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Sugar concentration and timing of feeding affect feeding characteristics and survival of a parasitic wasp

Livy Williams III [a,*], Pauline Deschodt [b], Olivia Pointurier [b], Kris A.G. Wyckhuys [c]

[a] USDA-ARS European Biological Control Laboratory, Montferrier sur Lez, France
[b] Montpellier SupAgro, Montpellier, France
[c] CGIAR-CIAT, Hanoi, Viet Nam

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ABSTRACT

The availability of food sources is important for parasitoid survival, especially for those that inhabit ecosystems where nectar and honeydew are spatially or temporally scarce. Therefore, the value of even a single meal can be crucial for survival. *Psyttalia lounsburyi* is a parasitoid, and biological control agent, of the olive fruit fly, *Bactrocera oleae*. In order to improve our understanding of the basic nutritional ecology of *P. lounsburyi* and its role in survival we evaluated the effect of a single sucrose meal on the longevity of female and male wasps. We measured the duration of feeding, volume ingested, sucrose consumption, energy content, and longevity of wasps provided with different concentrations of sucrose (0.5, 1, and 2 M) at different times after emergence (0, 1, 2 or 3 days after emergence). Our results showed that longevity was significantly influenced by sucrose concentration and timing of feeding. For females, feeding on sucrose increased the likelihood of survival to varying degrees, ranging from 32.3% to 95.4%, compared to water-only controls. The longest duration of feeding was observed for the highest sucrose concentrations and oldest wasps. The amount of sugar ingested and energy uptake increased, up to a point, as sugar concentration increased. Our results suggest that *P. lounsburyi* derived greatest benefit from the intermediate concentration (1 M) of sucrose provided 2 or 3 days after emergence. Our study emphasizes the importance of finding balance between increasing longevity and limiting the duration of feeding, and concomitant uptake of nutrients, that is fundamental for survival of the wasp in nature.

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1. Introduction

Many species of parasitic wasps require carbohydrate food sources to satisfy metabolic energy needs. Floral and extrafloral nectar, and honeydew excreted by homopteran insects, provide sugar-foraging wasps with rich sources of carbohydrates which generally increase longevity and subsequent rates of parasitism (Géneau et al., 2013; Irvin et al., 2007; Jamont et al., 2014; Lee et al., 2004; Lee and Heimpel, 2008a; Sivinski et al., 2006; Winkler et al., 2006; Wyckhuys et al., 2008). Theoretical and empirical evidence suggests that parasitoid food sources play an important role in regulation of host population dynamics (Heimpel and Jervis, 2005; Jervis et al., 1996; Sabelis et al., 2005). Consequently, the presence of suitable carbohydrates for foraging parasitoids is an important factor in the development of habitat management strategies aimed at enhancing the effectiveness of biological control agents against agricultural pests (Heimpel and Jervis, 2005; Landis et al., 2000; Orre Gordon et al., 2012).

Successful sugar foraging depends on the availability of suitable nectar or honeydew at the time of foraging. However, considerable variability exists in the spatial and temporal availability of food sources, especially in manipulated environments, such as agricultural systems. Despite this, most studies evaluating food resources of parasitoids have permitted feeding *ad libitum*; little work has been done where feeding frequencies are limited. Most studies that have examined feeding frequency suggest that longevity is optimized when sugar-feeding occurs daily (Azzouz et al., 2004; Fadamiro et al., 2005; Lee and Heimpel, 2008b; Siekmann et al., 2001; but see Dyer and Landis, 1996; Fadamiro and Heimpel, 2001; Krugner et al., 2005). Sugar concentration and composition in nectar and honeydew can also vary greatly within and between individual plants and over time (Baker and Baker, 1983a,b; Byrne et al., 2003; Heil, 2011). The viscosity of a sugar solution generally increases as the concentration increases, (Chirife and Buera, 1997; Kingsolver and Daniel, 1995; Nithiyanantham and Palaniappan,

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* Corresponding author at: USDA-ARS European Biological Control Laboratory, Campus International de Baillarguet, CS 90013 Montferrier-sur-Lez, 34988 St. Gely du Fesc Cedex, France.

E-mail address: lwilliams@ars-ebcl.org (L. Williams III).

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2013); in turn, increased viscosity usually slows a parasitoid's rate of feeding, i.e., the rate of sugar uptake (Siekmann et al., 2001; Wyckhuys et al., 2008). Also, increased time spent feeding or resting afterwards may put a parasitoid at greater risk of attack by natural enemies (Lightle et al., 2010; Völkl and Kroupa, 1997; Wäckers, 2005), and may decrease the time spent searching for and attacking hosts. Thus, the concentration-viscosity relationship represents complicated tradeoffs that will influence a parasitoid's rate of nutrient uptake and survival, as well as vulnerability to natural enemies and reproductive success.

Psyttalia lounsburyi (Silvestri) (Hymenoptera: Braconidae) is an African parasitoid of the olive fruit fly, *Bactrocera oleae* Gmelin (Diptera: Tephritidae) (Copeland et al., 2004; Wharton and Gilstrap, 1983) that has been imported into California as part of a classical biological control program (Daane et al., 2008). *P. lounsburyi* is synovigenic, and adults do not host feed. Little is known about the nutritional ecology of this parasitoid. Although observations of adult *P. lounsburyi* feeding in the field are lacking, wasps apparently rely on food sources such as nectar and honeydew. However, commercial olive orchards are characterized by a paucity of food resources for *P. lounsburyi*, and thus opportunities for successful foraging are at a premium. Given the low likelihood of food encounters in an olive orchard, the benefit to *P. lounsburyi* from even a single meal may be crucial for survival.

