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Dietary sugars and amino acids increase longevity and enhance reproductive parameters of *Bracon cephi* and *B. lissogaster*, two parasitoids that specialise on wheat stem sawfly

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Abstract

The wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), is a major pest of wheat grown on the Northern Great Plains and Canadian Prairies of North America, causing economic losses of as much as $350 million annually. Two species that parasitize wheat stem sawfly larvae in wheat stems are *Bracon cephi* (Gahan) and *B. lissogaster* Muesebeck (Hymenoptera: Braconidae). Carbohydrate-rich diets increase adult parasitoid longevity and reproductive parameters, enhancing their success as natural enemies. In previous studies, these species had increased longevity, egg load, and volume when fed sucrose solutions, encouraging further research on their nutritional needs. Therefore, we conducted experiments with artificial diets using adult females fed sucrose, glucose, and fructose solutions. Females were also fed these sugars in combination with a mixture of amino acids. We assessed individuals daily for longevity. Both species benefited from diets containing carbohydrates, with an increase in longevity from an average of 9 to 51 days for *B. cephi*, and from 6 to 34 days for *B. lissogaster*. Additional experiments assessed egg load and volume after 2, 5, and 10 days of cumulative feeding in *B. cephi*. These females produced 1.4-fold more eggs when fed amino acids, 2.5-fold more with sugar, and 2.7-fold more when fed sugar with amino acids. They had a 1.3-fold increase in egg volume when fed amino acids, 1.9-fold with sugar, and 2.1-fold when fed sugar with amino acids. Our study reveals the nutritional requirements of these braconid parasitoids and the benefits of nutritional sources when implementing conservation biological control strategies.

KEYWORDS
Cephus cinctus, conservation biological control, egg load, egg size, integrated pest management, nutrition, reproductive physiology, synovigenic

INTRODUCTION

Food sources can play an important role for hymenopteran parasitoids, by increasing their longevity, development, fecundity, and rates of parasitism (Lee et al., 2004; Lee & Heimpel, 2008; Tunçbilek et al., 2012). Plant species that improve parasitoid performance as biological control agents by providing food supplies, such as floral and extrafloral nectar are desirable, especially in agricultural systems (Bianchi et al., 2006; Gurr et al., 2017; Heimpel & Jervis, 2005; Simpson et al., 2011; Stephens et al., 1998; Tena et al., 2015). Supplemental nutrition plays a critical role in insect metabolism. However, fundamental knowledge is required on the relative use of mono and

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disaccharides, as well as supplementary amino acids in species that have specific dietary limitations as adults, such as the parasitoids *Bracon cephi* (Gahan) and *Bracon lissogaster* Muesebeck (Hymenoptera: Braconidae), which do not host feed (Reis et al., 2019). These two biological control agents are well known for suppressing locally damaging populations of the wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae), which causes severe economic losses in the Northern Great Plains of North America (Bekkerman & Weaver, 2018; Beres et al., 2011; Morrill et al., 1998; Peterson et al., 2011; Weaver et al., 2005). In addition, the availability of supplemental food sources has been shown to benefit both *B. cephi* and *B. lissogaster* (Rand & Waters, 2020; Reis et al., 2019).

The life cycles and biology of *B. cephi* and *B. lissogaster* are similar, with two generations per year (Criddle, 1923; Morrill, 1997; Nelson & Farstad, 1953), where the second generation has a lengthy diapause period during fall and winter (Holmes et al., 1963; Somsen & Luginbill, 1956). The immature parasitoids feed on and kill the wheat stem sawfly larvae, completing their development in approximately 10 days (Holmes et al., 1963; Nelson & Farstad, 1953). After completing the life cycle, *B. cephi* and *B. lissogaster* adults emerge by chewing a hole through the wheat stem, moving to the exterior (Nelson & Farstad, 1953). Both species are specialist ectoparasitoids (Nelson & Farstad, 1953; Somsen & Luginbill, 1956). However, *B. lissogaster* is gregarious and lays as many as four eggs per host (Somsen & Luginbill, 1956), while *B. cephi* is solitary and lays one egg per host (Nelson & Farstad, 1953). Both species are synovigenic, so they do not have a full complement of mature eggs before beginning oviposition (Flanders, 1950). Synovigenic individuals can benefit from nectar availability by increasing longevity and fecundity, thus increasing their success as biological control agents (Jervis et al., 2008; Manandhar & Wright, 2016). This is even more important for those species that are not known to host feed, as is the case for *B. cephi* and *B. lissogaster* (Reis et al., 2019).

Even though parasitoid larvae feed on their arthropod hosts (Balzan & Wäckers, 2013; Godfray, 1994), the majority of adult parasitoids use supplemental carbohydrates, such as floral and extrafloral nectar (Wäckers, 2005), as a primary source of energy (Benelli et al., 2017; Casas et al., 2005; Heimpel & Jervis, 2005; Jervis et al., 2008; Rivero & Casas, 1999a; Wäckers, 2005). Under lab conditions, *B. lissogaster* was initially reared with honey and sugar solutions to increase longevity as part of general biological investigations (Somsen & Luginbill, 1956). Reis et al. (2019) reported that a sucrose solution had a positive effect on the reproductive success of these braconid parasitoids, in which *B. cephi* and *B. lissogaster* both experienced increased longevity and greater egg loads, while egg volume in *B. cephi* also increased when feeding on sucrose.

