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Comparing the effects of five naturally occurring monosaccharide and oligosaccharide sugars on longevity and carbohydrate nutrient levels of a parasitic phorid fly, *Pseudacteon tricuspis*

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Abstract. The longevity and nutrient levels of *Pseudacteon tricuspis* provided with 1 M solutions of five naturally occurring sugars, fructose, glucose, sucrose, trehalose and melezitose, are compared. All but melezitose, result in significant increases in the longevity of *P. tricuspis* in comparison with sugar-starved flies (flies provided with water only). Sugar-starved female and male *P. tricuspis* have an average longevity of 3.3 and 4.1 days, respectively. Provision of free water in addition to sugar solution is necessary for optimum longevity by female and male flies. Longevity is increased by 2.4–2.6-fold by the two monosaccharides, fructose and glucose, and by 2.6–2.8-fold by the disaccharides, sucrose and trehalose. Phorid flies provided with the trisaccharide sugar, melezitose, had a marginal increase in lifespan (approximately 1 day), but this is not significantly different from the longevity of sugar-starved flies. Significantly greater levels of total sugars are detected in *P. tricuspis* fed the disaccharide sugars (sucrose, trehalose) or the monosaccharide sugars (fructose, glucose), compared with flies provided with melezitose (trisaccharide), or to sugar-starved flies. Fructose is not detected in sugar-starved flies, or in flies fed glucose or trehalose. However, high levels of fructose are detected in flies fed sucrose or fructose, whereas levels of fructose in melezitose-fed flies are intermediate. In general, significantly greater glycogen levels are detected in *P. tricuspis* fed sucrose, glucose, trehalose or fructose, compared with melezitose-fed or sugar-starved flies. Levels of total sugars and glycogen in sugar-fed flies are positively correlated with wing length, possibly indicating a higher accumulation of storage sugars by larger flies. These results are discussed in relation to the nutritional ecology of the phorid fly.

Key words. Biological control, fructose, glycogen, melezitose, *Pseudacteon tricuspis*, sucrose, sugar feeding, trehalose.

Introduction

Adult parasitoids of many species are known to require carbohydrate meals to achieve maximum longevity and reproduction (Jervis *et al.*, 1996; Heimpel *et al.*, 1997; Fadamiro & Heimpel, 2001). In field situations, various species of hymenopteran parasitoids have been observed feeding on naturally occurring sugar sources, such as floral and extrafloral nectar (Leius, 1960; Syme, 1975; Bugg *et al.*, 1989; Jervis *et al.*, 1993) and homopteran honeydew (Idoine & Ferro, 1988; Evans, 1993). Several studies have demonstrated that feeding on floral nectar and aphid honeydew can increase adult parasitoids longevity and/or fecundity (Hagley & Barber, 1992; Idris & Grafius, 1995; Jervis *et al.*, 1996; England & Evans, 1997; Stapel *et al.*,

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1997; Wäckers, 2001; Lee *et al*., 2004). This basic knowledge is now commonly applied in biological control programmes in many agroecosystems in the form of habitat management involving planting of nectar-producing floral vegetation within or near crops, or application of artificial sugar sources (Baggen & Gurr, 1998; Jacob & Evans, 1998; Rogers & Potter, 2004; Heimpel & Jervis, 2005). However, naturally occurring sugar sources may differ in their suitability to different species of natural enemies.

Several studies have identified significant variations in sugar composition, quality, quantity and concentration between the principal naturally occurring sugar sources in the field (van Handel *et al*., 1972; Baker & Baker, 1983; Wäckers, 2001). Sucrose and its two monosaccharide components, glucose and fructose, are key components of nectar and honeydew (van Handel *et al*., 1972; Baker & Baker, 1983; Wäckers, 2001). However, honeydew also contains some signature sugars, including the disaccharide, trehalose, and the trisaccharides, melezitose and erlose (Baker & Baker, 1983; Rhodes *et al*., 1997; Wäckers, 2001). The presence of these complex sugars in honeydew may influence its utilization by different insect species. In addition, some honeydew-specific sugars, such as melezitose, also have a tendency to crystallize within a short time (Wäckers, 2000, 2001), and this property may further impact their utilization by parasitoids.

Under field conditions, parasitoids have been demonstrated to feed both on nectar and on honeydew sources. The relative contribution of these naturally occurring sugar sources to the diet of parasitoids is likely dependent on the species and on the relative availability of nectar and honeydew sources. Studies comparing the relative suitability and utilization of floral nectar and honeydew by parasitoids have yielded different results for different species. Several hymenopteran species have been found to utilize floral nectar more than honeydew as sugar sources (Avidov *et al*., 1970; McDougall & Mills, 1997; Lee *et al*., 2004). In a recent study, Lee *et al*. (2004) compared the effects of floral nectar from buckwheat, *Fagopyrum esculentum*, and honeydew produced by the soybean aphid, *Aphis glycines*, on longevity, nutrient levels and egg loads of the parasitoid *Diadegma insulare*. The authors reported a significant increase in longevity for wasps fed on aphid honeydew compared with sugar-starved wasps; however, buckwheat nectar was superior to aphid honeydew, resulting in significantly greater longevity and body nutrient levels. By contrast, Idoine & Ferro (1988) reported that the egg parasitoid *Edovum puttler* utilizes aphid honeydew rather than flower nectar as a food source in the field. Similar results indicating utilization of honeydew have also been recorded for several other parasitoids including *Bathyplectes curculionis* (England & Evans, 1997) and *Cotesia glomerata* (Wäckers & Steppuhn, 2003).

