5.17 (±2.5	5.17 (±2.5	6	22.50 (±5.49)	6.34 (±1.15)	4.57 (±1.85)	10.18 (±2.83)	1.50 (±0.40)	2.46 (±0.74)	2.10 (±0.89)	13.09 (±8.03)	4.76 (±1.41)	5.41 (±1.87)	6.31 (±1.49)
$100 \ (\pm 101)$ $78 \ (\pm 13.6)$ $622 \ (\pm 13.6)$ $3457 \ (\pm 13.6)$ $347 \ (\pm 13.6)$ $3457 \ (\pm 13.6)$ $3458 \ (\pm 14.6)$ $3467 \ (\pm 13.6)$ $3458 \ (\pm 13.6)$ $200 \ (\pm 20.6)$ $2057 \ (\pm 20.6)$ $3267 \ (\pm 13.6)$ $2107 \ (\pm 12.6)$ $2107 \ (\pm 12.6)$ $2107 \ (\pm 12.6)$ $2076 \ (\pm 20.6)$ $3467 \ (\pm 13.6)$ <td< td=""><td>6.97 (±1.63) 10.69 (±3.24) 1.93 (±1.06)</td><td>10.69 (±3.24) 1.93 (±1.06)</td><td>1.93 (±1.06)</td><td>-</td><td>9.85 (±2.66)</td><td>2.03 (±0.54)</td><td>2.05 (±0.78)</td><td>32.99 (±10.20)</td><td>4.02 (±1.83)</td><td>2.96 (±2.30)</td><td>0.23 (±0.08)</td><td>9.20 (±2.40)</td><td>194.20 (±39.85)</td><td>10.66 (±1.39)</td><td>144.32 (±7.67)</td></td<>	6.97 (±1.63) 10.69 (±3.24) 1.93 (±1.06)	10.69 (±3.24) 1.93 (±1.06)	1.93 (±1.06)	-	9.85 (±2.66)	2.03 (±0.54)	2.05 (±0.78)	32.99 (±10.20)	4.02 (±1.83)	2.96 (±2.30)	0.23 (±0.08)	9.20 (±2.40)	194.20 (±39.85)	10.66 (±1.39)	144.32 (±7.67)
3:00(±148) 14.44(±3.06) 3:45.4(±10.34) 15.47(±7.124) 3.06(±0.61) 3.16(±0.134) 15.46(±3.263) 3.33:12(±10.54) 2.41(±5.72) 15.446(±3.264) 5.99(±2.06) 0.78(±0.01) 3.14(±0.03) 3.16(±88) 1.50(±0.92) 1.01(±0.42) 2.64(±3.11) 2.66(±1.84) 1.60(±0.71) 2.61(±3.11) 2.66(±1.64) 9.88(±1.64) 9.88(±1.64) 9.88(±1.64) 9.88(±1.64) 9.98(±1.64) 9.98(±1.64) 9.98(±1.64) 9.98(±1.64) 9.98(±1.64) 9.98(±1.64) 9.98(±1.64) 9.98(±1.64) 9.99(±1.64)	2.57 (±1.77) 80.34 (±9.75) 3.86 (±1.07)	80.34 (±9.75) 3.86 (±1.07)	3.86 (±1.07)		1.08 (±1.01)	4.78 (±1.36)	6.22 (±3.30)	135.79 (±13.95)	4.42 (±1.50)	3.45 (±1.43)	2.01 (±0.56)	36.59 (±7.97)	555.87 (±68.96)	19.65 (±1.35)	59.94 (±10.91)
599 (±2.60) 0.78 (±0.016) 3.13 (±2.06) 3.165 (±8.88) 1.50 (±0.92) 1.01 (±0.42) 0.26 (±1.31) 2.66 (±1.84) 0.26 (±1.31) 2.66 (±1.84) 0.20 (±1.31) 2.66 (±1.41) 2.86 (±1.44) 9.88 (±3.12) 9.88 (±3.12) 9.88 (±3.12) 9.89 (±3.12) 9.89 (±3.12) 9.89 (±3.12) 9.70 (±1.83)	1.98 (±0.66) 128.04 (±44.90) 7.72 (±1.13)	128.04 (±44.90) 7.72 (±1.13)	7.72 (±1.13)		3.30 (±1.48)	14.24 (±3.60)	43.26 (±10.33)	145.47 (±17.26)	3.06 (±0.81)	3.48 (±0.43)	4.92 (±2.22)	231.92 (±25.83)	333.12 (±110.96)	26.17 (±7.22)	194.46 (±32.60)
28.2 (±14.%) 20.7 (±1.83) 49.2 6 (±1.11) 20.31 (±4.74) 5.6 (±1.84) 7.6 (±1.33) 7.20 (±1.63) 43.01 (±1.112) 9.88 (±3.12) 20.7 (±1.63) 2.04 (±2.04) 2.05 (±2.05) 3.26 (±3.24) 2.64 (±1.63) 2.18 (±2.18) 1.00 (±0.77) 2.73 (±1.53) 12.93 (±8.03) 2.03 (±1.2.0) 2.67 (±1.53) 2.04 (±2.04) 1.40 (±1.02) 1.48 (±9.64) 2.49 (±16.54) 19.66 (±16.53) 2.57 (±1.20) 183 (±1.11) 49.92 (±17.51) 2.03 (±2.05) 2.03 (±1.50) 2.57 (±1.50) 2.03 (±1.50) 2.57 (±1.50) 2.67 (±1.51) 2.05 (±2.044) 1.11 (±0.64) 4.81 (±1.92) 5.27 (±0.92) 13.81 (±5.63) 3.56 (±1.63) 3.55 (±1.63) 3.56 (±1.63) 2.57 (±2.04) 2.57 (±2.04) 2.57 (±2.04) 2.57 (±2.04) 2.57 (±2.04) 2.57 (±2.04) 2.57 (±2.04) 2.57 (±2.04) 2.57 (±2.04) 2.57 (±2.04) 2.57 (±2.04) 2.56 (±2.04) 2.56 (±2.04) 2.56 (±2.04) 2.56 (±2.04) 2.56 (±2.04) 2.56 (±2.04) 2.56 (±2.04) 2.56 (±2.04) 2.56 (±2.04) 2.56 (±2.04) 2.56 (±2.04) 2.56 (±2.04) 2.57	4.54 (±1.85) 9.65 (±2.67) 1.54 (±0.95)	9.65 (±2.67) 1.54 (±0.95)	1.54 (±0.95)		5.99 (±2.60)	0.78 (±0.16)	3.31 (±2.06)	31.65 (±8.89)	1.50 (±0.92)	1.01 (±0.42)	0.29 (±0.12)	7.61 (±3.11)	28.68 (±4.62)	10.10 (±5.70)	8.98 (±1.41)