Carbohydrates can be used in the process of lipogenesis to synthesise lipids, the main constituent of the fat body in insects (Arrese & Soulages, 2010). The fat body is the tissue involved in key metabolic processes like transamination, and vitellogenin production, forming the proteins that will become the yolk protein in eggs (Hoshizaki, 2013). However, some parasitoid species have been shown to be unable to synthesise lipids from excess carbohydrates (Casas et al., 2003; Giron & Casas, 2003; Olson et al., 2000; Rivero & West, 2002; Visser & Ellers, 2008). Other studies with parasitoids have suggested that their performance changes when they feed on different carbohydrates. Luo et al. (2010) reported that *Microplitis mediator* (Haliday) (Hymenoptera: Braconidae) females accumulate fructose and total sugars, in addition to having greater longevity and egg reproduction when fed fructose, glucose, and sucrose. Similarly, the non-hymenopteran parasitoid *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae), accumulates higher concentrations of sugars and glycogen when fed sucrose, trehalose, glucose, and fructose, with these sugars being related to an increase in its longevity (Chen & Fadamiro, 2006). Therefore, individual mono- or polysaccharides can play different roles in parasitoid physiology. Honeydew with higher concentrations of monosaccharides (glucose and fructose) increases *Aphidius ervi* Haliday (Hymenoptera: Braconidae) longevity when compared with honeydew with higher concentrations of the disaccharide sucrose (Hogervorst et al., 2007), while *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) has greater longevity when fed the disaccharide sucrose, followed by other monosaccharides (Özalp & Emre, 2001). When it comes to dietary amino acids, their role in insects is not completely understood, however, it is known that glutamate is critical to transamination reactions (Douglas & Simpson, 2013), while tyrosine is used for cuticle sclerotization (Hoshizaki, 2013). Interestingly, some amino acids confer specific taste and scent to nectar, which are responsible for pollinator attraction or repulsion (Gijbels et al., 2015). More specifically, the amino acid proline is reported to be attractive to honeybees and is one of the most important energy sources for them, being rapidly consumed during flight (Carter et al., 2006). For adult *B. cephi* and *B. lissogaster*, there is limited information to date about their dietary needs and associated behaviours.

To improve biological control of the wheat stem sawfly, it is critical to better understand the benefits of nutritional resources on the reproductive biology of these sympatric parasitoids. Therefore, we further investigate the importance that supplemental food sources might have for *B. cephi* and *B. lissogaster*, which already have been shown to benefit from sucrose (Reis et al., 2019). Here, we are evaluating their longevity, egg load, and egg volume after feeding on artificial diets containing mono- and disaccharides, mixed or not with amino acids. We expect sugars to improve all the parameters being tested, with the addition of amino acids boosting longevity, egg load, and egg volume. Our findings will provide a better understanding of the dietary needs of these species as well as other non-host feeding adult parasitoids. In addition, practical adaptation of this knowledge may provide a powerful opportunity to increase parasitoid populations at the field level and allow for the implementation of a more resilient biological control programme for the wheat stem sawfly.

MATERIALS AND METHODS

We conducted laboratory experiments with adult females of both *B. cephi* and *B. lissogaster* reared on individual sucrose, fructose, and

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glucose solutions at 0.5 M. These individual diets were tested in isolation and combined with amino acids (Supplemental Material, Table 1). Newly emerged first-generation females were used to quantify the effects of sucrose, glucose, and fructose, with or without supplemental amino acids on female longevity. In addition, we also observed the effects of these diets on *B. cephi* reproductive parameters, where eggs were counted and assayed after feeding for 2, 5, or 10 days.

Parasitoids

Parasitoid cocoons of both species were collected from field samples, as described below, and were dissected in 2019 and 2020 and stored in plastic bags (16.5 × 14.9 cm) at 0–4°C to prevent development and emergence until use. About 3 weeks before experiments, parasitoid cocoons were placed in glass jars (236 ml) and maintained in a growth chamber under controlled conditions (25 ± 2°C, 40%–60% humidity, and photoperiod LD 12:12 h) for several weeks until adult parasitoids emerged.

Row samples of wheat stems and stubs were field collected in Montana, USA. In 2019, these were from near Amsterdam, Montana, USA (45° 45′32.4′N, 111° 23′49.2′W) and in 2020, these were again from near Amsterdam (45° 45′32.4′N, 111° 23′49.2′W), and also from near Big Sandy, Montana, USA (48° 10′51.6′N, 110° 20′34.8′W), and from near Conrad, Montana, USA (48° 18′11.9′N, 111° 55′22.2′W). Stems and stubs were placed inside black plastic barrels (121 L, 56 × 63 × 71 cm) with lids that had a circular hole (11 cm diameter) in the centre, where a clear plastic jar (500 ml; S-22855; Uline, Pleasant Prairie, Wisconsin) was fixed to the top to trap emerging adults. To assist parasitoid movement from the inside of the barrel to the plastic jar, a tapered cone made from a clear laminate sheet glued with silicone sealant (GE5000; GE, Boston, Massachusetts) was used. Barrels were kept at room temperature until parasitoid emergence ended. During this interval, the row-sample residue was occasionally misted with water using a hand sprayer to facilitate development and, more importantly, emergence from desiccated stems.

