Effects of sugar feeding on carbohydrate and lipid metabolism in a parasitoid wasp

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Abstract. Lifetime patterns of carbohydrate and lipid metabolism were compared in starved and sucrose-fed adults of the parasitoid *Macrocentrus grandii* (Goidanich) (Hymenoptera: Braconidae). As expected, sucrose-fed individuals lived longer than did starved individuals. *Macrocentrus grandii* males and females eclosed with levels of simple storage sugars (presumably primarily trehalose) and glycogen that were below maximum levels recorded from sucrose-fed parasitoids. Both of these nutrients dropped to very low levels in starved individuals within 4 days post-emergence and were maintained at high levels in sucrose-fed individuals throughout their lives. Lipid reserves at emergence represented the highest lipid levels for both sexes in the two diet treatments, with levels declining over the lifetimes of males and females from both diet treatments. Our results therefore suggest that dietary sucrose is used to synthesize trehalose and glycogen, but not lipids in *M. grandii*. Also, in contrast to the patterns observed for the simple sugars and glycogen, lipid levels in starved individuals did not drop below levels observed in sugar-fed individuals. The average number of mature eggs carried by females at emergence was 33 and increased to approximately 85 in sucrose-fed and 130 in starved females by the age of 5 d in the absence of hosts. The egg maturation rate was therefore higher in starved than in sugar-fed females. Potential explanations for this unexpected result are discussed.

Key words. Anthrone tests, Braconidae, carbohydrate metabolism, lipid metabolism, *Macrocentrus grandii*, parasitoid, sugar feeding.

Introduction

The adults of many insect species require sugar meals to achieve maximum longevity (House, 1974; Chapman, 1982). Parasitoid wasps are especially sensitive to sugar deprivation as adults; the laboratory lifespan of many parasitoid species is typically less than 5 days in the absence of sugar but exceeds 2 to 3 weeks when sugar meals are provided (Hagen, 1986; van Lenteren *et al*., 1987; Heimpel *et al*., 1997; Quicke, 1997; Olson & Andow, 1998). In the field, potential sugar sources for

parasitoids include nectar and homopteran honeydew (Rogers, 1985; Hagen, 1986; Jervis *et al*., 1992, 1993; Evans, 1993; Jervis & Kidd, 1996), which provide primarily the disaccharide sucrose and its two monosaccharide components, glucose and fructose (van Handel *et al*., 1972; Magnarelli, 1979; Harborne, 1988). These sugar meals supplement carbohydrate-based resources carried over from immature feeding stages and can be used immediately to generate energy for metabolic purposes or stored for later use by conversion to trehalose and glycogen (Friedman, 1985; Rivero & Casas, 1999). *De novo* production of lipids from simple sugars has been documented from some insects as well, but is apparently less universal than production of trehalose or glycogen (Downer & Matthews, 1976; van Handel, 1984).

Although it is known that insect parasitoids utilize sugar sources in the field (Hagen, 1986; Evans, 1993; Jervis *et al*., 1993, 1996; Jervis & Kidd, 1996), a detailed understanding of the conditions under which sugar is used in the field has been

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hindered in part by a lack of the application of appropriate methodologies to parasitoids. By contrast, tools for the study of sugar feeding and carbohydrate and lipid use based on simple biochemical assays have been used for decades with various dipteran species in both the laboratory and the field (e.g. Bidlingmayer & Hem, 1973; Magnarelli, 1979; Magnarelli & Anderson, 1981; van Handel, 1984; Yuval & Schlein, 1986; van Handel & Day, 1988; Yuval *et al.*, 1994; Warburg & Yuval, 1996). One goal of this study is to develop these techniques for parasitoids.

Here we use a series of biochemical assays developed by van Handel (1985a, 1985b) to document lifetime trajectories of simple storage sugars, glycogen and lipids of starved and sugar-fed adults of the parasitoid *M. grandii* (Goidanich) (Hymenoptera: Braconidae) (also known as *M. cingulum*; see van Achterberg & Haeselbarth, 1983). In particular, our goals are to quantify the differences between emergence and maximum levels of nutrients under the two feeding regimes, and to determine if *M. grandii* are capable of lipogenesis from sugar meals. We also compare lifetime egg maturation patterns of sucrose-fed and starved females in the absence of hosts.