$2.04 (\pm 2.04)$ $2.05 (\pm 2.05)$ $3.26 (\pm 3.26)$ $2.08 (\pm 1.06)$ $2.18 (\pm 2.18)$ $1.00 (\pm 0.77)$ $2.73 (\pm 1.56)$ $19.34 (\pm 8.05)$ $2.03 (\pm 1.30)$ $2.03 (\pm 1.20)$ $5.76 (\pm 5.74)$ $2.28 (\pm 1.52)$ $1.40 (\pm 1.02)$ $1.48 (\pm 0.94)$ $2.19 (\pm 1.65)$ $1.95 (\pm 1.65)$ $1.95 (\pm 1.65)$ $1.95 (\pm 1.65)$ $2.57 (\pm 1.20)$ $1.83 (\pm 1.11)$ $4.992 (\pm 1.751)$ $207.32 (\pm 23.64)$ $2.97 (\pm 20.44)$ $5.76 (\pm 5.06)$ $1.11 (\pm 0.64)$ $4.81 (\pm 1.92)$ $5.27 (\pm 9.92)$ $1.38 (\pm 1.65)$ $3.36 (\pm 1.65)$ $2.97 (\pm 20.44)$ $4.585 (\pm 20.64)$ $4.585 (\pm 20.64)$ $1.11 (\pm 0.64)$ $4.81 (\pm 1.92)$ $5.12 (\pm 10.62)$ $3.36 (\pm 1.65)$ $3.86 (\pm 7.410)$ $8.55 (\pm 20.64)$ $2.75 (\pm 1.65)$ $4.28 (\pm 1.27)$ $3.46 (\pm 1.98)$ $3.56 (\pm 1.65)$ $3.86 (\pm 7.410)$ $8.55 (\pm 20.64)$ $3.53 (\pm 1.65)$ $2.97 (\pm 20.64)$ $4.28 (\pm 1.25)$ $0.78 (\pm 0.12)$ $0.78 (\pm 0.12)$ $0.74 (\pm 0.23)$ $1.44 (\pm 3.38)$ $6.66 7 (\pm 1.02)$ $2.95 (\pm 2.06)$ $4.24 (\pm 1.27)$ $0.78 (\pm 0.12)$ $0.78 (\pm 0.12)$ $0.71 (\pm 0.2)$ $1.44 (\pm 2.33)$ $6.67 (\pm 1.02)$ $2.95 (\pm 0.20)$ $4.24 (\pm 1.27)$ $0.78 (\pm 0.12)$ $0.78 (\pm 0.12)$ $0.74 (\pm 0.2)$ $0.74 (\pm 0.2)$ $0.74 (\pm 0.2)$ $2.95 (\pm 0.2)$ $4.24 (\pm 1.27)$ $0.84 (\pm 0.18)$ $0.84 (\pm 0.18)$ $0.86 (\pm 0.18)$ $0.86 (\pm 0.18)$ $0.86 (\pm 0.18)$ $0.97 (\pm 1.26)$ $0.74 (\pm 0.2)$ $4.24 (\pm 1.26)$ $0.84 (\pm 0.18)$ $0.84 (\pm 0.18)$ $0.84 (\pm 0.18)$ $0.84 (\pm 0.18)$ $0.94 (\pm 0.2)$	3.16 (±0.51) 26.47 (±7.87) 19.59 (±6.41) :	26.47 (±7.87) 19.59 (±6.41)	19.59 (±6.41)		28.22 (±14.96)	20.72 (±1.83)	49.26 (±8.11)	20.31 (±4.74)	5.68 (±1.84)	7.64 (±1.33)	7.20 (±1.83)	12.12 (±3.25)	43.01 (±11.12)	9.88 (±3.12)	20.79 (±1.62)
2.58 (±1.52) 1.40 (±102) 1.48 (±0.94) 24.94 (±9.65) 1.57 (±1.53) 2.57 (±1.51) 2.73 (±2.1.56) 3.97 (±2.4.18) 2.252 (±50.44) 1.11 (±0.64) 4.81 (±1.92) 5.27 (±0.95) 1.381 (±5.63) 3.56 (±1.67) 3.36 (±1.67) 3.36 (±1.67) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66) 3.53 (±1.66)	0.95 (±0.65) 1.50 (±1.50) 1.28 (±1.21)	1.50 (±1.50) 1.28 (±1.21)	1.28 (±1.21)		2.04 (±2.04)	2.05 (±2.05)	3.26 (±3.26)	2.69 (±1.98)	2.18 (±2.18)	1.00 (±0.77)	2.73 (±1.58)	19.34 (±8.05)	6.03 (±3.05)	2.03 (±1.20)	5.76 (±5.71)
111 (±0.64) 481 (±1.92) 5.27 (±0.95) 1381 (±5.63) 3.56 (±1.67) 3.31 (±1.867) 3.31 (±1.867) 3.31 (±1.867) 3.53 (±1.68) 3.53 (±1.867) 3.53	0.68 (±0.49) 3.23 (±2.69) 125.60 (±46.61)	3.23 (±2.69) 125.60 (±46.61)	125.60 (±46.61)		2.58 (±1.52)	1.40 (±1.02)	1.48 (±0.94)	24.94 (±9.65)	19.66 (±16.36)	2.57 (±) 2.00	1.83 (±1.11)	49.92 (±17.51)	207.32 (±23.68)	39.97 (±24.18)	225.20 (±50.44)
2.8 (±37.7) 364.7 (±6.8.52) 61.48 (±9.85) 23.4 (±9.68) 172.58 (±4.4.3) 25.28 (±4.4.0) 257.12 (±7.8.07) 14.48 (±3.38) 60.67 (±10.18) 56.70 (±20.19) 353.1 (±8.67) 0.41 (±015) 0.78 (±015) 0.93 (±028) 3.17 (±122) 0.41 (±010) 1.35 (±0.52) 0.74 (±0.33) 1.86 (±0.83) 7.32 (±0.93) 256 (±0.93) 258 (±0.91) 2.42 (±759) 0.84 (±0.44) 2.09 (±0.68) 5.61 (±1.52) 1.06 (±0.49) 2.75 (±1.05) 2.17 (±0.96) 3.83 (±1.46) 0.29 (±0.05) 4.52 (±1.25) 2.42 (±759) 0.84 (±0.12) 2.96 (±1.87) 1.06 (±0.49) 2.75 (±1.05) 2.17 (±0.96) 3.83 (±1.46) 0.29 (±0.05) 4.52 (±1.25) 2.42 (±0.70) 0.84 (±0.12) 2.96 (±1.87) 0.54 (±0.20) 0.54 (±0.20) 0.54 (±0.20) 0.54 (±0.20) 1.96 (±0.60) 4.52 (±1.25) 1.96 (±0.75) 1.96 (±0.75) 1.97 (±0.57) 1.94 (±0.56) 4.52 (±1.25) 2.41 (±0.01) 0.46 (±0.12) 0.56 (±0.01) 0.55 (±0.11) 0.41 (±0.09) 1.63 (±0.15) 1.96 (±0.57) 1.94 (±0.56) 