Both jars and barrels were checked daily to collect all overwintering and newly emerged adults, with females immediately randomly assigned to the treatments. The number of females available for use in each experiment differed based on variation in population density and species composition for different locations, so the assignment of individuals to treatments followed the approach used by Reis et al. (2019). Adult parasitoid identification was based on several distinguishing morphological characters (Runyon et al., 2001), and any individuals not readily assigned to species were discarded.

Longevity and body size

Newly emerged females of *B. cephi* and *B. lissogaster* were fed artificial diets to assess the influence of nutrient components on their longevity. These experiments were conducted in growth chambers (25 ± 2°C, 40%–60% humidity, and a LD 12:12 h photoperiod), where individual females were held in plastic petri dishes along with nutrients provisioned via a piece of dental cotton roll soaked in the treatment solutions (Reis et al., 2019). Individuals were randomly assigned to one of eight treatments: sucrose (S), glucose (G), fructose (F), and water (W) in isolation or combined with amino acids (S + AA, G + AA, F + AA, W + AA) (Supplemental Material, Table 1), with a total of 30 females per treatment. The diet compositions were chosen because they have been used in other studies using dietary amino acids (Mevi-Schütz & Erhardt, 2017). The petri dishes were placed inside the growth chamber, and the females were inspected daily to record mortality and to provision with new diet-soaked cotton rolls.

After death, as an estimate of parasitoid body size (Jervis et al., 2003), we measured the hind tibiae using a Dino-Lite microscope (model AM7115MZT-Edge, Dino-Lite, Hsinchu, Taiwan) at ×50 magnification using the associated computer software (DinoXcope version 1.5.15). These measurements are needed because female size can influence longevity, egg load, and egg volume (Ellers & Jervis, 2003; Jervis et al., 2003; Reis et al., 2019; Saeki & Crowley, 2013).

Egg load and volume – *B. cephi*

To assess egg load and volume, newly emerged females were fed artificial diets comprising distilled water, amino acids, sugars (sucrose, fructose, and glucose) at 0.5 M, or sugars combined with amino acids (Supplemental Material, Table 1), where 15 *B. cephi* females were tested for each treatment. Given the dependency on population density and species composition in the field, we were unable to conduct these experiments with *B. lissogaster*. In previous lab observations, we noticed that females consuming only water died after an average of 10 days, meaning that this was the maximum number of days we conducted experiments to evaluate egg load and volume. Females were randomly assigned to the different treatments and placed in petri dishes under growth chamber conditions (25 ± 2°C, 40%–60% humidity, and a LD 12:12 h photoperiod). These assays were made after feeding on the diet for 2, 5, and 10 days. Later, female parasitoids were freeze-killed and stored at −30°C until being dissected in distilled water using microdissection forceps to isolate and count eggs under a stereomicroscope (MZ95; Leica, Germany) at ×2 magnification. Egg size was measured using a Dino-Lite digital camera (AM7115MZT-Edge, Dino-Lite, AnMo Electronics Corp., Hsinchu, Taiwan), at ×50 magnification, by measuring the length and the diameter at the midpoint of its long axis for each mature egg. Only mature eggs were considered while counting and measuring the eggs, which were identified based on a curved sausage shape (Iwata, 1959). Egg volume (μl) was estimated using the formula $V = (\pi\ r^2)\ (L-2r) + (4/3)\ (\pi\ r^3)$, where L = length, D = diameter, and r = D/2 (O'Neill et al., 2014).

Statistical analysis

The statistical analyses were conducted using R (R Core Team, 2019) in the integrated development environment RStudio (version 3.6.1).

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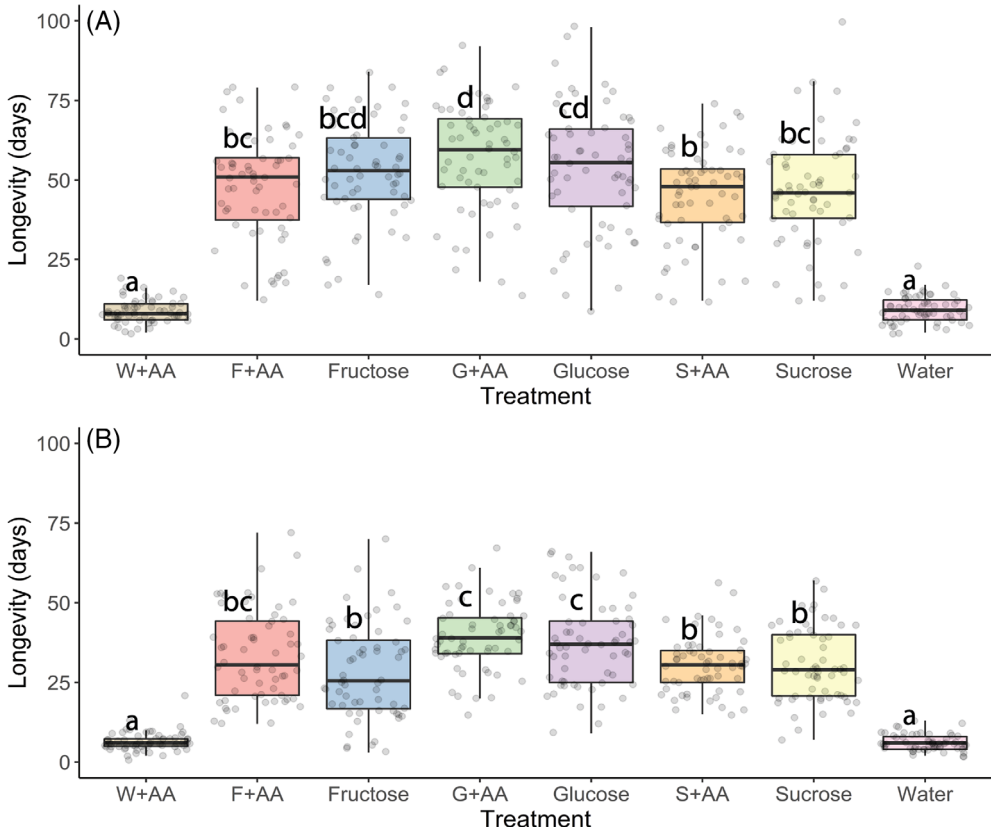