The majority of the research comparing the acceptance or utilization of individual sugar components by parasitoids has focused on hymenopterans (Leatemia *et al*., 1995; Morales-Ramos *et al*., 1996; McDougall & Mills, 1997; Wäckers, 2001; Beach *et al*., 2003). Beach *et al*. (2003) reported that several individual components of nectar and honeydew sugars are readily accepted by *Anaphes iole*. Higher longevity is recorded for *C. glomerata* females provided with individual solutions of sucrose, glucose or fructose than females provided with the honeydew-specific sugars, melezitose or erlose, possibly indicating that honeydew sugars may be less suitable to the species (Wäckers, 1999). Given this indication of variation in the preferences and utilization of sugars by different parasitoid species, an evaluation of the relative suitability of individual sugar components may provide insights regarding the suitability and field utilization of different naturally occurring sugar sources by different parasitoid species. However, our current knowledge of the ability of several parasitoid species to utilize different types, sources and components of sugar remains weak.

In one of the few studies on the utilization of naturally occurring sugar sources by a nonhymenopteran parasitoid, the suitability of nectar and homopteran honeydew as food sources for the phorid fly, *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae) has been investigated (Fadamiro & Chen, 2005). *Pseudacteon tricuspis* is a species of phorid fly currently being released and monitored in the southern U.S.A. for the biological control of the red imported fire ant, *Solenopsis invicta* (Gilbert, 1996; Porter *et al*., 1999, 2004). Fadamiro & Chen (2005) reported that female and male *P. tricuspis* are capable of utilizing cotton aphid honeydew with a modest but significant increases in adult longevity and body nutrient levels. An approximately 1-day increase in longevity is recorded for phorid flies provided with cotton aphid honeydew compared with sugar-starved flies. However, this modest increase in longevity recorded for cotton aphid honeydew (Fadamiro & Chen, 2005) is significantly lower than the approximately 4–5-day increase in longevity recorded for flies fed sucrose solution in earlier studies (Chen *et al*., 2005; Fadamiro *et al*., 2005). To better understand the possible reasons for the reduced utilization of cotton aphid honeydew by *P. tricuspis* compared with sucrose solution, the present study tests the effects of some individual components of nectar and honeydew sugars on the longevity and body nutrient levels of the parasitic phorid fly. The quantification of the body nutrient levels of flies provided different types of sugar will allow the patterns of production of carbohydrate energy resources and reserves (e.g. glycogen) from the different sugar types to be documented and also provide additional data on their suitability as food sources for *P. tricuspis*. It is hoped that the results will contribute to the growing body of literature on the nutritional ecology and food preferences of this important parasitoid.

Materials and methods

Parasitoids

Pseudacteon tricuspis were reared on workers of red imported fire ant, *Solenopsis invicta*, at the fire ant

rearing facility of the USDA-ARS, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, Florida, U.S.A. as described by Porter *et al.* (1997). Parasitized fire ant worker heads were supplied by this facility in batches, and held in a plastic jar (25 × 13 cm) with a lid until emergence. No food or water source was provided in the jar. Newly emerged flies were removed promptly with an aspirator and sexed immediately under a dissecting microscope by the presence or absence of the distinct female ovipositor.

Sugars

The sugar types tested in this study were: D-fructose (fructose), D-glucose (glucose or dextrose), Sucrose (saccharose), D(+)-trehalose (trehalose) and D(+)-melezitose monohydrate (melezitose). Fructose, glucose and sucrose were purchased from Fisher (Fairlawn, New Jersey), whereas trehalose and melezitose were purchased from Sigma Chemical Co. (St Louis, Missouri). The sugars were labelled by the manufacturers as >99% pure. Solutions of each sugar were made in distilled water.

Longevity

The longevity of female and male *P. tricuspis* provided with six different diet treatments was compared: (i) water only (starved); (ii) fructose; (iii) glucose; (iv) sucrose; (v) trehalose; and (vi) melezitose. In the first experiment, a separate water source was not provided in the sugar treatments to test if phorid flies could obtain their water requirement from sugar solution. In the second experiment, water was provided in all treatments by filling a 0.5-mL microcentrifuge tube with distilled water and threading a cotton string through a hole in the cap of the tube, and water tubes were refilled as needed. Newly emerged flies were placed in groups of two individuals of the same sex in a 6-cm diameter plastic Petri dish. For the sugar treatments, approximately five drops (each drop approximately 1 μL) of 1 M of each sugar solution were smeared on the inside of the Petri dish cover with a cotton-tipped applicator. Petri dishes were kept in a growth chamber at 25 ± 1 °C, 75 ± 5% relative humidity (RH), under an LD 14 : 10 h photoperiod. Saturated brine was used to maintain a constant 75 ± 5% RH in the chamber (Winston & Bates, 1960). Female and male *P. tricuspis*, emerging on the same day, were distributed evenly across the treatment combinations. Fresh sugar solution was smeared on the dish cover every 3 days for all sugar treatments, with the exception of melezitose. Because of its tendency to crystallize, fresh melezitose solution was smeared on the dish daily. Crystallization of other sugars was rarely observed, possibly due to their hygroscopic nature and the high humidity conditions of the test chambers. Petri dishes were checked every day for parasitoid survival and dead flies were promptly removed. To determine the effect of size on longevity of flies provided with the different diets with or without free water source, one forewing was pulled from each dead fly, slide mounted and measured to the nearest 0.05 mm. Measurements were taken from the outer edge of the anal cell to the outer edge of the tip of the wing. In each experiment, 20 females and 16 males were tested for each diet treatment. Data were initially analysed by using proportional hazard modelling to test for the effects of diet, sex, wing length and interactions among the variables on survivorship (SAS Institute, 1998). Further data analysis was conducted for each sex by using analysis of variance (ANOVA) followed by the Tukey–Kramer HSD test for multiple comparisons of means at $P < 0.05$ (SAS Institute, 1998).