Materials and Methods

Macrocentrus grandii was introduced from Europe and Asia to the United States in the 1920s as a biological control agent of the European corn borer, *Ostrinia nubilalis* Hübner (Baker *et al.*, 1949; Edwards & Hopper, 1998). Female *M. grandii* oviposit one or a few eggs into the haemocoel of second to fourth instar *O. nubilalis* and each egg probably divides into 6–10 larvae by polyembryony (Parker, 1931). The larvae feed within the host body and emerge while the host is in the fifth instar. After a brief period of external feeding, they spin cocoons in masses of between three and 50 individuals adjacent to the carcass of the host (Parker, 1931). Adult females do not host feed and the maximum adult lifespan of healthy sugar-fed *M. grandii* is approximately 3 weeks (Andreadis, 1980; Siegel *et al.*, 1986; Orr & Pleasants, 1996; see below). Substantially lower lifespans occur in the laboratory when wasps are infected by the microsporidian *Nosema pyrausta* (Pailot) (Andreadis, 1980; Siegel *et al.*, 1986) or deprived of a source of sugar (Orr & Pleasants, 1996).

Adult parasitoids used in the assays came from 92 broods, 57 of which had eclosed from diapausing *O. nubilalis*, which had been collected as larvae from fields throughout 12 counties of Minnesota between August and October 1997. The remaining 35 broods came from a colony that had been maintained on *O. nubilalis* larvae for one to four generations at the time of the experiments at 27°C, LD 16 : 8 h and 75 ± 5% RH (hosts maintained as described by Leahy & Andow, 1994). Brood composition was either all-male (26%), all-female (52%) or mixed sex (22%) and ranged in size from three to 48 eclosing adults. Upon emergence, one or two wasps from each brood were tested for *Nosema* spp. infection by slide-mounting whole-insect homogenates in 50 μl of Ringer's saline solution, and checking for *Nosema* spores at 400×. Parasitoids were only used in experiments if they developed as part of a brood

from which the sampled individuals were free from *Nosema* infection. Andreadis (1980) showed that *Nosema pyrausta* could be detected in all members of infected broods.

Sugar meals and survivorship

We compared survivorship of *M. grandii* males and females that were provided sucrose and water, or water only. Individual broods were divided in half within 3 h of emergence and randomly assigned to treatments of either sucrose and water, or water only. For mixed-sex broods, the males and females were equally divided between each treatment. Parasitoids were housed in groups of two wasps of the same sex within 10 cm diameter plastic Petri dishes. Parasitoids emerging from single-sex broods were therefore unmated and parasitoids emerging from mixed-sex broods were potentially mated to their siblings (Parker, 1931). Water was provided 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. The tubes were refilled as needed (i.e. every 5–7 days). Sucrose was provided as a 50% solution in distilled water and was smeared on the inside of the Petri dish cover with a cotton-tipped applicator. Petri dishes were checked once daily for survival. To determine the effect of size on the probability of survival, the forewings of each wasp were slide-mounted and measured to the nearest 0.02 mm. Measurements were taken from the outer edge of the anal cell to the outer edge of the tip of both wings for most parasitoids and averaged. For several wasps, a single wing was measured twice and these measurements were averaged. A total of 124 females and 105 males was used for the survivorship analysis, stemming collectively from 15 broods. Some winglength measurements were lost, so that winglengths were obtained for a total of 96 females and 76 males. The effects of the food treatment, the sex of the wasp, winglength and their interactions on survivorship were tested using Cox's proportional hazard model (SAS Institute, 1995).

Sugar, glycogen and lipid assays

Parasitoids used in the nutrient assays came from 57 broods that had eclosed from diapausing *O. nubilalis* larvae, and 20 laboratory-reared broods. Members of each brood were split evenly across treatments and age levels. Two wasps from each brood were placed in a Petri dish with sucrose and water or water only, as described above. A single parasitoid was chosen randomly from each Petri dish for the assay. Parasitoids were assayed daily from ages 1–7 days and every other day thereafter until the age of 21 days in the sucrose-fed treatment, and daily from ages 0–6 days in the starvation treatment. A total of 10 females was assayed per age treatment, except for the water-only treatment, where lower numbers were used for the two oldest age groups because of low survival to these ages. Between five and 10 males were used for each time period for both diet treatments, with the exception of the oldest age groups. The forewings of each insect were removed and

measured as described above immediately before nutrient analyses were carried out.