1.94 (±0.56) <td< td=""><td>1.19 (±0.46) 1.25 (±0.86) 19.57 (±7.22)</td><td>1.25 (±0.86) 19.57 (±7.22)</td><td>19.57 (±7.22)</td><td></td><td>1.11 (±0.64)</td><td>4.81 (±1.92)</td><td>5.27 (±0.95)</td><td>13.81 (±5.63)</td><td>3.56 (±1.67)</td><td>3.36 (±1.34)</td><td>6.94 (±5.44)</td><td>63.55 (±8.83)</td><td>386.95 (±20.64)</td><td>45.85 (±10.53)</td><td>209.06 (±20.67)</td></td<>	1.19 (±0.46) 1.25 (±0.86) 19.57 (±7.22)	1.25 (±0.86) 19.57 (±7.22)	19.57 (±7.22)		1.11 (±0.64)	4.81 (±1.92)	5.27 (±0.95)	13.81 (±5.63)	3.56 (±1.67)	3.36 (±1.34)	6.94 (±5.44)	63.55 (±8.83)	386.95 (±20.64)	45.85 (±10.53)	209.06 (±20.67)
0.41 (±0.15) 0.78 (±0.15) 0.93 (±0.28) 3.17 (±1.22) 0.41 (±0.10) 1.35 (±0.52) 0.74 (±0.33) 1.86 (±0.83) 7.32 (±0.93) 1.90 (±0.93) 2.85 (±0.91) 42.42 (±7.59) 0.84 (±0.44) 2.09 (±0.68) 5.61 (±1.52) 1.06 (±0.49) 2.75 (±1.05) 2.17 (±0.96) 3.83 (±1.46) 43.98 (±13.97) 0.29 (±0.05) 452 (±1.25) 8.24 (±0.40) 0.46 (±0.12) 3.96 (±1.87) 0.96 (±0.04) 0.54 (±0.20) 0.25 (±0.11) 0.41 (±0.09) 1.63 (±0.15) 1.94 (±0.59) 8.24 (±0.02) 0.46 (±0.12) 3.96 (±1.87) 0.96 (±0.04) 0.54 (±0.20) 0.25 (±0.11) 0.41 (±0.09) 1.63 (±0.15) 1.94 (±0.57) 8.24 (±0.03) 5.89 (±0.01) 6.58 (±0.01) 5.64 (±0.04) 5.60 (±0.01) 5.88 (±0.01) 6.58 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02) 6.33 (±0.02)	4.42 (±0.55) 21.56 (±6.11) 45.27 (±14.14)	21.56 (±6.11) 45.27 (±14.14)	45.27 (±14.14)	•	42.86 (±37.79)	364.76 (±68.52)	61.48 (±9.85)	23.46 (±9.68)	172.58 (±44.39)	25.28 (±4.40)	257.12 (±78.07)	14.48 (±3.38)	60.67 (±10.18)	56.70 (±20.19)	35.31 (±8.67)
42.42(±7.59) 0.84(±0.44) 2.09(±0.68) 5.61(±1.52) 1.06(±0.49) 2.75(±1.05) 2.17(±0.96) 3.83(±1.46) 43.98(±13.97) 0.29(±0.05) 45.2(±1.25) 8.24(±0.40) 0.46(±0.12) 3.96(±1.87) 0.96(±0.32) 0.14(±0.04) 0.54(±0.20) 0.25(±0.11) 0.41(±0.09) 1.63(±0.18) 1.02(±0.57) 1.94(±0.59) 8.27(±0.02) 6.60(±0.03) 5.89(±0.01) 6.56(±0.01) 5.54(±0.01) 5.64(±0.03) 5.64(±0.03) 5.64(±0.03) 5.64(±0.03) 5.64(±0.03) 5.64(±0.03) 5.64(±0.03) 5.64(±0.03) 5.64(±0.01) 5.88(±0.01) 6.58(±0.02) 6.59(±0.02) 6.59(±0.02) 6.59(±0.02) 6.58(±0.02) 6.59(±0.02) 6.58(±0.02) 6.5	0.28 (±0.22) 1.06 (±0.89) 2.85 (±1.49)	1.06 (±0.89) 2.85 (±1.49)	2.85 (±1.49)		0.41 (±0.15)	0.78 (±0.15)	0.93 (±0.28)	3.17 (±1.22)	0.41 (±0.10)	1.35 (±0.52)	0.74 (±0.33)	1.86 (±0.83)	7.32 (±0.93)	1.90 (±0.93)	2.85 (±0.91)
8.24 (±0.40) 0.46 (±0.12) 3.96 (±1.87) 0.96 (±0.32) 0.14 (±0.04) 0.54 (±0.20) 0.25 (±0.11) 0.41 (±0.09) 1.63 (±0.18) 1.02 (±0.57) 1.94 (±0.57) 6.57 (±0.02) 6.60 (±0.03) 5.89 (±0.01) 6.58 (±0.05) 5.64 (±0.04) 5.60 (±0.03) 5.94 (±0.01) 5.88 (±0.01) 6.59 (±0.02) 6.33 (±0.02)	0.63 (±0.20) 0.70 (±0.23) 3.72 (±1.85)	0.70 (±0.23) 3.72 (±1.85)	3.72 (±1.85)		42.42 (±7.59)	0.84 (±0.44)	2.09 (±0.68)	5.61 (±1.52)	1.06 (±0.49)	2.75 (±1.05)	2.17 (±0.96)	3.83 (±1.46)	43.98 (±13.97)	0.29 (±0.05)	4.52 (±1.25)
6.57 (±0.02) 6.60 (±0.03) 5.89 (±0.01) 6.58 (±0.05) 5.64 (±0.04) 5.60 (±0.01) 5.21 (±0.03) 5.94 (±0.01) 5.88 (±0.01) 6.59 (±0.02) 6.33 (±0.02)	3.73 (±1.68) 0.55 (±0.40) 0.96 (±0.51)	0.55 (±0.40) 0.96 (±0.51)	0.96 (±0.51)		8.24 (±0.40)	0.46 (±0.12)	3.96 (±1.87)	0.96 (±0.32)	0.14 (±0.04)	0.54 (±0.20)	0.25 (±0.11)	0.41 (±0.09)	1.63 (±0.18)	1.02 (±0.57)	1.94 (±0.59)
	5.73 (±0.03) 5.69 (±0.01) 6.62 (±0.01)	5.69 (±0.01) 6.62 (±0.01)	6.62 (±0.01)		6.57 (±0.02)	6.60 (±0.03)	5.89 (±0.01)	6.58 (±0.05)	5.64 (±0.04)	5.60 (±0.01)	5.21 (±0.03)	5.94 (±0.01)	5.88 (±0.01)	6.59 (±0.02)	6.33 (±0.02)