Body nutrient analyses

To document the temporal patterns of production of carbohydrate energy resources from different sugar types, daily changes in the levels of carbohydrate nutrients (fructose, total sugars and glycogen) were quantified in female and male *P. tricuspis* provided with the six diet treatments evaluated in the longevity experiment. It was hypothesized that the sugar types (diet treatments) that result in optimum longevity of *P. tricuspis* were also likely to result in the accumulation of optimal levels of carbohydrate energy resources (including glycogen reserves) in the flies. Subsequent to the test protocols described in the longevity experiment, newly emerged adult flies were placed in groups of two individuals of the same sex within 6-cm diameter plastic Petri dishes. Individuals emerging on the same day from the same batch were randomly assigned to the six treatments. For the sugar treatments, 1 M sugar solution was smeared (five drops) on the inside of the Petri dish cover with a cotton-tipped applicator. A separate water source was provided in all sugar treatments, as described in the longevity experiment. Petri dishes were kept in a growth chamber at 25 ± 1 °C, 75 ± 5% RH, under an LD 14 : 10 h photoperiod. Live flies from each treatment were collected, frozen at −20 °C and assayed daily from ages 1–5 and 7 days in all sugar treatments, with the exception of melezitose. Melezitose-fed flies rarely lived beyond 4 days under these test conditions; hence, nutrient assays were conducted only for 1–4-day-old flies provided with this trisaccharide sugar. Similarly, nutrient assays were conducted only for 1–3-day-old flies in the water-only treatment because flies provided with water only had a mean longevity of approximately 3 days under these test conditions. Before nutrient analyses, fly size was estimated using forewing length as described in the longevity experiment. Nutrient levels were quantified in newly emerged unfed (day 0) female ($n = 14$) and male ($n = 13$) phorid flies. The mean amount of each nutrient was calculated and considered as the teneral amount present in flies of each sex at emergence. Nutrient analyses were conducted on at least 10 individuals of each sex per day for each diet treatment.

Levels of carbohydrate nutrients (fructose, total sugars and glycogen) were biochemically quantified in individual flies using a series of biochemical tests originally developed by van Handel (1965, 1985a, b) for mosquitoes, and recently adapted for phorid fly by Fadamiro *et al.* (2005). Each fly was crushed with a plastic pestle in a 1.5-mL microcentrifuge tube containing 50 μL of 2% sodium sulphate solution and placed on ice. The dissolved nutrients were then extracted with 450 μL of chloroform-methanol (1 : 2), after which the tube was vortexed. The mixture was centrifuged at 17 090 ***g*** for 2 min and the supernatant was divided into two tubes each containing 200 μL, one for sugar assay and the other for lipid assay. However, lipid levels of flies used in this study were not analysed because adult *P. tricuspis* are not capable of converting dietary sucrose to lipids (Fadamiro *et al.*, 2005). The precipitate was left in the microcentrifuge tube for the glycogen assay. All tubes were heated at 90 °C until approximately 50 μL of solution was left in the sugar tube and all solution was evaporated from the glycogen tube.

To analyse fructose, 950 μL of anthrone reagent was added to the sugar tube, vortexed and left to react at room temperature for 1.5 h (cold anthrone test). After the reaction time had elapsed, the solution was poured into a 1.5-mL methacrylate cuvette and the optical density (absorbance) measured at 625 nm using a spectrophotometer. Absorbance readings were converted to absolute fructose amounts (μg), using fructose standard curves generated by determining the cold anthrone absorbance (at 625 nm) of different amounts (1–50 μg; three replicates per dose) of pure fructose (Fisher). This amount was then multiplied by a factor of 2.5 because only 200 μL of the original 500 μL was used for the fructose assay. The same solution used for the cold anthrone test was poured back into a glass tube, heated at 90 °C for 10 min and cooled on ice. The absorbance was again read at 625 nm to give an estimate of total sugars (hot anthrone test). Absorbance readings were converted to absolute amounts (μg), using sucrose standard curves generated by determining the hot anthrone absorbance (at 625 nm) of different amounts (1–50 μg; three replicates per dose) of pure sucrose (Fisher). The absolute amount of total sugars present in each fly was estimated by multiplying the amount of sugars from the hot anthrone test by 2.5 because only 200 μL of the original 500 μL was used for the hot anthrone assay.

For glycogen analysis, 1 mL of anthrone reagent was added to the microcentrifuge tube containing the glycogen precipitate. After vortexing, the tube was heated at 90 °C for 10 min and then cooled on ice and the absorbance read at 625 nm. Absorbance readings were converted to absolute glycogen amounts (μg), using glycogen standard curves generated by determining the absorbance (at 625 nm) of different amounts (1–50 μg; three replicates per dose) of oyster glycogen (ICN Biomedicals, Aurora, Ohio). The amount of glycogen estimated above was considered to be representative of the whole fly because all glycogen in the sample is presumed to precipitate to the bottom of the tube.

Data were log-transformed to equalize variances (SAS Institute, 1998), and analysed using multiple regression analysis to test the effects of diet, age, wing length and any two- or three-factor interactions on nutrient levels. Each sex was tested separately because male *P. tricuspis* are smaller and tended to have lower nutrient levels than females (Fadamiro *et al.*, 2005). Nutrient data were further analysed by using analysis of variance and Tukey–Kramer HSD for multiple comparisons (SAS Institute, 1998) to compare mean nutrient levels among flies of the same sex and age provided with the different diet treatments. Newly emerged flies were not included in the statistical analyses because they were not part of any of the diet treatments. Statistical analyses were run on absolute nutrient amounts rather than on absorbance values.