The presence of eggs in female parasitoids could potentially complicate interpretation of the nutrient assay because the nutrients held in eggs are presumably unavailable for adult maintenance. Even if eggs are resorbed, oosorption probably occurs only under some conditions and is likely to be metabolically costly (Bell & Bohm, 1975). We therefore report nutrient levels for females from which the eggs were removed by dissection. Females were dissected on a microscope slide cover slip in 50 μl of a 2% sodium sulphate solution and their ovaries were removed. The number of mature eggs present at the time of dissection (the 'egg load') was recorded. The ovaries were transferred to 50 μl of Ringer's saline, slide-mounted and viewed at 100×. Eggs for which the complete chorion was clearly visible were considered mature and counted.

Post-dissection females were transferred singly to 1.5-ml microcentrifuge tubes. The cover-slip from which the dissections had been done was washed into the tubes with 450 μl of chloroform-methanol (1 : 2) to recover as much material lost during the dissection as possible and females were crushed with a plastic pestle. Males were placed singly in 1.5-ml microcentrifuge tubes with 50 μl sodium sulphate, crushed with a plastic pestle and 450 μl of chloroform-methanol (1 : 2) was used to wash the pestle. The tubes were then vortexed and centrifuged at 16 000 *g* for 2 min. After centrifuging, 200 μl of the supernatant was transferred to a glass test tube (12 mm × 75 mm) for the sugar assays and 200 μl was transferred to a similar glass tube for the lipid assay. The precipitate was left in the microcentrifuge tube for the glycogen assay. All tubes were heated at 90°C until all solution was evaporated from the lipid and glycogen tubes and approximately 50 μl of solution remained in the sugar tubes.

Glycogen assay. Anthrone reagent (1 ml), prepared as in van Handel (1985a), was added to the tubes containing the precipitate and heated at 90°C for 15 min. The tubes were cooled on ice, mixed and contents were placed in 1.5-ml methacrylate cuvettes. The absorbance at 625 nm was read using a spectrophotometer.

Lipid assay. Sulphuric acid (40 μl) was added to the tubes containing the lipid precipitate and heated for 2 min at 90°C. The tubes were cooled on ice and 960 μl of a vanillin-phosphoric acid reagent (prepared as in van Handel, 1985b)
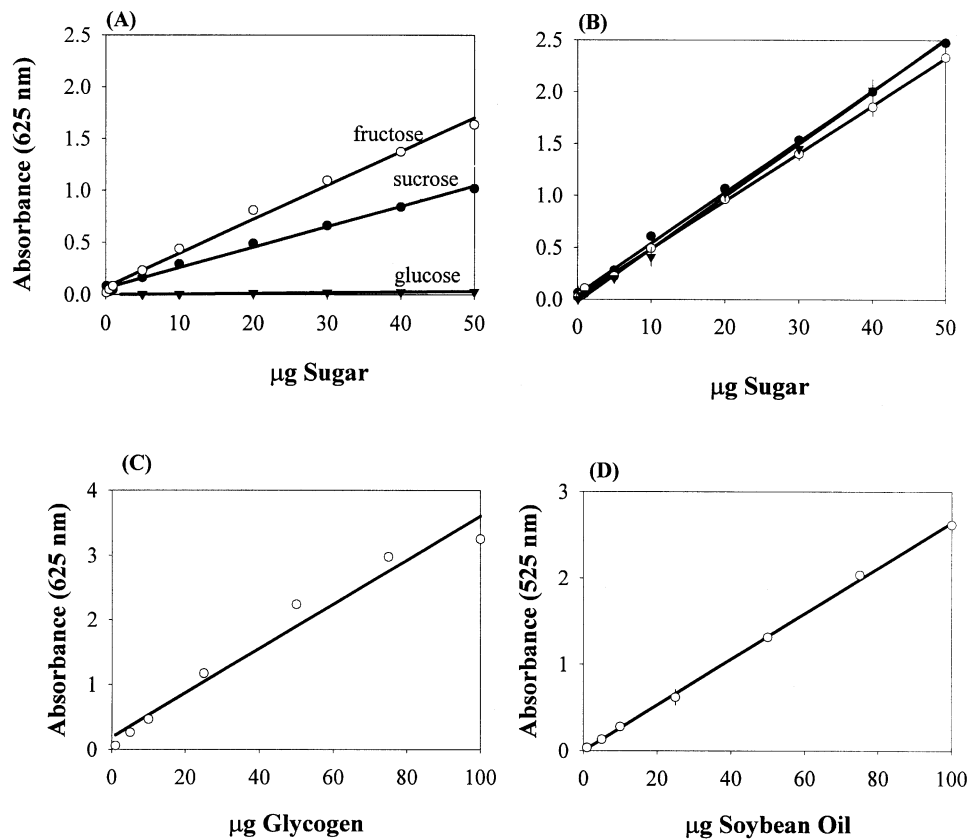