FIGURE 1 Boxplots of longevity in number of days of (A) *Bracon cephi* and (B) *B. lissogaster*. Females were fed until death with either water (control), amino acids (W + AA), fructose, sucrose, glucose, or the respective sugars combined with amino acids: Fructose plus amino acids (F + AA), sucrose plus amino acids (S + AA), or glucose plus amino acids (G + AA). Groups with the same letter are not different by the pairwise comparison tests, with 5% family wise significance level. The shaded dots are showing the distribution of the data set. The thick horizontal lines in the boxplots are median values for longevity, while the box represents the interquartile range spanning 50% of the data. The whiskers are data variability outside the interquartile range, representing the upper and lower 25 percentiles, extending to minimum and maximum data points within 1.5 times the interquartile range.

Assumptions were checked by using diagnostic plots generated with the 'car' package (Fox & Weisberg, 2019). Models were simplified through stepwise deletion of terms based on the *p*-value ($p < 0.05$); interactions that did not meet the criteria were dropped from the model. The effect of artificial diets on longevity was analysed using the Poisson Generalised Linear Model, where we tested the effects of different experiment years (2019 and 2020) and body size (tibia length). Based on Reis et al. (2019), there is a priori expectation that different parasitoid species (*B. cephi* and *B. lissogaster*) will have different longevity responses; therefore, we also tested for a species effect to confirm this. To test the effects of diet over time on egg load, we followed the procedures as described for longevity. For egg volume, we used linear models, and the assumptions of normality and homoscedasticity were met, therefore, transformation on the data was not needed, as was true for the other parameters. We used the fitted models to generate figures with accurate predictions for egg load and egg volume. In these figures, the error bars are representing 95% confidence interval for the predicted values. Multiple comparisons were made using Tukey's post-hoc test at $p \leq 0.05$. We used the function 'contrast' from the 'emmeans' package (Russell, 2021) to compare a pooled sample containing all treatments with amino acids with those without amino acids at $p \leq 0.05$. For longevity, contrasts were controlled for year, body size, and species, while for egg load and volume had contrasts controlled for the parameters body size and cumulative days of feeding.

RESULTS

Longevity

We found weak evidence for an interaction between treatment and species on longevity ($F_{7,\ 896} = 1.93$, $p = 0.06$), with both species increasing longevity further with glucose when compared with other treatments (Figure 1a,b). However, there was a noticeable difference in patterns between *B. cephi* and *B. lissogaster*, with *B. lissogaster* females benefitting further from the monosaccharides glucose and fructose combined with amino acids (Figure 1b) when compared with *B. cephi* (Figure 1a). In addition, *B. lissogaster* females fed fructose combined with amino acids had slightly increased longevity when

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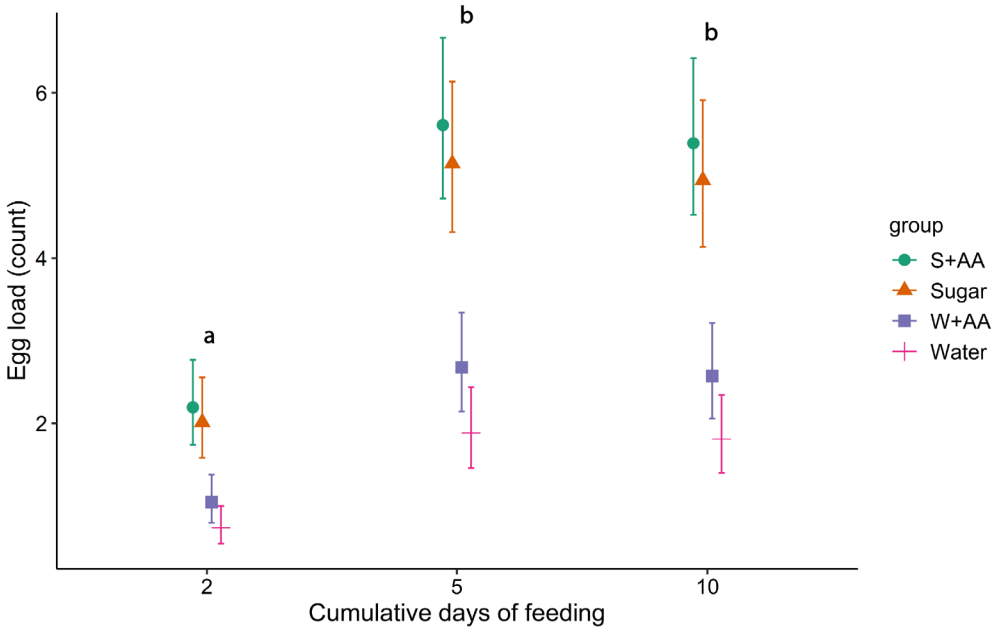