Concentration (mean \pm SE, N = 4) of sugar metabolites, expressed in ng/mg of dry weight of synthetic nectar fermented by the 14 distinct bacterial isolates. **TABLE 1**

epidemidis SAAF 22.3.11, Stho = Staphylococcus hominis SAAF 22.3.10 and Te.sa = Terribacillus saccharophilus SAAF 22.2.3; for Pseudomonadota—Pa.ag = Pantoea agglomerans SAAF 22.4.2, Pa.di = Pantoea dispersa SAAF 22.3.3, Pa.sp1 = Pantoea sp1 SAAF 22.4.1, for Actinomycetota—Ce.sp. = Cellulosimicrobium sp. SAAF 22.4.18. Abbreviations: RI, retention index; RT, retention time. Not

(a) Bacillota

8













FIGURE 3 Projection to latent structures discriminant analysis (PLS-DA) of synthetic nectar fermented by the different bacterial isolates. The synthetic nectar was fermented by: for Bacillota-Bacillus sp1 SAAF 22.2.6. Bacillus sp2 SAAF 22.2.27, Brevibacillus sp. SAAF 22.4.13, Brevibacterium frigoritolerans SAAF 22.2.4, Saccharibacillus endophyticus SAAF 22.4.25, Staphylococcus epidermidis SAAF 22.3.11, Staphylococcus hominis SAAF 22.3.10, and Terribacillus saccharophilus SAAF 22.2.3; for Pseudomonadota-Pantoea agglomerans SAAF 22.4.2, Pantoea dispersa SAAF 22.3.3, Pantoea sp1 SAAF 22.4.5, and Pantoea sp2 SAAF 22.4.17; for Actinomycetota-Cellulosimicrobium sp. SAAF 22.3.25 and Curtobacterium sp. SAAF 22.4.18. Comparisons were carried out among isolates belonging to the same phylum: (a) Bacillota (8 isolates); (b) Pseudomonadota (4 isolates); (c) Actinomycetota (2 isolates). Left panels: Score plot visualizing the grouping pattern of the samples according to the first two principal components (PCs) with the explained variance in parenthesis. Right panels: Compounds ranked according to values of Variable Important for the Projection (VIP). All biological replicates (n = 4) indicate cell-free nectars.



be important for the longevity of a wide range of insects from this taxonomic group (Goelen et al., 2018; Hirose et al., 2009; Xia et al., 2021; Zhang et al., 2014). These sugars are immediately directed towards energy generation or are transformed into glycogen and/or trehalose within the fat body around the gut of most insects (Jervis et al., 2008; Rivero & Casas, 1999; Wyatt, 1967). There are indications that the accumulation of sugar and glycogen reserves as a result of fructose- and glucose-rich diets correlate with parasitoid longevity enhancement (Luo et al., 2010).

While glucose and fructose are known to positively affect the longevity of parasitoids, it is less clear how the other sugars may affect parasitoid performance. In general, monosaccharides (glucose, fructose) do not crystallize as fast as oligosaccharides such as trehalose (Wäckers, 2000) and raffinose (Luo et al., 2010). Therefore glucose and fructose are more palatable, and parasitoids are more likely to feed on them compared to oligosaccharides such as raffinose (Wäckers, 2000). There is evidence that feeding parasitoids with glucose or fructose can increase their glycogen levels compared to galactose (Luo et al., 2010). Galactose has been shown to marginally enhance the longevity of parasitoids (Goelen et al., 2018; Wäckers, 2001). Moreover, it has been suggested that erythrose may offer limited nutritional value for insects (Choi et al., 2017), while sophorose and galactinol derivatives are associated with antimicrobial properties. Sophorose lipids are known for their antifungal properties (Kulakovskaya et al., 2014), whereas galactinol, a D-galactosecontaining oligosaccharide, is known for its role in plant resistance to microbial infections (Cho et al., 2010; Kim et al., 2008). Additionally, 1-kestose, a fructan sugar that can be found in the nectar of flowers utilized in CBC programmes (van Laere & van den Ende, 2002), has been observed to be consumed by microbial communities colonizing the nectar (Lenaerts et al., 2016).

Future research efforts are needed to ascertain the impact of such sugars and sugar alcohols on the longevity of the egg parasitoid species investigated in this study. Within the Actinomycetota phylum, multivariate analysis also showed differences between strains. Nevertheless, no parasitoid species showed increased or decreased longevity when fed with nectar fermented by Actinomycetota isolates, making it difficult to discuss how the chemistry of sugars and sugar alcohols correlates with parasitoid performance.

Further investigations should explore how bacterial fermentation affects the concentration of sucrose in the nectar media which could not be quantified in this study. A higher ratio of sucrose to hexose (glucose and fructose) has been hypothesized as preferable for parasitoids, since it gradually increases the osmotic pressure in their bodies, maintaining the water balance (Vattala et al., 2006). Lenaerts et al. (2017) showed that bacterial fermentation significantly reduced sucrose, while increasing fructose and glucose concentrations, possibly pointing to competition for sugar resources between nectarinhabiting bacteria and parasitoids. Moreover, even though nectar is a sugar-rich solution, it contains in much lower amounts amino acids and secondary metabolites, such as alkaloids, that might also affect parasitoid longevity (Lo & Hwang, 2024; Nicolson, 2022) There are indications that nectar may also contain compounds that are not directly associated with its nutritional and energetic value, such as non-protein amino acids (Nepi, 2014). How such compounds affect T. basalis and O. telenomicida should be further investigated.