Results

Longevity

In the first experiment in which a separate water source was not provided in the sugar treatments, no significant effects of diet ($\chi^2 = 2.9$, d.f. $= 5$, $P = 0.71$), sex ($\chi^2 = 0.01$, d.f. $= 1$, $P = 0.95$), diet × sex interaction ($\chi^2 = 5.6$, d.f. $= 5$, $P = 0.34$), diet × wing length interaction ($\chi^2 = 2.8$, d.f. $= 5$, $P = 0.73$), sex × wing length interaction ($\chi^2 = 0.06$, d.f. $= 1$, $P = 0.81$) or diet × sex × wing length interaction ($\chi^2 = 2.4$, d.f. $= 5$, $P = 0.79$) were recorded on longevity. For example, mean longevity of sucrose fed females (3.2 ± 0.3 days) was not significantly different from the mean longevity of females provided with water only (3.6 ± 0.3 days) (Table 1). Similar results were obtained for male phorid flies; mean longevity was not different among the diet treatments (Table 1), indicating that phorid flies require a free water source to achieve optimum longevity. However, wing length had a significant effect on longevity ($\chi^2 = 7.9$, d.f. $= 1$, $P = 0.005$) of adult *P. tricuspis*. Further analysis of the data showed a significant positive linear relationship between wing length and longevity ($F = 11$, d.f. $= 1$, $P = 0.004$, $r^2 = 0.38$) only for female *P. tricuspis* provided with water only. No significant effect of wing length was recorded on longevity of male flies provided with water only ($F = 3.9$, d.f. $= 1$, $P = 0.07$,

Table 1. Mean ± SE longevity (days) of female and male *Pseudacteon tricuspis* provided with different sugar treatments without a free water source.

Diet	Females	Males
Water only	3.6 ± 0.3	3.3 ± 0.4
Fructose	2.7 ± 0.3	2.6 ± 0.3
Glucose	3.2 ± 0.4	2.3 ± 0.3
Sucrose	3.2 ± 0.3	2.4 ± 0.3
Trehalose	3.1 ± 0.3	2.3 ± 0.3
Melezitose	2.6 ± 0.3	2.2 ± 0.2
F	1.3	1.9
P	0.28	0.1

A total of 20 female and 16 male *P. tricuspis* were tested with each diet.

$r^2 = 0.23$). Similarly, the relationship between wing length and longevity was not significant for female and male flies in the sugar treatments.

In the second experiment in which a separate water source was provided in the sugar treatments, proportional hazard analysis showed that longevity of adult *P. tricuspis* was significantly affected only by diet ($\chi^2 = 63.5$, d.f. = 5, $P < 0.001$). No significant effects of sex ($\chi^2 = 2.9$, d.f. = 1, $P = 0.09$) or wing length ($\chi^2 = 0.6$, d.f. = 1, $P = 0.45$) were recorded on longevity. Furthermore, the interactions between diet and sex ($\chi^2 = 1.8$, d.f. = 5, $P = 0.88$), diet and wing length ($\chi^2 = 3.3$, d.f. = 5, $P = 0.65$), sex and wing length ($\chi^2 = 0.05$, d.f. = 1, $P = 0.82$) or diet, sex and wing length ($\chi^2 = 9.0$, d.f. = 5, $P = 0.10$) were not significant. ANOVA testing for the effect of diet on longevity of each sex separately showed a significant effect of diet on longevity of female ($F = 8.2$, d.f. = 5, $P < 0.001$) and male ($F = 9.2$, d.f. = 5, $P < 0.001$) *P. tricuspis*. All sugar treatments, with the exception of melezitose, resulted in significantly greater longevity of female and male *P. tricuspis* compared with flies provided water only (Table 2). The longevity of flies fed dissacharide sucrose or trehalose was not significantly different from longevity of flies fed either of the two monosaccharide sugars (fructose or glucose). Flies fed melezitose increased their longevity by approximately 1 day relative to those provided with water only, but this was not significant (Table 2). Survivorship curves showing longevity of flies fed the different diet treatments are shown in Figure 1. *Pseudacteon tricuspis* fed either of the two tested

Table 2. Mean ± SE longevity (days) of female and male *Pseudacteon tricuspis* provided with different diet treatments (free water source was provided in each sugar treatment).

Diet	Females	Males
Water only	3.3 ± 0.2^{b}	4.1 ± 0.2^{b}
Fructose	7.9 ± 0.9^{a}	9.9 ± 1.0^{a}
Glucose	8.5 ± 1.0^{a}	9.9 ± 1.3^{a}
Sucrose	8.6 ± 1.3^{a}	11.4 ± 1.4^{a}
Trehalose	8.9 ± 0.9^{a}	10.6 ± 1.1^{a}
Melezitose	4.3 ± 0.2^{b}	5.2 ± 0.5^{b}
F	8.2	9.2
P	<0.001	<0.001

A total of 20 female and 16 male *P. tricuspis* were tested with each diet. Means within the same column having different superscript letters are significant (Tukey–Kramer HSD, $P < 0.05$).