FIGURE 2 Predicted values for the mean egg load of *Bracon cephi* with the interaction between cumulative days of feeding and treatments. Diets were offered ad libitum over 2, 5, and 10 days and consisted of either water, water + amino acids (W + AA), sugar, or sugar + amino acids (S + AA). Letters represent cumulative feeding effects on egg load. Groups with the same letter are not different in pairwise comparison tests, with a 5% familywise significance level. Error bars represent the 95% confidence interval for the predicted values based on the fitted model described in the statistical analysis.

compared with either the water control or a fructose solution, but *B. cephi* had the opposite outcome with this diet—a slight decrease in longevity when amino acids were combined with fructose (Figure 1a,b).

There is also weak evidence for an interaction between body size and species ($F_{1, 896} = 3.53$, $p = 0.06$), with *B. cephi* females tending to be larger (Supplemental Material, Figure 1). When comparing experiments conducted in 2019 and 2020 with both parasitoid species, we measured a strong influence on longevity based on the different treatments ($F_{7, 896} = 285.41$, $p < 0.0001$) and different species ($F_{1, 896} = 315.90$, $p < 0.0001$), with *B. cephi* living longer than *B. lissogaster* (Figure 1a,b). In both cases, the water control and amino acid only diets had the lowest longevity.

Egg load and volume – *B. cephi*

There were effects of diet ($F_{3, 173} = 31.06$, $p < 0.0001$) and cumulative days of feeding ($F_{2, 173} = 38.13$, $p < 0.0001$) on egg load. Overall, there was an increase in egg load from Day 2 to Day 5, which was then similar on Day 10 (Figure 2). Our water control had the smallest egg load in isolation, as well as when combined with amino acids. The contrasts using additive models controlling for cumulative days of feeding and body size revealed that the addition of amino acids improves egg load ($p = 0.020$, Figure 2 and Supplemental Material, Table 2). We also noticed that there is an influence of body size on egg load for *B. cephi* ($F_{1, 173} = 13.14$, $p < 0.004$), with larger females having more eggs (Figure 3a).

There is strong statistical evidence to support the hypothesis that egg volume differs based on the different treatments ($F_{3, 173} = 24.05$, $p < 0.0001$), with our water controls and isolated amino acids solution having the lowest volumes (Figure 4). Our results showed an influence of cumulative days of feeding ($F_{2, 173} = 11.39$, $p < 0.0001$), with egg volume having a significant increase from day 2 to day 5 (Figure 4). We also observed an influence of body size on egg volume ($F_{1, 173} = 13.97$, $p < 0.004$), in which larger females had larger eggs (Figure 3b). Even though we did not find strong evidence suggesting an interaction between 'treatment' and 'day', our contrasts show weak evidence suggesting that *B. cephi* females also increase egg volume when both water and sugars were combined with amino acids ($p = 0.0557$; Figure 4 and Supplemental Material, Table 2).

DISCUSSION

Sugar feeding is essential to increase parasitoid reproductive success (Siekmann et al., 2001), and several studies have shown that supplemental nutrition can benefit longevity, fecundity, and rates of parasitism (Heimpel et al., 1997; Lee et al., 2004; Lee & Heimpel, 2008; Lewis et al., 1998; Tunçbilek et al., 2012). Even though it is not yet well known how adults of *B. cephi* and *B. lissogaster* can benefit from broad supplemental nutrition, Reis et al. (2019) demonstrated the benefits of dietary sucrose on the adults of these parasitoids. Sucrose increased both *B. cephi* and *B. lissogaster* longevity and egg load while also increasing egg volume for *B. cephi* (Reis et al., 2019). In our study, we observed that feeding on either monosaccharides (fructose and