Our study aims at evaluating the effect of a single sucrose meal on the longevity of *P. lounsburyi* under laboratory conditions. Since wasps can encounter varying concentrations of sugar in the field, meals were offered at three concentrations representative of those found in nature. Also, we tested the temporal effects of sugar meals by provisioning the wasps on different days after emergence. We predicted that optimal beneficial effects of sugar-feeding would occur at a sucrose concentration that permitted maximum rate of energy uptake, and at a wasp age >1 day post-emergence (after depletion of energy reserves accumulated during the larval period).

2. Materials and methods

2.1. Insect rearing

The stock of *P. lounsburyi* used in this study originated from *B. oleae* on wild olive, *Olea europaea* L. subsp. *cuspidata* (Wall. ex G. Don), in the Burguret Forest, Kenya in 2005. Since then, the parasitoids have been maintained as a laboratory colony in the quarantine facility of the USDA-ARS European Biological Control Laboratory, Montferrier-sur-Lez, France. After the initial collection in Africa, parasitoids were reared for a few months on *B. oleae* in olives, but in May 2005 the parasitoids were reared continuously (90 generations) using a factitious host, *Ceratitis capitata* (Diptera: Tephritidae) on artificial diet (Wong and Ramadan, 1992) (23 °C ± 1, 45% r.h., L:D 16:8). This colony of *P. lounsburyi* is infected with two variants of *Wolbachia* endosymbionts (Cheyppe-Buchmann et al., 2011).

After emergence from rearing chambers, male and female parasitoids were placed together (ca. 30 total) to allow mating in a 1-l plastic food container (No. DM32, SOLO Cups Co., Urbana, IL) containing a cotton wick soaked with distilled water. When only females emerged, older males were added to the cage. The food container was covered with women's hosiery (L'eggs Knee Highs, Sara Lee Hosiery, Rural Hall, NC) to allow ventilation. The parasitoids were provided with only distilled water until experimentation.

2.2. Experimental procedure

Three concentrations of sucrose (Fluka Analytical, Inc., catalog No. 84097, >99.5%) solutions were used as food treatments in the experiments: 0.5, 1 and 2 M (17.1, 34.2, and 68.5% w/w, respectively). Sucrose is one of the major components of nectar and honeydew (Baker and Baker, 1983a,b; Byrne et al., 2003). Test solutions were prepared in 20 ml glass scintillation vials and were stored at 4 °C. Fresh test solutions were prepared weekly. The study was conducted with female and male wasps. Parasitoids were fed with sucrose only once after emergence, either on the day of emergence (day 0) or 1, 2, or 3 days after emergence (after 4 days without food most wasps would have starved to death). Thus, days 0, 1, 2, and 3 are days of feeding, hereafter referred to as 'wasp age'. For each wasp age, wasps (ca. 20 females; ca. 17 males) were assigned to each food treatment, i.e., to one of the three sucrose concentrations and to distilled water only controls.

For each assay, a known volume, ranging from 2 to 10 µl of sucrose solution (warmed to 23 °C) was pipetted into a 45 ml plastic vial (12 dram, No. 55-12, Thornton Plastics Co., Salt Lake City, UT). A wasp was aspirated into the vial, after which the vial was capped, and wasp behavior was observed at 20× under a dissecting scope. Each wasp was allowed 3 min to explore the vial and encounter the sugar droplet; after this time a wasp that had not encountered the droplet was discarded and a new trial was begun. Feeding was observed as uninterrupted ingestion, i.e., movement of the mouthparts on the droplet and distention of the abdomen. Wasps were allowed to feed uninterrupted until satiated. Termination of feeding was defined as withdrawing the mouthparts from the droplet, followed by walking away from the droplet and/or grooming. Sometimes wasps fed in several bouts; in this case a wasp would withdraw its mouthparts but remain oriented toward the droplet for several seconds before resuming ingestion. For each trial, we recorded the total duration of feeding (measured to the nearest second with a lab timer).

After a trial was terminated, the wasp was gently aspirated from the vial, and was transferred alone into a ventilated 1-l plastic food container as described above, where it was provided with distilled water-soaked cotton and held in a climate-controlled room (23 °C ± 1, 45% r.h., L:D 16:8) until death. Water was added to the cotton twice a day (0900–1000 h and 1700–1800 h) when wasp mortality was also recorded. Wasps not exhibiting repetitive (non-reflex) movement were considered dead.

The volume of sugar solution ingested was determined for ca. 70 of the 2 day old female wasps by measuring the amount of solution which remained in the vial after cessation of feeding. This was accomplished by using a micropipet (10- or 20-µl) to recover the remaining solution not ingested by the wasp. The length of the solution column was measured under the dissecting scope using a graduated reticle. This number was then divided by the length of the micropipet from the tip to the calibration mark and multiplied by the capacity of the micropipet to give volume (µl) sugar solution collected. The difference between the volumes of sugar solution at the beginning and end of each trial represented the volume ingested by the wasp. A clean, dry micropipet was used for each trial. From these data, the amount of sucrose consumption (mg sucrose = molarity × µl ingested × molecular weight × 0.001) and energy content (Joule (J) ingested = 16.8 J × mg sucrose ingested) were calculated for each wasp. Value for energy content (16.8 J/mg sucrose) reported by Dafni (1992).

2.3. Data analysis

Survival data were analyzed by the Cox Proportional Hazards Analysis, where sucrose concentration and wasp age were tested as covariates (i.e., explanatory variables) (SAS Institute, 2003). After the initial analysis establishing differences for gender, wasp age and sucrose concentration, survival analyses were conducted for each gender × wasp age combination. Differences in survival curves for each day were analyzed by likelihood ratio tests. After this,