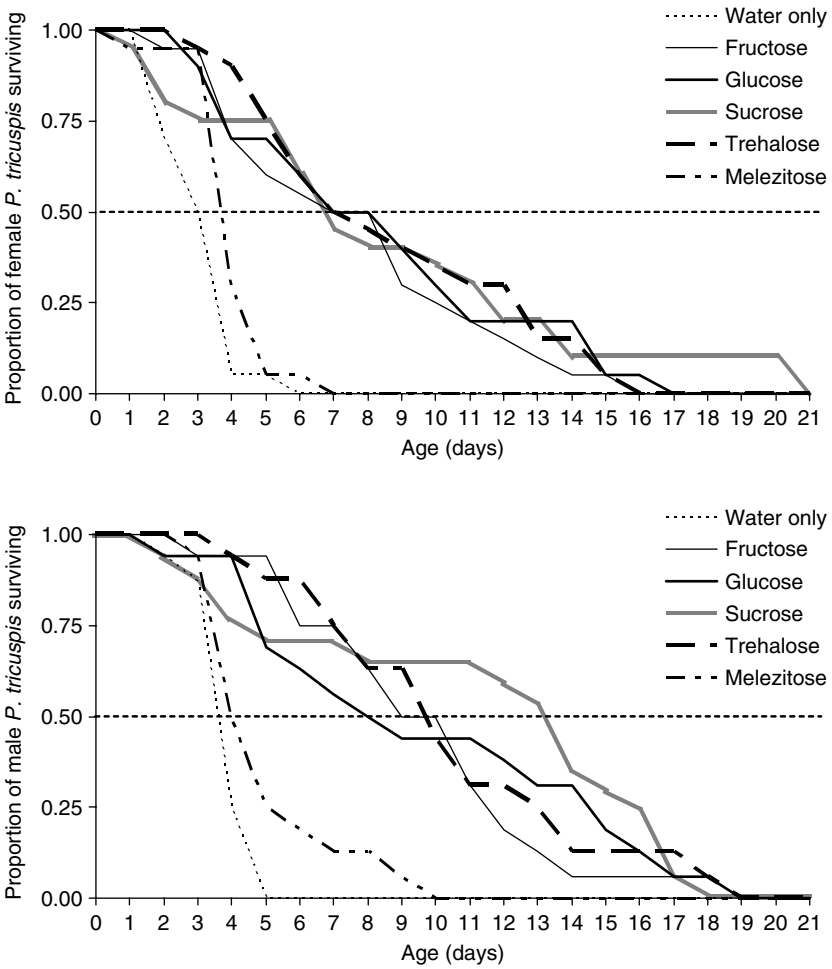

Fig. 1. Survivorship curves for female (A) and male (B) *Pseudacteon tricuspis* provided with different diet treatments. The dashed line at 0.5 survivorship indicates median longevity for each treatment.

disaccharide sugars (sucrose or trehalose) survived longest, with some individual flies surviving for up to 20 days compared with the maximum longevity of 4–5 days recorded for flies in the water-only treatment (Fig. 1).

Body nutrient analyses

Diet had a significant effect on fructose levels of female and male *P. tricuspis* (Table 3). No significant effect of age or wing length was recorded on the fructose levels of either sex, but a significant diet × age interaction was recorded on fructose levels of female flies (Table 3). As expected, fructose was not detected in sugar-starved flies (water-only treatment). Similarly, fructose was not detected in flies fed glucose or trehalose (a glucose-glucose dimer) (Fig. 2). However, significantly greater fructose levels were detected in female and male flies fed fructose or sucrose from day 1–7. Compared with levels in sugar-starved females or those fed glucose or trehalose, fructose levels in melezitose-fed females were not different on days 1 and 2, but were significantly greater on subsequent days, resulting in the recorded significant diet × age interaction (Fig. 2A). Generally, fructose levels in males fed melezitose were significantly lower than levels in sucrose- or fructose-fed males, and slightly higher than levels in sugar-starved males (Fig. 2B).

Multivariate analysis also showed significant effects of diet (female and male), age (female only) and wing length (female and male) on total sugar levels of *P. tricuspis* (Table 3). In general, significantly greater total sugar levels were detected in flies fed sucrose, trehalose, glucose or fructose, compared with flies provided with water only (Fig. 3). Females fed sucrose had significantly greater total sugar levels than females fed fructose or melezitose only on days 1 and 2, but not on subsequent days, leading to a significant diet × age interaction (Table 3; Fig. 3A). Endpoint (day 7) total sugar levels in females were significantly greater in the sucrose than in the trehalose treatment. Total sugar levels detected in melezitose fed males were consistently significantly below levels in males fed sucrose, trehalose, fructose or glucose, but greater than levels in sugar-starved males on day 1 (Fig. 3B). Data were further analysed to determine the source of the recorded significant relationship between wing length and total sugar levels. A significant linear relationship between wing length and total sugar levels was recorded only for females fed melezitose ($F = 15.2$, d.f. $= 1$, $P = 0.001$, $r^2 = 0.29$) or trehalose ($F = 3.9$, d.f. $= 1$, $P = 0.05$, $r^2 = 0.06$). Furthermore, wing length was positively correlated with total sugar levels of males fed fructose ($F = 4.6$, d.f. $= 1$, $P = 0.04$, $r^2 = 0.07$), glucose ($F = 5.4$, d.f. $= 1$, $P = 0.02$, $r^2 = 0.09$), sucrose ($F = 9.4$, d.f. $= 1$, $P = 0.003$, $r^2 = 0.14$) and trehalose ($F = 13.4$, d.f. $= 1$, $P = 0.001$, $r^2 = 0.19$) (Table 3).

Table 3. Multiple regression analyses testing for effects of diet, age, wing length (WL), the interaction between diet and age, diet and WL, age and WL, and diet, age and WL (D × A × WL) on nutrient levels of female and male *Pseudacteon tricuspis*.