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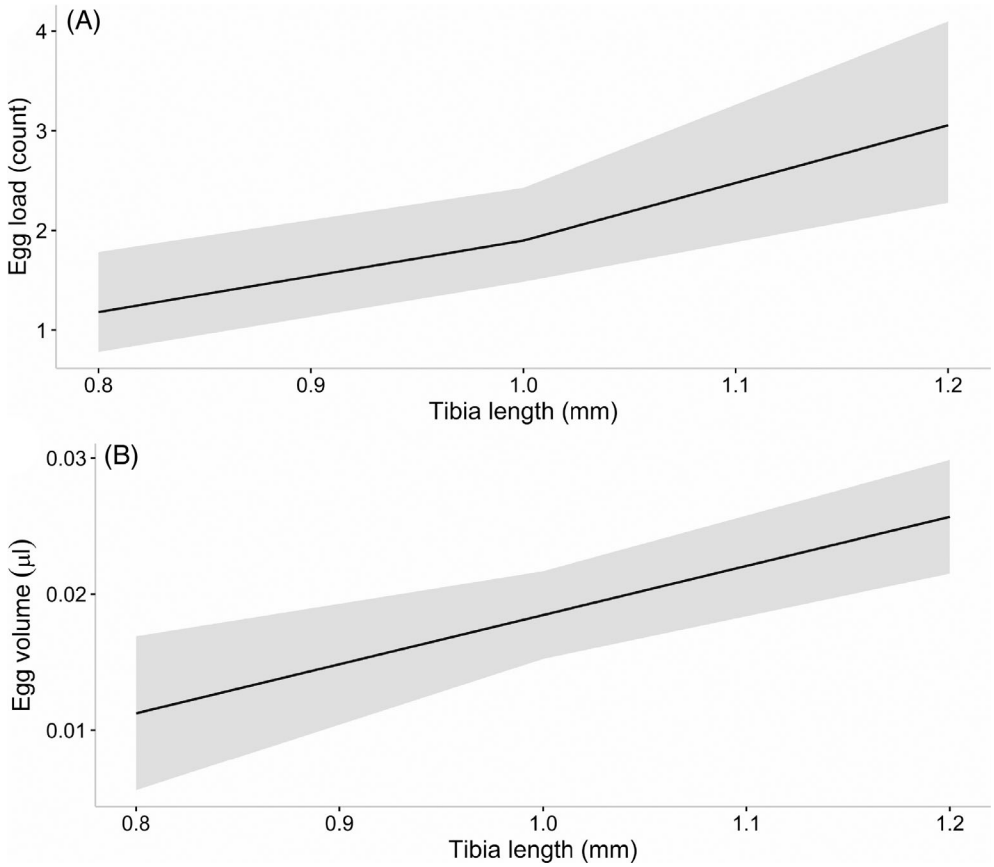

FIGURE 3 Relationship between the predicted values of *Bracon cephi* (A) mean egg load and body size (tibia length in mm), and (B) mean egg volume (µl) and body size (tibia length in mm). Diets consisted of either water, water + amino acids (W + AA), sugar, or sugar + amino acids (S + AA). Shading represents a 95% confidence interval.

glucose) or disaccharide (sucrose) was key to increasing adult female longevity. We also observed an influence of carbohydrates on the increases in egg load and egg volume in *B. cephi*. Therefore, supplemental nutrition with saccharides positively affects these female parasitoids, benefiting their metabolism and reproductive biology, with differing effects depending on the molecular structure.

Our results indicate that both species, when fed carbohydrates, had increased longevity when compared with water and a water solution of amino acids. These braconid parasitoids are able to use provisioned carbohydrates, indicating the influence of the digestive enzyme α-glucosidase, which is involved in the hydrolyses of certain saccharides (Chippendale, 1978), which are, in turn, key to the successful use of these energy sources. Our observations corroborate results observed for these species when fed sucrose (Reis et al., 2019), and other studies have indicated that carbohydrate-rich diets can increase longevity and fecundity in parasitic hymenopterans (Heimpel et al., 1997; Lewis et al., 1998). In addition, Xiong et al. (2020) observed a correlation between longevity and the expression of certain lifespan-related genes when comparing parasitic wasps fed different concentrations of sucrose and honey, suggesting that not only the concentrations but also the type of saccharide molecule can change gene expression and how these determine insect longevity.

When comparing the monosaccharide sugars tested in our research, there was a tendency for glucose to better benefit longevity when compared with the monosaccharide fructose or the disaccharide sucrose, which is composed of combined glucose and fructose. This is an interesting result because insect hemolymph contains mostly trehalose (which is two molecules of glucose combined together) and is synthesised in the fat body as an important carbohydrate reserve (Arrese & Soulages, 2010; Rivero & Casas, 1999a). As the amount of trehalose increases to a critical concentration in the hemolymph, synthesis is interrupted, and glycogen synthesis takes place instead (Arrese & Soulages, 2010). Chen and Fadamiro (2006) reported that age plays an important role in glycogen levels, with the levels decreasing in older individuals. In addition to age, the diets also influenced glycogen content, with sugars helping to maintain glycogen levels (Chen & Fadamiro, 2006; Luo et al., 2010). The parasitoid *Diglyphus isaea* Walker (Hymenoptera: Eulophidae) was able to use sucrose and convert it to glycogen and body sugars, but it could not convert it into lipids (Zhang et al., 2011). Many parasitoid species are not able to synthesise lipids from carbohydrates (Akman Gündüz et al., 2010; Olson et al., 2000; Rivero & West, 2002; Zhang et al., 2011), and parasitoids emerge with a considerable reserve of lipids that decrease with age (Akman Gündüz et al., 2010).