To increase the efficiency of parasitoids in CBC, nectar-inhabiting microbes should not only enhance parasitoid performance, but also attract the parasitoids to nectar sources, to ensure high visitation rates of floral resources (Colazza et al., 2023). In a study focused on olfactory responses, it was found that several nectar-inhabiting bacteria attracted *T. basalis*, including *T. saccharophilus* SAAF 22.2.3 (Cusumano et al., 2023). This bacterial isolate is therefore particularly interesting for CBC as it has the potential to enhance not only the longevity of the parasitoid, but also its attraction to flowering plants in the fields, such as buckwheat. This flowering plant has received considerable attention in CBC as it provides high-quality and easily accessible nectar to several parasitoids, including species attacking stink bug eggs (Araj et al., 2019; Blassioli-Moraes et al., 2022; Campbell et al., 2015; van Rijn & Wäckers, 2010; Winkler et al., 2006). The beneficial outcomes seen in CBC might be, at least partially, attributed to the bacterial fermentation of floral nectar.

Given the importance of nectariferous plants for sustainable crop protection (Gurr et al., 2016), further research should be undertaken to investigate how nectar-inhabiting bacteria of buckwheat and other plants used in CBC programmes affect the performance of parasitoids, a novel scenario that deserves to be further investigated. In particular, the effect of the most promising nectar-inhabiting bacteria should be confirmed in field conditions. We foresee two main strategies by which nectar-inhabiting bacteria could be taken into account in CBC programmes: (1) flowering plants could be selected based on their likelihood to host beneficial nectar-inhabiting bacteria; (2) candidate bacterial isolates could be delivered into crops with the aid of spray applications in order to support beneficial arthropods (Colda et al., 2021).

Further research efforts should be made to extend the number of case studies in order to understand under which conditions nectarinhabiting microbes act as beneficial partners for parasitoids (Cusumano & Lievens, 2023). In addition, a topic that remains largely unexplored is whether nectar-inhabiting microbes may also affect other natural enemies, including predators such as syrphids and lacewings which are known to rely on floral nectar and other sugar-rich resources at the adult stage (Leroy et al., 2011). Undoubtedly, fungi and bacteria ubiquitously colonize the nectar in nature and therefore, the impact of their interactions should not be neglected (Álvarez-Pérez et al., 2019), as the correct choice of nectar-inhabiting microbes is likely to be contingent on the beneficial organism's identity and its relative abundance.

AUTHOR CONTRIBUTIONS

Ezio Peri: conceptualization, methodology, writing-review & editing, supervision. Stefano Colazza: conceptualization, methodology, writing-review & editing, supervision. Bart Lievens: conceptualization, methodology, writing-review & editing. Michael Rostás: conceptualization, methodology, writing-review & editing. Antonino Cusumano: conceptualization, methodology, writing-review & editing. Antonino Cusumano: conceptualization, methodology, writing-review & editing. Antonino Cusumano: conceptualization, methodology, writing-review & editing. Nethodology, writing-review & editing. Jay Darryl L. Ermio: investigation, visualization. Mirella Lo Pinto: investigation. Alfonso Agrò: investigation. Shahinoor Rahman: investigation, formal analysis. Evgenia Sarakatsani: investigation, formal analysis, visualization, writing-original draft, writing-review & editing.

ACKNOWLEDGEMENTS

 \perp Wii fy_

This research was funded by the PRIN project 2020–Prot. 2020T58TA3–Bio-inspired Plant Protection (BiPP).

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain studies with human participants performed by any of the authors.

ORCID

Evgenia Sarakatsani b https://orcid.org/0000-0003-4493-8618 Jay Darryl L. Ermio b https://orcid.org/0000-0002-7832-1010 Shahinoor Rahman b https://orcid.org/0000-0001-5536-8521 Patrizia Bella b https://orcid.org/0000-0002-7215-184X Alfonso Agrò b https://orcid.org/0000-0002-0067-7682 Mirella Lo Pinto b https://orcid.org/0000-0003-4314-2650 Ezio Peri b https://orcid.org/0000-0002-1095-7258 Stefano Colazza b https://orcid.org/0000-0001-8023-7814 Bart Lievens b https://orcid.org/0000-0002-7698-6641 Michael Rostás b https://orcid.org/0000-0001-9722-6513 Antonino Cusumano b https://orcid.org/0000-0001-9663-9164

REFERENCES

- Álvarez-Pérez, S., Herrera, C. M., & de Vega, C. (2012). Zooming-in on floral nectar: A first exploration of nectar-associated bacteria in wild plant communities. *FEMS Microbiology Ecology*, 80, 591–602.
- Álvarez-Pérez, S., Lievens, B., & Fukami, T. (2019). Yeast-bacterium interactions: The next frontier in nectar research. *Trends in Plant Science*, 24, 393–401.
- Araj, S. E., Shields, M. W., & Wratten, S. D. (2019). Weed floral resources and commonly used insectary plants to increase the efficacy of a whitefly parasitoid. *BioControl*, 64, 553–561.
- Araj, S. E., & Wratten, S. D. (2015). Comparing existing weeds and commonly used insectary plants as floral resources for a parasitoid. *Biological Control*, 81, 15–20.
- Blassioli-Moraes, M. C., Venzon, M., Silveira, L. C. P., Gontijo, L. M., Togni, P. H. B., Sujii, E. R., Haro, M. M., Borges, M., Michereff, M. F. F., de Aquino, M. F. S., Laumann, R. A., Caufield, J., & Birkett, M. (2022). Companion and smart plants: Scientific background to promote conservation biological control. *Neotropical Entomology*, *51*, 171–187.
- Cadotte, M. W., Carscadden, K., & Mirotchnick, N. (2011). Beyond species: Functional diversity and the maintenance of ecological processes and services. *Journal of Applied Ecology*, 48, 1079–1087.
- Caltagirone, L. (1981). Landmark examples in classical biological control. Annual Review of Entomology, 26, 213–232.
- Campbell, J. W., Irvin, A., Irvin, H., Stanley-Stahr, C., & Ellis, J. D. (2016). Insect visitors to flowering buckwheat, *Fagopyrum esculentum*

(Polygonales: Polygonaceae), in north-central Florida. *Florida Entomologist*, 99, 264–268.