		Females			Males		
	d.f.	MS	*F*	*P*	MS	*FP*	
Fructose							
Diet	5	76	55.3	<0.001	120	131	<0.001
Age	1	4.7	3.4	0.07	0.04	0.05	0.83
WL	1	0.46	0.34	0.56	2.2	2.4	0.12
Diet × Age	5	5.49	3.9	0.002	0.95	1.0	0.39
Diet × WL	5	1.3	0.93	0.46	0.88	0.96	0.44
Age × WL	1	0.40	0.29	0.59	0.01	0.01	0.94
D × A × WL	5	1.1	0.80	0.55	0.16	0.18	0.97
Total sugars							
Diet	5	14.6	16.8	<0.001	14.7	34.2	<0.001
Age	1	5.6	6.5	0.01	0.01	0.01	0.97
WL	1	11.3	13.0	<0.001	10.0	23.1	<0.001
Diet × Age	5	3.4	4.0	0.002	0.51	1.2	0.32
Diet × WL	5	0.84	0.97	0.44	0.56	1.3	0.26
Age × WL	1	1.1	1.3	0.25	1.9	4.3	0.04
D × A × WL	5	1.2	1.4	0.23	0.40	0.93	0.46
Glycogen							
Diet	5	4.0	8.8	<0.001	3.5	7.7	<0.001
Age	1	5.6	12.5	<0.001	2.7	5.9	0.02
WL	1	2.7	6.0	0.01	5.2	11.4	<0.001
Diet × Age	5	0.34	0.74	0.59	0.38	0.8	0.50
Diet × WL	5	0.40	0.87	0.50	0.26	0.55	0.74
Age × WL	1	2.3	5.0	0.03	1.7	3.7	0.06
D × A × WL	5	1.0	2.2	0.05	0.86	1.9	0.10

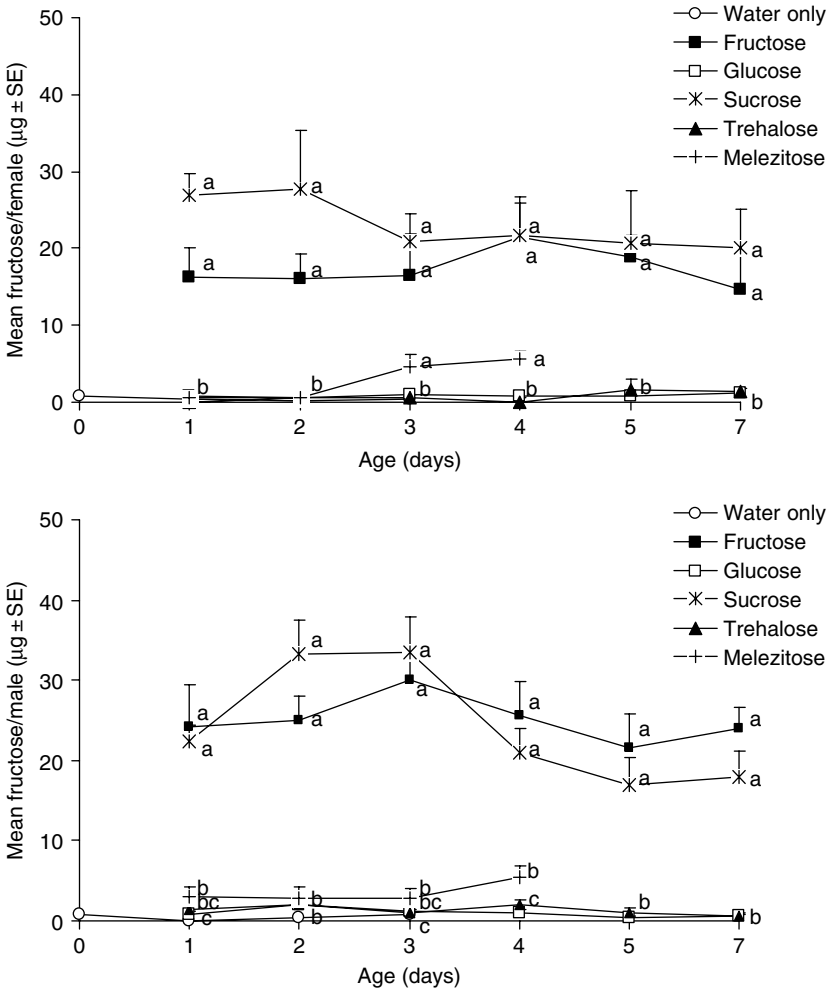

Fig. 2. Mean amounts (µg ± SE) of fructose in female (A) and male (B) *Pseudacteon tricuspis* provided with different diet treatments. Means for the same fly age having different letters are significant (Tukey–Kramer HSD, $P < 0.05$).

Glycogen levels were significantly affected by diet, age, and wing length in female and male *P. tricuspis* (Table 3). Significantly greater glycogen amounts were detected on days 1 and 2 in females fed sucrose, glucose, trehalose or fructose compared with females provided with water only (Fig. 4A). However, glycogen levels on day 3 were significantly greater only in sucrose-fed or trehalose-fed females than in females provided with water only. The highest glycogen levels were recorded on day 1, and decreased gradually thereafter, resulting in the recorded significant age effect. For males, glycogen levels in sugar-starved flies were significantly lower than levels in males fed sucrose, fructose, glucose, or trehalose (Fig. 4B). Levels of glycogen were slightly higher in melezitose-fed males than in sugar-starved males, but this was not significant (Fig. 4B). In general, the highest levels of glycogen were recorded in the sugar treatments on day 2 (Fig. 4B). The glycogen level was positively correlated with wing length only for females fed sucrose ($F = 11.3$, d.f. $= 1$, $P = 0.001$, $r^2 = 0.16$) or trehalose ($F = 10.2$, d.f. $= 1$, $P = 0.002$, $r^2 = 0.15$), and only for males fed sucrose ($F = 7.7$, d.f. $= 1$, $P = 0.007$, $r^2 = 0.12$).

