MEASURING PARASITOID CARBOHYDRATE LEVELS TO IMPROVE BIOLOGICAL CONTROL

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ABSTRACT

Lifetime carbohydrate levels were compared in the laboratory between unfed and honey-fed *Microctonus hyperodae* (Hymenoptera: Braconidae), a parasitoid of the Argentine stem weevil, *Listronotus bonariensis* (Coleoptera: Curculionidae). High performance liquid chromatography (HPLC) was used to analyse sugars in individual parasitoids and showed that fed and unfed parasitoids can be distinguished by measuring the total sugar levels. Furthermore, the fructose/total sugar ratio (f/T) of honey-fed *M. hyperodae* was always higher than that of the unfed individuals. This method should prove very useful for determining the nutritional status of field-collected parasitoids, and for evaluating the benefits gained by parasitoids through the provision of floral resources in the field.

INTRODUCTION

Carbohydrates are the main energy source for adult parasitoids, which feed on several different sugar sources, such as floral and extrafloral nectar, and aphid honeydew (Jervis et al. 1993). If the energy reserves that parasitoid adults carry over from their larval stage are not supplemented by additional sugar sources, then their activities could be curtailed. Many laboratory experiments have shown that sugar can increase the longevity and fecundity of parasitoids (Wäckers 2001; Gurr et al. 2004; Vattala et al. 2004), and can even change their sex ratios (Berndt & Wratten 2005). Also, parasitoid host searching activity (Takasu & Lewis 1995; Landis et al. 2000) and parasitism rates (Leius 1967) can be increased in the field by provision of sugar sources.

Several studies have examined changes in nutrient levels during the lifetimes of parasitoid adults. Olson et al. (2000) used the anthrone test (van Handel 1967) to quantify changes in the levels of fructose, glycogen and total sugars in fed and unfed parasitoids. Wäckers & Steppuhn (2003) and Steppuhn & Wäckers (2004) developed a more sensitive method using HPLC to identify and quantify a range of sugars in individual parasitoids. These studies showed that fed parasitoids had higher sugar levels than unfed parasitoids, at least for the initial part of their adult life, and that they lived longer.

The endoparasitoid *Microctonus hyperodae* Loan (Hymenoptera: Braconidae) investigated in this study is an introduced biological control agent of *Listronotus bonariensis* (Kuschel) (Coleoptera: Curculionidae) (McNeill et al. 2002). Previous studies have shown that the longevity of *M. hyperodae* adults can be significantly increased by provision of either glucose or honey solutions (Hodgson et al. 1993; Phillips 1998). Unfed *M. hyperodae* adults lived 10 days with water in the laboratory (Hodgson et al. 1993; Vattala et al. 2004), which was an unexpectedly long time because many other parasitoids live only 2–3 days without sugar. More recent laboratory experiments have shown that *M. hyperodae* will feed from flowers and that nectar can

increase its longevity to 21 days (Vattala et al. 2004). A field study of *M. hyperodae* and *L. bonariensis* population dynamics showed that adult parasitoids are abundant but hosts are scarce during early summer (Phillips et al. 1998). Analysis indicated that enhancing *M. hyperodae* longevity during this period, perhaps through provision of nectar sources, could improve suppression of *L. bonariensis* (Phillips et al. 1998).

To exploit this opportunity to improve biological control, it is important to gain a better understanding of how sugar sources influence the energy reserves of *M. hyperodae* adults. Accordingly, this study addressed the following questions: What is the mean body sugar level in recently eclosed *M. hyperodae* adults and how does this change over time if they do not have access to sugar? What are the effects of sugar feeding on *M. hyperodae* body sugar levels? Is it possible to develop a biochemical method to discriminate between fed and unfed *M. hyperodae*?

MATERIALS AND METHODS

Adult *L. bonariensis* were collected by sweeping grasses at night, either at the Lincoln University Dairy Farm, or from roadside verges near Lincoln. Weevils were maintained in the laboratory at 20°C ($\pm 2^\circ\text{C}$) and 16:8 h light:dark photoperiod to allow parasitoids to emerge and develop to the pupal stage. Rearing methods for parasitoids followed Goldson et al. (1993). Cages were checked for cocoons every 24 h and all cocoons were transferred to Petri dishes that were kept under the conditions noted above. A cotton bud soaked in water was placed in each Petri dish to maintain high humidity. The dishes were checked every 24 h for newly emerged parasitoids.

Newly emerged parasitoid adults were maintained separately in individual Petri dishes. The two treatments involved providing parasitoid adults with *ad libitum* access either to 50% honey solution or water only. These liquids were made available on a piece of cotton wool placed in each Petri dish and were replenished every 2 days. As they emerged, parasitoid adults were randomly assigned to a treatment using a table of random numbers. A sub-sample of parasitoids from the honey treatment was frozen daily at -80°C from age 1 to 9 days, and every second day thereafter, up to the maximum longevity of 19 days. In the water treatment, a sub-sample of parasitoids was frozen at -80°C daily from age 1 to the maximum longevity of 8 days. The number of replicates for each age category ranged from 3 to 10 parasitoids per treatment. In addition, twelve recently emerged parasitoids were not subjected to the experimental treatments, but were frozen at -80°C for subsequent analysis of sugar levels.