- Cho, S. M., Kang, E. Y., Kim, M. S., Yoo, S. J., Im, Y. J., Kim, Y. C., Yang, K. Y., Kim, K. Y., Kim, K. S., & Choi, Y. S. (2010). Jasmonatedependent expression of a galactinol synthase gene is involved in priming of systemic fungal resistance in *Arabidopsis thaliana*. *Botany*, 88, 452–461.
- Choi, M. Y., Tang, S. B., Ahn, S. J., Amarasekare, K. G., Shearer, P., & Lee, J. C. (2017). Effect of non-nutritive sugars to decrease the survivorship of spotted wing drosophila, *Drosophila suzukii*. *Journal of Insect Physiology*, 99, 86–94.
- Colazza, S., Peri, E., & Cusumano, A. (2023). Chemical ecology of floral resources in conservation biological control. *Annual Review of Entomol*ogy, 68, 13–29.
- Colda, A., Bossaert, S., Verreth, C., Vanhoutte, B., Honnay, O., Keulemans, W., & Lievens, B. (2021). Inoculation of pear flowers with Metschnikowia reukaufii and Acinetobacter nectaris enhances attraction of honeybees and hoverflies, but does not increase fruit and seed set. PLoS One, 16, e0250203.
- Conti, E., Avila, G., Barratt, B., Cingolani, F., Colazza, S., Guarino, S., Hoelmer, K., Laumann, R. A., Maistrello, L., Martel, G., Peri, E., Rodriguez-Saona, C., Rondoni, G., Rostás, M., Roversi, P. F., Sforza, R. F. H., Tavella, L., & Wajnberg, E. (2021). Biological control of invasive stink bugs: Review of global state and future prospects. *Entomologia Experimentalis et Applicata*, 169, 28–51.
- Conti, E., & Colazza, S. (2012). Chemical ecology of egg parasitoids associated with true bugs. *Psyche*, 2012, 651015. https://doi.org/10. 1155/2012/651015
- Crawley, M. J. (2007). The R book. John Wiley & Sons Ltd.
- Cusumano, A., Bella, P., Peri, E., Rostás, M., Guarino, S., Lievens, B., & Colazza, S. (2023). Nectar-inhabiting bacteria affect olfactory responses of an insect parasitoid by altering nectar odors. *Microbial Ecology*, 86, 364–376.
- Cusumano, A., & Lievens, B. (2023). Microbe-mediated alterations in floral nectar: Consequences for insect parasitoids. *Current Opinion in Insect Science*, 60, 101116.
- Cusumano, A., Peri, E., Alınç, T., & Colazza, S. (2022). Contrasting reproductive traits of competing parasitoids facilitate coexistence on a shared host pest in a biological control perspective. *Pest Management Science*, 78, 3376–3383.
- Cusumano, A., Peri, E., Vinson, S. B., & Colazza, S. (2012). The ovipositing female of *Ooencyrtus telenomicida* relies on physiological mechanisms to mediate intrinsic competition with *Trissolcus basalis*. *Entomologia Experimentalis et Applicata*, 143, 155–163.
- Ehlers, B. K., & Olesen, J. M. (1997). The fruit-wasp route to toxic nectar in *Epipactis* orchids? *Flora*, 192, 223–229.
- Ermio, J. D., Peri, E., Bella, P., Rostás, M., Sobhy, I. S., Wenseleers, T., Colazza, S., Lievens, B., & Cusumano, A. (2024). The indirect effect of nectar-inhabiting yeasts on olfactory responses and longevity of two stink bug egg parasitoids. *BioControl*, 69, 575–588. https://doi.org/10. 1007/s10526-023-10237-y
- Foti, M. C., Peri, E., Wajnberg, E., Colazza, S., & Rostás, M. (2019). Contrasting olfactory responses of two egg parasitoids to buckwheat floral scent are reflected in field parasitism rates. *Journal of Pest Science*, 92, 747–756.
- Foti, M. C., Rostás, M., Peri, E., Park, K. C., Slimani, T., Wratten, S. D., & Colazza, S. (2017). Chemical ecology meets conservation biological control: Identifying plant volatiles as predictors of floral resource suitability for an egg parasitoid of stink bugs. *Journal of Pest Science*, 90, 299–310.
- Fridman, S., Izhaki, I., Gerchman, Y., & Halpern, M. (2012). Bacterial communities in floral nectar. *Environmental Microbiology Reports*, 4, 97–104.
- Goelen, T., Baets, D., Kos, M., Paulussen, C., Lenaerts, M., Rediers, H., Wäckers, F., Jacquemyn, H., & Lievens, B. (2018). Gustatory response and longevity in *Aphidius* parasitoids and their hyperparasitoid *Dendrocerus aphidum*. *Journal of Pest Science*, 91, 351–360.

17447348, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/aab.12959 by Bart Lievens

- Ku Leuven, Wiley Online Library on [12/02/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.

) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

- Griffiths, G. J. K., Holland, J. M., Bailey, A., & Thomas, M. B. (2008). Efficacy and economics of shelter habitats for conservation biological control. *Biological Control*, 45, 200–209.
- Gurr, G. M., Liu, J., & Pogrebna, G. (2024). Harnessing artificial intelligence for analysing the impacts of nectar and pollen feeding in conservation biological control. *Current Opinion in Insect Science*, 62, 101176.
- Gurr, G. M., Lu, Z., Zheng, X., Xu, H., Zhu, P., Chen, G., Yao, X., Cheng, J., Zhu, Z., Catindig, J. L., Villareal, S., Chien, H. V., Cuong, L. Q., Chanoo, C., Chengwattana, N., Lan, L. P., Hai, L. H., Chaiwong, J., Nicol, H. I., ... Heong, K. L. (2016). Multi-country evidence that crop diversification promotes ecological intensification of agriculture. *Nature Plants*, *2*, 16014.
- Herrera, C. M., Canto, A., Pozo, M. I., & Bazaga, P. (2010). Inhospitable sweetness: Nectar filtering of pollinator-borne inocula leads to impoverished, phylogenetically clustered yeast communities. *Proceedings of the Royal Society B: Biological Sciences*, 277, 747–754.
- Herrera, C. M., García, I. M., & Pérez, R. (2008). Invisible floral larcenies: Microbial communities degrade floral nectar of bumble bee-pollinated plants. *Ecology*, 89, 2369–2376.
- Hirose, Y., Mitsunaga, T., Yano, E., & Goto, C. (2009). Effects of sugars on the longevity of adult females of *Eretmocerus eremicus* and *Encarsia formosa* (Hymenoptera: Aphelinidae), parasitoids of *Bemisia tabaci* and *Trialeurodes vaporariorum* (Hemiptera: Alyerodidae), as related to their honeydew feeding and host feeding. *Applied Entomology and Zoology*, 44, 175–181.
- Hunt, L. G., Dively, G., & Hooks, C. R. (2021). Flowering Chamaecrista fasciculata borders enhance natural enemy populations and improve grain quality in field com. Agriculture, Ecosystems & Environment, 306, 107193.
- Jervis, M. A., Ellers, J., & Harvey, J. A. (2008). Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. Annual Review of Entomology, 53, 361–385.
- Kim, M. S., Cho, S. M., Kang, E. Y., Im, Y. J., Hwangboo, H., Kim, Y. C., Ryu, C. M., Yang, K. Y., Chung, G. C., & Cho, B. H. (2008). Galactinol is a signaling component of the induced systemic resistance caused by *Pseudomonas chlororaphis* O6 root colonization. *Molecular Plant-Microbe Interactions*, 21, 1643–1653.
- Kulakovskaya, E., Baskunov, B., & Zvonarev, A. (2014). The antibiotic and membrane-damaging activities of cellobiose lipids and sophorose lipids. *Journal of Oleo Science*, 63, 701–707.
- Lee, J., & Heimpel, G. (2003). Nectar availability and parasitoid sugar feeding. USDA Forest Service Morgantown, West Virginia, pp. 220–225.
- Lenaerts, M., Goelen, T., Paulussen, C., Herrera-Malaver, B., Steensels, J., van den Ende, W., Verstrepen, K. J., Wäckers, F., Jacquemyn, H., & Lievens, B. (2017). Nectar bacteria affect life history of a generalist aphid parasitoid by altering nectar chemistry. *Functional Ecology*, *31*, 2061–2069.
- Lenaerts, M., Pozo, M. I., Wäckers, F., van den Ende, W., Jacquemyn, H., & Lievens, B. (2016). Impact of microbial communities on floral nectar chemistry: Potential implications for biological control of pest insects. *Basic and Applied Ecology*, 17, 189–198.
- Leroy, P. D., Sabri, A., Heuskin, S., Thonart, P., Lognay, G., Verheggen, F. J., Francis, F., Brostaux, Y., Felton, G. W., & Haubruge, E. (2011). Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies. *Nature Communications*, 2, 348.
- Lo, Y., & Hwang, S. (2024). Enhancing natural enemy performance through plant secondary metabolites: The role of caffeine for the parasitoid *Snellenius manilae. Journal of Applied Entomology*, 148, 330–338.
- Lu, Z. X., Zhu, P. Y., Gurr, G. M., Zeng, X. S., Read, D. M. Y., Heong, K. L., Yang, Y. J., & Xu, H. X. (2014). Mechanisms for flowering plants to benefit arthropod natural enemies of insect pests: Prospects for enhanced use in agriculture. *Insect Science*, 21, 1–12.
- Luo, S., Li, J., Liu, X., Lu, Z., Pan, W., Zhang, Q., & Zhao, Z. (2010). Effects of six sugars on the longevity, fecundity and nutrient reserves of *Microplitis mediator*. *Biological Control*, 52, 51–57.
- Martin, V. N., Schaeffer, R. N., & Fukami, T. (2022). Potential effects of nectar microbes on pollinator health. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 377, 20210155.

- McIntosh, H. R., Skillman, V. P., Galindo, G., & Lee, J. C. (2020). Floral resources for *Trissolcus japonicus*, a parasitoid of *Halyomorpha halys*. *Insects*, 11, 413.
- Mele, A., Scaccini, D., Zanolli, P., & Pozzebon, A. (2022). Semi-natural habitats promote biological control of *Halyomorpha halys* (Stål) by the egg parasitoid *Trissolcus mitsukurii* (Ashmead). *Biological Control*, 166, 104833.
- Nepi, M. (2014). Beyond nectar sweetness: The hidden ecological role of non-protein amino acids in nectar. *Journal of Ecology*, 102, 108–115.
- Nicolson, S. W. (2022). Sweet solutions: Nectar chemistry and quality. Philosophical Transactions of the Royal Society B: Biological Sciences, 377, 20210163.
- Peay, K. G., Belisle, M., & Fukami, T. (2012). Phylogenetic relatedness predicts priority effects in nectar. Proceedings of the Royal Society B: Biological Sciences, 279(1729), 749–758.
- Peri, E., Cusumano, A., Agrò, A., & Colazza, S. (2011). Behavioral response of the egg parasitoid *Ooencyrtus telenomicida* to host-related chemical cues in a tritrophic perspective. *BioControl*, *56*, 163–171.
- Pollier, A., Tricault, Y., Plantegenest, M., & Bischoff, A. (2019). Sowing of margin strips rich in floral resources improves herbivore control in adjacent crop fields. *Agricultural and Forest Entomology*, 21, 119–129.
- Pozo, M. I., Lievens, B., & Jacquemyn, H. (2015). Impact of microorganisms on nectar chemistry, pollinator attraction and plant fitness. In R. L. Peck (Ed.), *Nectar: Production, chemical composition and benefits to animals and plants* (pp. 1–40). Nova Publishers.
- Rahat, S., Gurr, G. M., Wratten, S. D., Mo, J., & Neeson, R. (2005). Effect of plant nectars on adult longevity of the stinkbug parasitoid, *Trissolcus* basalis. International Journal of Pest Management, 51, 321–324.
- Rering, C. C., Beck, J. J., Hall, G. W., McCartney, M. M., & Vannette, R. L. (2018). Nectar-inhabiting microorganisms influence nectar volatile composition and attractiveness to a generalist pollinator. *New Phytologist*, 220, 750–759.
- Rivero, A., & Casas, J. (1999). Incorporating physiology into parasitoid behavioral ecology: The allocation of nutritional resources. *Researches on Population Ecology*, 41, 39–45.
- Rosenheim, J. A. (2011). Stochasticity in reproductive opportunity and the evolution of egg limitation in insects. *Evolution*, 65, 2300–2312.
- Russell, K. A., & McFrederick, Q. S. (2022). Elevated temperature may affect nectar microbes, nectar sugars, and bumble bee foraging preference. *Microbial Ecology*, 84, 473–482.
- Russell, M. (2015). A meta-analysis of physiological and behavioral responses of parasitoid wasps to flowers of individual plant species. *Biological Control*, *82*, 96–103.
- Schleifer, K. H. (2009). Phylum XIII. Firmicutes Gibbons and Murray 1978, 5. In Bergey's manual of systematic bacteriology (pp. 19–1317). Springer.
- Sobhy, I. S., Baets, D., Goelen, T., Herrera-Malaver, B., Bosmans, L., van den Ende, W., Verstrepen, K. J., Wäckers, F., Jacquemyn, H., & Lievens, B. (2018). Sweet scents: Nectar specialist yeasts enhance nectar attraction of a generalist aphid parasitoid without affecting survival. *Frontiers in Plant Science*, *9*, 345098.
- Srinatha, H. S., Jalali, S. K., Sriram, S., & Chakravarthy, A. K. (2015). Isolation of microbes associated with field-collected populations of the egg parasitoid, *Trichogramma chilonis* capable of enhancing biotic fitness. *Biocontrol Science and Technology*, 25, 789–802.
- Urbaneja-Bernat, P., Tena, A., González-Cabrera, J., & Rodriguez-Saona, C. (2020). Plant guttation provides nutrient-rich food for insects. Proceeding of the Royal Society B: Biological Sciences, 287, 20201080.
- van Laere, A., & van den Ende, W. (2002). Inulin metabolism in dicots: Chicory as a model system. *Plant, Cell & Environment, 25,* 803–813.
- van Rijn, P. C. J., & Wäckers, F. L. (2010). The suitability of field margin flowers as food source for zoophagous hoverflies. *IOBC/WPRS Bulletin*, *56*, 125–128.
- van Rijn, P. C. J., & Wäckers, F. L. (2016). Nectar accessibility determines fitness, flower choice and abundance of hoverflies that provide natural pest control. *Journal of Applied Ecology*, 53, 925–933.