Discussion

The results show that female and male *P. tricuspis* are able to feed on all the five sugars tested in this study. When a separate free water source is provided in the treatments, a significant increase in lifespan is recorded for flies fed solutions of fructose, glucose, sucrose or trehalose. However, the trisaccharide melezitose provides only a marginal (approximately 1 day), but insignificant increase in longevity compared with sugar-starved flies. The results show no effect of sugar feeding on longevity in the absence of a free water source. This suggests that this is necessary for *P. tricuspis* to achieve optimum longevity. Leatemia *et al.* (1995) compared utilization of solutions of sucrose or fructose by adult *Trichogramma minutum* and showed no significant differences in the longevity of wasps provided with each sugar solution. However, the mushroom phorid fly, *Megaselia halterata*, is reported to prefer certain honeydew sugars, such as sucrose, fructose and melezitose to lactose, maltose, glucose or D-mannitol (Binns, 1980). In one of the few studies investigating the utilization of sugars by nonparasitic Diptera, Kircher & Al-zawi (1985) reported

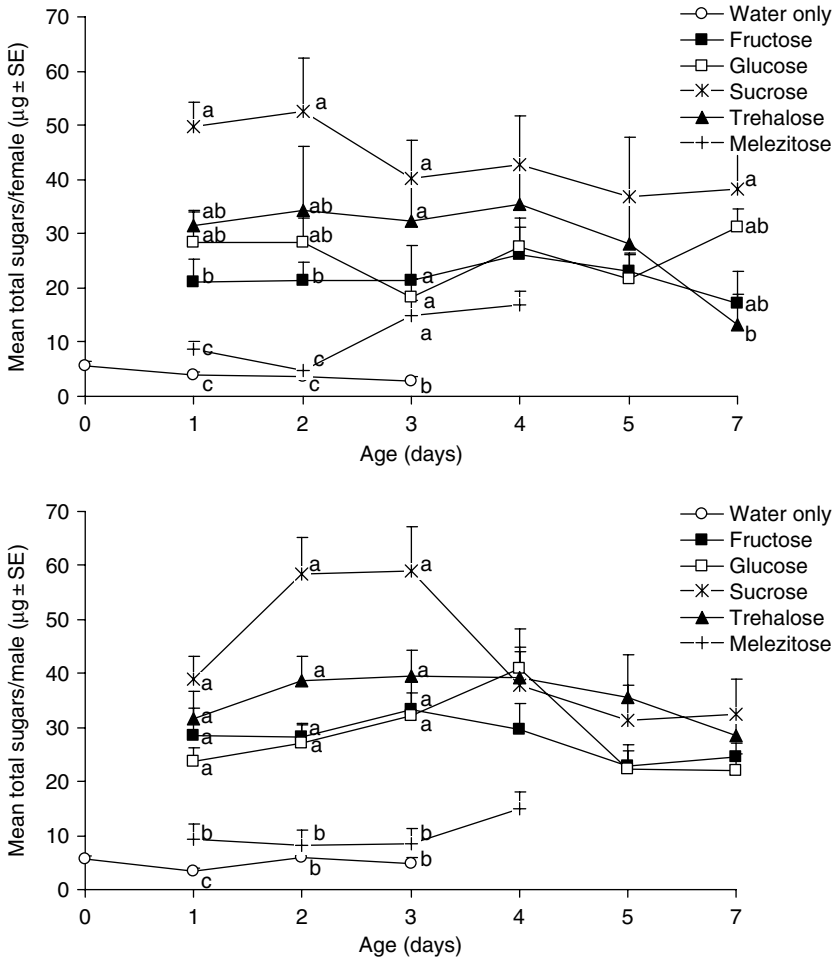

Fig. 3. Mean amounts (μg ± SE) of total sugars in female (A) and male (B) *Pseudacteon tricuspis* provided with different diet treatments. Means for the same fly age having different letters are significant (Tukey–Kramer HSD, $P < 0.05$).

that several species of *Drosophila* are able to utilize a wide range of sugars (including those tested in the current study) with significant increases in their longevity.

In one of the very few comprehensive evaluations of sugar utilization by parasitoids, Wäckers (2001) tested the effect of 14 naturally occurring individual sugars on longevity of *C. glomerata*. The longest lifespan was recorded with sucrose, fructose and glucose. Melezitose provides an intermediate increase in longevity, whereas trehalose provides only a marginally significant increase in lifespan. Furthermore, some oligosaccharide sugars, such as lactose and raffinose, are not utilized by the parasitoid, whereas rhamnose significantly reduces longevity compared with sugar-starved wasps. Similar negative effects of some sugars, including melezitose, on parasitoid longevity have been reported previously (Leius, 1961a, b; Avidov *et al.*, 1970). However, no negative effects of melezitose on longevity are observed in the current study, suggesting a moderate suitability of the trisaccharide to *P. tricuspis*. Sex has no significant effect on fly longevity, in accordance with previous studies (Fadamiro & Chen, 2005; Chen *et al.*, 2005; Fadamiro *et al.*, 2005). A positive correlation between longevity and wing length is recorded in the first longevity experiment in which free water is not provided in the sugar treatments, but this is only for female *P. tricuspis* provided with water only, possibly suggesting that, when sugar-starved, larger females could survive longer than smaller females by drinking more water.

The pattern of relative sugar utilization observed in the longevity experiment is further confirmed by the results of the body nutrient analyses of phorid flies provided the different sugar components. The greatest levels of body fructose are detected in flies fed sucrose or fructose. As expected, fructose is not detected in glucose-fed flies. This is because the cold anthrone test cannot detect glucose, because this monosaccharide does not react at room temperature. Similarly, fructose was not detected in trehalose-fed phorid flies. This is possibly due to trehalose, the dominant sugar in insect haemolymph and other tissues (Wyatt, 1967; van Handel, 1969), not being present in significant amounts in the gut. This may explain why significantly higher levels of total sugars are detected in trehalose-fed individuals. The total sugar estimate is a quantification of the amounts of the different sugars present in the insect, including gut sugars and the sugars in the haemolypmh and other body tissues. Feeding on sucrose, trehalose, glucose and fructose