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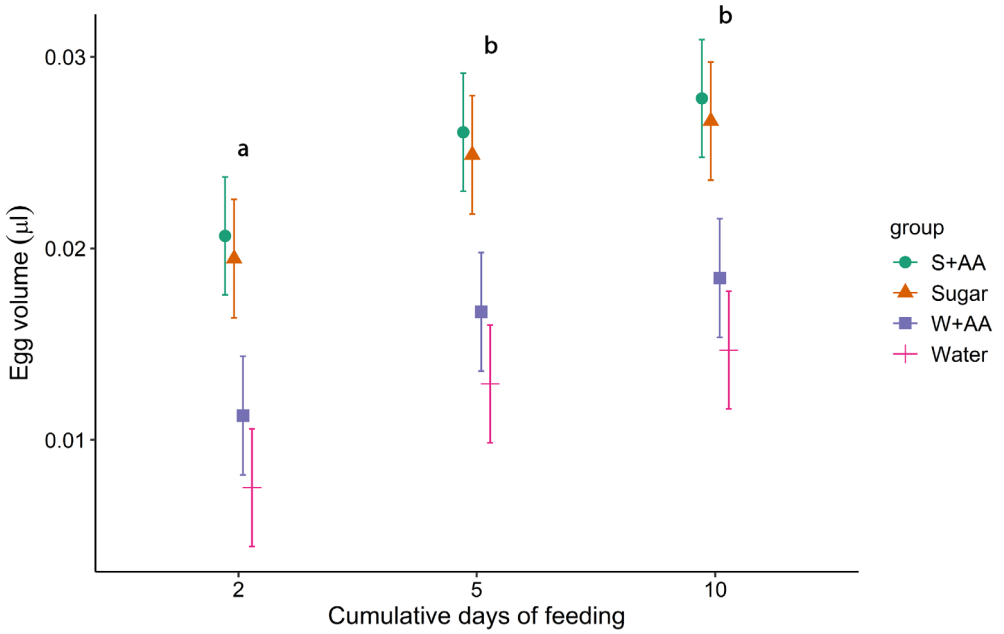

FIGURE 4 Predicted values for mean egg volume (µl) of *Bracon cephi* and the interaction of cumulative days of feeding and treatments. Treatments consisted of either water, water + amino acids (W + AA), sugar, or sugar + amino acids (S + AA). Diets were offered ad libitum over 2, 5, and 10 days. Letters represent cumulative feeding effects on egg volume. Groups with the same letter are not different in pairwise comparison tests, with a 5% familywise significance level. Error bars represent 95% confidence interval for the predicted values based on the fitted model described in the statistical analysis.

Dietary fructose can be combined with other molecules, such as biphosphate, and act on the regulation of glycolysis (Arrese & Soulages, 2010). Knowing that insects absorb carbohydrates in their guts as monosaccharide sugars, and that all insects can use metabolic intermediates from lipids and amino acids to synthesise glucose by gluconeogenesis (Douglas & Simpson, 2013), we hypothesise that females feeding on glucose can rapidly use this sugar or have it easily combined to yield trehalose, avoiding or decreasing the gluconeogenesis process. As a consequence, they may be conserving energy and preserving metabolic resources that might be crucial for other physiological processes, perhaps increasing their longevity. Other dietary compounds in addition to carbohydrates can be important for the survival and reproductive success of insects (Levin et al., 2017). Another objective of our study was to determine if amino acids could further increase *B. cephi* and *B. lissogaster* longevity, as has been found for certain other nectar-feeding species (Berndt & Wratten, 2005; Tompkins et al., 2010). Our results show that females fed on water combined with amino acids did not have increased longevity, confirming that carbohydrates are critical in maintaining homeostasis and optimal metabolism. In addition, carbohydrates combined with amino acids did not benefit female longevity. Insects can convert carbohydrates into certain proteins, but the opposite is not true. Even though this process is costly and the rate of occurrence is limited (Hoshizaki, 2013), females could be benefitting by this process when fed carbohydrates, but not when amino acids were present in the diet. In addition, the food intake by hymenopteran parasitoids is limited by their stomach volume and not caloric consumption (Ellers et al., 2011); therefore, the parasitoids used in our study might need to feed on a broader range of nutrient concentrations to show a significant increase in longevity when comparing diets containing sugars with amino acids. Our results for longevity could also indicate an allocation of these amino acids to the reproductive system, as observed in studies with lepidopterans (Levin et al., 2017; Mevi-Schütz & Erhardt, 2017) or vitellogenin synthesis and fecundity in the crop pest *Nilaparvata lugens* (Stål) (Lu et al., 2016). Perhaps consequently, we do not see an increase in longevity when carbohydrates are combined with amino acids.

To better clarify and investigate how carbohydrates and amino acids benefit female parasitoids, we analysed egg load and volume with *B. cephi* females fed for 2, 5, and 10 days on artificial diets. These different diets contained water, amino acids only, sugars (fructose, sucrose, and glucose), or sugars combined with amino acids. Our results showed that there is an influence of sugars and sugars combined with amino acids on egg load and volume. Levin et al. (2017) noticed that adult moths allocate amino acids to flight muscles and eggs, which are also detectable in their offspring (1st and 2nd instars), showing that dietary amino acids are essential to the quality of eggs and young offspring (Levin et al., 2017). Although the Levin et al. (2017) experiments were conducted with Lepidoptera, it is possible that our braconid female parasitoids are also allocating similar nutrient resources to their eggs to improve offspring quality and fitness. This could be an important strategy being used by our synovigenic parasitoids, especially because the adults are not known to host feed on *C. cinctus* (Reis et al., 2019), which could otherwise be a dietary source of amino acids. There are studies showing that only host-feed parasitoids can replenish protein reserves associated with egg production,