[7447348, 0, Downloaded from https://onlinelibrary.wiley.com/doi/10.11111/aab.12959 by Bart Lievens

- Ku Leuven , Wiley Online Library on [12/02/2025]. See the Terms and Conditions

(https://onlin

elibrary.wiley.com

) on Wiley Online Library

for rules

use; OA articles

are governed by the applicable Creative Commons

- 12
- Vannette, R. L., & Fukami, T. (2014). Historical contingency in species interactions: Towards niche-based predictions. *Ecology Letters*, 17, 115–124.
- Vannette, R. L., & Fukami, T. (2016). Nectar microbes can reduce secondary metabolites in nectar and alter effects on nectar consumption by pollinators. *Ecology*, 97, 1410–1419.
- Vannette, R. L., Gauthier, M.-P. L., & Fukami, T. (2013). Nectar bacteria, but not yeast, weaken a plant–pollinator mutualism. *Proceedings of the Royal Society B: Biological Sciences*, 280, 20122601.
- Vattala, H., Wratten, S., Phillips, C., & Wäckers, F. (2006). The influence of flower morphology and nectar quality on the longevity of a parasitoid biological control agent. *Biological Control*, 39, 179–185.
- Vinson, S. B. (1998). The general host selection behavior of parasitoid Hymenoptera and a comparison of initial strategies utilized by larvaphagous and oophagous species. *Biological Control*, 11, 79–96.
- Wäckers, F. L. (2000). Do oligosaccharides reduce the suitability of honeydew for predators and parasitoids? A further facet to the function of insect-synthesized honeydew sugars. Oikos, 90, 197–201.
- Wäckers, F. L. (2001). A comparison of nectar- and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata. Journal of Insect Physiology*, 47, 1077–1084.
- Wang, Z., Tang, P., Shi, M., Huang, J., & Chen, X. (2022). Flowering plants and entomophagous arthropods in the agricultural landscape: A practise-oriented summary of a complex relationship. *Frontiers of Agricultural Science and Engineering*, 9, 63–74.
- Winkler, K., Wäckers, F., Bukovinszkine-Kiss, G., & van Lenteren, J. (2006). Sugar resources are vital for *Diadegma semiclausum* fecundity under field conditions. *Basic Applied Ecology*, 7, 133–140.
- Wold, S. (2001). Personal memories of the early PLS development. Chemometrics and Intelligent Laboratory Systems, 58, 83–84.
- Wood, S. A., Karp, D. S., DeClerck, F., Kremen, C., Naeem, S., & Palm, C. A. (2015). Functional traits in agriculture: Agrobiodiversity and ecosystem services. *Trends in Ecology & Evolution*, 30, 531–539.

- Wyatt, G. (1967). The biochemistry of sugars and polysaccharides in insects. In *Advances in insect physiology* (pp. 287–360). Elsevier.
- Wyss, E. (1995). The effects of weed strips on aphids and aphidophagous predators in an apple orchard. *Entomologia Experimentalis et Applicata*, 75, 43–49.
- Xia, J., Psychogios, N., Young, N., & Wishart, D. S. (2009). MetaboAnalyst: A web server for metabolomic data analysis and interpretation. *Nucleic Acids Research*, 37(suppl_2), W652.
- Xia, S., Luo, S., Li, J., Yang, Q., Dai, C., Yang, Y., & Lu, Y. (2021). Fructose and glucose in buckwheat nectar enhance *Peristenus spretus* (Hymenoptera: Braconidae) survival and parasitism of the mirid *Apolygus lucorum. Biological Control*, 161, 104710.
- Zhang, Y., Yang, N., Wang, J., & Wan, F. (2014). Effect of six carbohydrate sources on the longevity of a whitefly parasitoid *Eretmocerus hayati* (Hymenoptera: Aphelinidae). *Journal of Asia-Pacific Entomology*, 17, 723–728.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Sarakatsani, E., Ermio, J. D. L., Rahman, S., Bella, P., Agrò, A., Pinto, M. L., Peri, E., Colazza, S., Lievens, B., Rostás, M., & Cusumano, A. (2024). Nectar-inhabiting bacteria differently affect the longevity of co-occurring egg parasitoid species by modifying nectar chemistry. *Annals of Applied Biology*, 1–12. <u>https://doi.org/10.</u> 1111/aab.12959