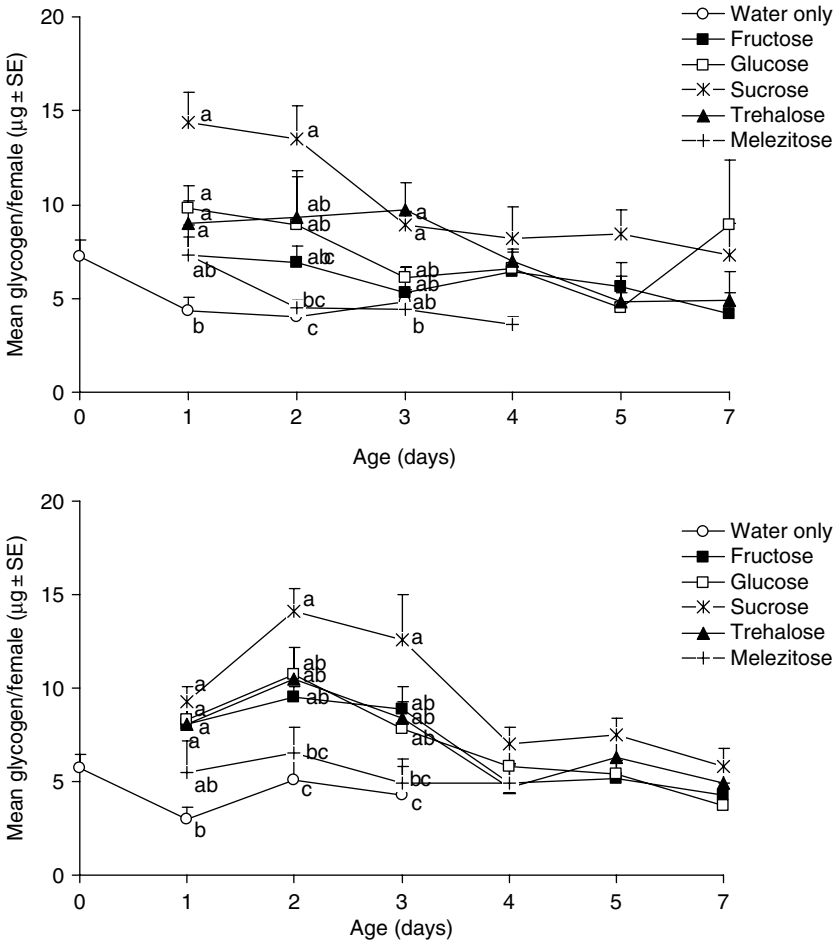

Fig. 4. Mean amounts (µg ± SE) of glycogen in female (A) and male (B) *Pseudacteon tricuspis* provided with different diet treatments. Means for the same fly age having different letters are significant (Tukey–Kramer HSD, $P < 0.05$).

(to a lesser degree) results in the accumulation of the greatest amounts of total sugars and glycogen. In general, very low amounts of fructose, total sugars, and glycogen are detected in melezitose-fed flies, corroborating the reduced utilization of this sugar by *P. tricuspis*. These results support the hypothesis that the sugar diet treatments that result in optimum longevity of *P. tricuspis* are also likely to result in the accumulation of optimal levels of carbohydrate energy resources. Similar patterns are recorded for females and males. Age has a significant effect on glycogen levels in female and male flies; glycogen levels gradually decrease with age. Levels of total sugars and glycogen in sugar-fed flies are positively correlated with wing length, possibly indicating higher accumulation of storage sugars by larger flies. These results are generally in agreement with previous reports on nutrient levels in *P. tricuspis* (Fadamiro & Chen, 2005; Fadamiro *et al.*, 2005).

The ability of *P. tricuspis* to utilize all the five sugars (including moderate utilization of melezitose) tested in this study may indicate the presence of the hydrolytic enzyme, α-glucosidase, a digestive enzyme commonly found in insects (Chippendale, 1978). This enzyme is typically involved in the hydrolysis of several dissacharide and oligosaccharide sugars with an α-glucosidic bond, including sucrose, melezitose, trehalose and maltose, and was also implicated in the utilization of several oligosaccharide sugars by *C. glomerata* (Wäckers, 2001). The utilization by *P. tricuspis* of trehalose, the main haemolymph sugar, is consistent with the observed host feeding behaviour of phorid flies (Disney, 1994). Given that feeding on trehalose results in a significant increase in longevity of *P. tricuspis*, it is also likely that host feeding will have a similar impact on its lifespan. Future studies will investigate this possibility.

These results suggest that *P. tricuspis* has the capability to utilize a broad range of sugar sources in the field, including nectar and honeydew sources. However, feeding on melezitose does not result in optimum longevity or accumulation of body nutrients. It is highly unlikely that the observed reduced utilization of melezitose by *P. tricuspis* is related to its rapid crystallization (Wäckers, 2000) because fresh melezitose solution was smeared on the Petri dish daily. The reduced utilization of melezitose, a honeydew-specific sugar, may partly explain the results of previous studies in which feeding on cotton aphid honeydew resulted in significant but modest increase in longevity of *P. tricuspis* (Fadamiro & Chen, 2005) compared with the relatively greater increase in lifespan recorded for flies fed sucrose solution in other studies (Chen *et al.*, 2005;

Fadamiro *et al*., 2005). However, the significant utilization of another honeydew sugar, trehalose, suggests that *P. tricuspis* is likely to prefer or fare better on honeydew sources with high relative concentration of trehalose or a high trehalose : melezitose component ratio. If host feeding has a major impact on the lifespan and fitness of *P. tricuspis*, it is possible that feeding on honeydew sugars (in particular those sources with a high relative trehalose concentration) could result in similar benefits and therefore provide an alternative to host feeding, as demonstrated for the whitefly parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) (Burger *et al*., 2004). Future studies will investigate this possibility, and compare the relative suitability of naturally occurring nectar and honeydew from different sources on lifespan and reproduction of *P. tricuspis*.

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