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while parasitoids that feed on nectar or honeydew (non-host feeders) do not enhance reproductive biology with supplemental nutrition, even though they increase longevity (Jervis et al., 2008; Rivero & Casas, 1999a). Rivero et al. (2001) reported that the host-feed parasitoid *Dinarmus basalis* Ashmead (Pteromalidae) can store amino acids to be used later in egg production. This parasitoid species is also reported to use larval reserves as the main nutrient source for egg production during the initial phases of adult life, but these were also being used throughout their lifespan (Rivero et al., 2001). Based on our results, it is likely that our parasitoids are using nutrients acquired as larvae for egg production, with supplemental nutrition substantially improving egg load and egg volume. We also noticed a tendency for amino acids combined with sugars to significantly increase egg load and volume in earlier stages (when comparing specific results from Day 2 to both days 5 and 10). On Day 2, there was an increase in both egg load and volume, reaching 67% and 46% greater, respectively. In addition, when carbohydrates were not present, amino acids seemed to boost these parameters when compared with our control, especially on Day 2, with an increase of 62% in egg load and 76% in egg volume. This suggests that these female parasitoids are using nutrients for metabolism as well as to improve their reproductive reserves. Females used in our experiments were of the first generation, and thus, they might be benefitting from nutrient restoration after overwintering (Nelson & Farstad, 1953). In addition, females might be incorporating some of these nutrients in their eggs (Levin et al., 2017). Amino acids are important for oocyte growth, yolk development, ovulation, and oviposition (Hegdekar, 1970), but logistical challenges in our system prevented us from experimental study of oviposition. Therefore, amino acid nutrition could additionally benefiting these female parasitoids, although this lies outside the scope of our study. In addition, it is well known that adult body size is related to the amounts of resources acquired during larval development (Jervis et al., 2001), and these nutritional reserves are used in somatic maintenance and can contribute to egg production (Jervis et al., 2001; Rivero et al., 2001). This corroborates our results showing that bigger *B. cephi* females have more and larger eggs, which is especially interesting given that, as discussed above, supplemental nutrition is further benefitting these reproductive parameters after 2 days of cumulative feeding. As noticed by Rivero and Casas (1999b), the maximum allocation of external nutrients to the eggs takes up to 3 days, however, these nutrients can also be stored and used during parasitoid lifespan.

Some of the amino acids used in our study are important to insects, such as glutamate that plays a central role in transamination reactions (Douglas & Simpson, 2013), or tyrosine that is used for cuticle sclerotization and is also a constituent of most proteins (Hoshizaki, 2013). Proline is an energy source used for flight (Douglas & Simpson, 2013), but in our study we did not analyse the flight performance of female parasitoids, and thus it is unknown if diets could be influencing parasitoid flight directly or indirectly via increased longevity. In addition, the compound ratios of diets can influence an insect's response. As noticed by Lee (2015), adult female Drosophila lived longer on a diet containing a 1:16 ratio of protein to carbohydrate, but produced more eggs on a 1:4 protein to carbohydrate ratio and laid more eggs on a 1:2 ratio. Interestingly, when offered the option to choose among diets containing different ratios, they preferred diets that were 1:4 protein to carbohydrate ratio (Lee, 2015). We also acknowledge that we tested lower ratios of carbohydrates to amino acids than those reported by Lee (2015); therefore, the braconid parasitoids in our experiments may have required a different ratio of carbohydrates to amino acids than was tested to display dietary benefits.

In conclusion, our research aimed to investigate the effects of carbohydrate-rich diets both in combination with amino acids and alone for both *B. cephi* and *B. lissogaster*, two synovigenic specialist ectoparasitoids. Both species benefited from glucose, especially when combined with amino acids. For *B. cephi*, we noticed a strong influence of carbohydrates on egg load and volume, especially during the initial 5 days of adult life, with the addition of amino acids tending to further increase these reproductive parameters. It is well known that in agricultural systems, parasitoids increase their performance as biological control agents when floral and extrafloral nectar are available (Berndt et al., 2006; Bianchi et al., 2006; Gurr et al., 2017; Heimpel & Jervis, 2005; Lee & Heimpel, 2003; Simpson et al., 2011; Stephens et al., 1998; Tena et al., 2015; Tylianakis et al., 2004). By exploring these parasitoids' specific nutritional requirements and behaviour, it is possible to improve the success of *B. cephi* and *B. lissogaster* as natural enemies and envision a more successful biological control programme for wheat stem sawfly Nevertheless, we encourage more studies to further investigate flight performance, egg composition, and fertility for these parasitoids. In addition, there is a need to explore how other more complex nutritional sources, such as floral and extrafloral nectar and their compositions, could affect these parasitoids. It would then be possible to better understand their nutritional needs and how they benefit the performance of these species as natural enemies of a key agricultural pest.

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CONFLICT OF INTEREST

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 paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

Human and animal rights

This work does not include studies with any animal (vertebrates) or human subjects performed by any of the authors.

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Permission to collect wheat samples

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SUPPORTING INFORMATION

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

Appendix S1. Supporting Information.

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