Before transferring parasitoids from the freezer to 70% ethanol for analysis of sugar levels, parasitoid size was estimated by measuring the length of the hind tibia (Phillips & Baird 2001). HPLC analysis was conducted as described by Steppuhn & Wäckers (2004) to detect the following sugars in *M. hyperodae*: sorbitol, manitol, trehalose, glucose, fructose, melibiose, sucrose, melezitose, raffinose, maltose and erlose.

SPSS 12 was used for statistical analysis. The ratio of fructose to total sugars (f/T) was not normally distributed and therefore non-parametric statistics were used. For parasitoids aged from 2 to 8 days, within day comparisons between the two treatments were made using the Mann-Whitney U-test.

RESULTS

The major fraction of total sugars of newly-emerged parasitoids was glucose (64%), followed by maltose (17%) and fructose (12%). In addition, small amounts of sucrose and sorbitol were detected. Trehalose was not detected in newly-emerged parasitoids.

The mean sugar level of newly emerged parasitoids was $17.3 \pm 2.55 \mu\text{g}$ ($\pm \text{SE}$; $n = 12$). The total sugar level declined with age in unfed parasitoids (Fig. 1) and had declined to 15% of that at emergence by the eighth day. Glucose was the main sugar detected in unfed parasitoids. *Ad libitum* honey feeding significantly increased the total sugar level of *M. hyperodae*. On day 2 (after honey had been available for 24 h) the total sugar level increased to $73.0 \mu\text{g}/\text{parasitoid}$. The sugar level reached its maximum of $154.7 \mu\text{g}/\text{parasitoid}$ on day six (Fig. 1). Total sugar levels subsequently declined to $12.3 \mu\text{g}/\text{parasitoid}$ on the nineteenth day.

FIGURE 1: Total mean sugar level (μg per parasitoid + SE) of fed ($y = -1.09x^2 + 17.53x + 31.31$ and $r^2=0.42$) and unfed ($y = -2.26x + 19.91$ and $r^2=0.40$) *M. hyperodae* from day 1 to day 19.

Fed parasitoids exhibited higher fructose levels than unfed parasitoids throughout their lifespans ($P<0.01$). Fructose levels were approximately equal to those of glucose in fed parasitoids, but were much lower in unfed parasitoids. The common insect haemolymph sugar, trehalose, was not detected in unfed *M. hyperodae*, but was detected in fed parasitoids after honey had been available for 24 h, and was thereafter continuously present until day 13. Trehalose levels in fed parasitoids remained extremely low compared with other sugars, and the maximum level recorded was $1.3 \mu\text{g}/\text{parasitoid}$ on day 9.

The ratio of fructose/total sugars (f/T) for newly-emerged parasitoids was 0.1157. The f/T ratio was always lower than 0.1157 for unfed parasitoids and always higher for fed parasitoids (Fig. 2). There was no significant relationship between total body sugar levels and tibia length ($P>0.05$ and $r^2 = 0.13$).

FIGURE 2: Fructose/total sugar (f/T) ratio of recently-emerged, unfed and fed parasitoids. Differences between fed and unfed parasitoids are significant by day 2 (24 h after feeding, $P<0.01$). Error bars are one standard error of the mean.

DISCUSSION

In previous laboratory experiments, unfed *M. hyperodae* lived 10 days with water only (Hodgson et al. 1993; Vattala et al 2004). This is of interest because other parasitoid species studied in similar experiments lived only 2-3 days (Steppuhn & Wäckers 2004; Scarratt 2002). HPLC analysis revealed that female *C. glomerata* and *M. mediator* had almost depleted their body sugars by the second day following emergence (Steppuhn & Wäckers 2004). In contrast, body sugar in unfed *M. hyperodae* declined to 50% of its initial level in about 5 days, and to 15% by the eighth day (Fig. 1). This apparently more gradual decline in total body sugar may facilitate the survival of *M. hyperodae* in the absence of sugar sources. This might be an interesting adaptation because the main habitats of this parasitoid are grasslands, which are often depauperate in nectar sources.

Fructose levels were clearly different between fed and unfed parasitoids. Newly-emerged and unfed parasitoids always had low fructose levels, thus their f/T ratios were also low. In contrast, fructose levels in fed parasitoids increased immediately after feeding, and thereafter remained equal to those of glucose, giving a f/T ratio high throughout their lifespans. This indicates the f/T ratio can provide a clear indication of whether or not a parasitoid has fed, and should prove very useful for evaluating the extent to which flowers are used by parasitoids in the field.

The limited data available on the rate of depletion of parasitoid carbohydrate reserves have all been derived from laboratory experiments (Scarratt 2002; Steppuhn & Wäckers 2004). In the present study, carbohydrate reserves were measured in parasitoids that were confined in Petri dishes, thus parasitoid flight did not occur, walking was greatly restricted and oviposition in hosts was impossible. In contrast, parasitoid activity should be greatly increased in the field because host searching must occur in a large and complex environment, and the act of ovipositing in hosts may demand significant additional energy. This indicates carbohydrate reserves in unfed parasitoids could become depleted much more quickly in the field than has been observed in laboratory experiments. In contrast, assuming the longevity of parasitoids is not strongly limited by predation and other mortality factors, it is plausible that parasitoids which have access to sugar in the field could replenish their carbohydrate reserves sufficiently frequently to live approximately as long as fed parasitoids in the laboratory. Thus, the beneficial effects of sugar feeding by parasitoids in the field could be even greater than those currently suggested by laboratory experiments. Additional laboratory experiments which more realistically simulate field conditions by using larger cages which contain plants and hosts could be used to make an initial assessment of this possibility.

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