Fig. 1. Standard curves for fructose, sucrose and glucose absorbance using the cold anthrone test (A), and hot anthrone tests (B), glycogen using the hot anthrone test (C), and soybean oil using the vanillin-phosphoric acid test (D). Bars are SEM (*n* = 3 for each data point) and are only visible if their length is greater than the diameter of the data point.

was added. The solution in each tube was left to react for 30 min at room temperature, mixed and transferred to 1.5-ml cuvettes. The absorbance at 525 nm was recorded.

Fructose assay (cold anthrone test). Anthrone reagent (950 μl) was added to the supernatant of the tubes containing the sugars, mixed and left to react for 1.5 h at room temperature. The absorbance at 625 nm was recorded.

Total sugars (hot anthrone test). The solutions from the fructose assay were heated for 15 min at 90°C, cooled on ice and the absorbance at 625 nm was recorded.

Samples were run in batches of between six and 12 insects, along with a single negative control sample without an insect. The absorbance values obtained for the negative control were subtracted from absorbance values of each of the members of a given batch of insects for each test.

Standard curves. Known amounts of glucose, fructose, sucrose, glycogen and lipid solutions (1 mg/ml) were prepared as in van Handel (1985a, 1985b). Soybean oil was used to generate the lipid curve and the source of glycogen was rabbit liver (Sigma, St. Louis, MO, U.S.A.). Glucose, fructose and sucrose solutions were prepared in amounts of 1, 5, 10, 20, 30, 40 and 50 μg and brought to a total volume of 1 ml with anthrone reagent. Glycogen and lipid solutions were prepared in amounts of 1, 5, 10, 25, 50, 75 and 100 μg and brought to a total volume of 1 ml with anthrone and vanillin reagents for glycogen and lipid standards, respectively. Absorbance was read at 625 nm for the sugars and glycogen and at 525 nm for the lipids. Three replicates per sample level were prepared and nutrient amounts were calculated from the resulting linear regression equations (Fig. 1).

To estimate the amount of sugars present in the body of individual parasitoids excluding the gut contents, we subtracted the gut sugars from the total sugars detected by the hot anthrone test. The cold anthrone reading represents half of the sugars present in the gut because it detects only fructose, which represents one half of the sugar present in the sucrose meals (Chapman, 1982; see Fig. 1a) and because fructose is not present in insect haemolymph (e.g. van Handel, 1984; see below). Body sugar levels were therefore estimated by subtracting two times the amount of fructose detected by the cold anthrone test from the sugars detected by the hot anthrone test.

Statistical analyses

Proportional hazards models were used to test for effects of sex, diet and winglength on survivorship. The effects of diet, age and winglength on absorbance values from assays of body sugars, glycogen and lipids were tested separately for males and females by multiple regression analysis in which the qualitative variable, diet, was coded as a binary variable (Neter *et al.*, 1990). The same test was used for the number of mature eggs in females. Transformations were necessary for the absorbance and egg load values to stabilize variances with respect to the diet treatment. Absorbance values associated with body sugar level estimates were square-root transformed. Absorbance values for the glycogen tests were transformed using a Box–Cox procedure (SAS Institute, 1995) that yielded the transformation $y' = (y^{0.6} - 1)/0.56$. An inverse square-root transformation was used on absorbance values from the lipid assays and on the egg load data.
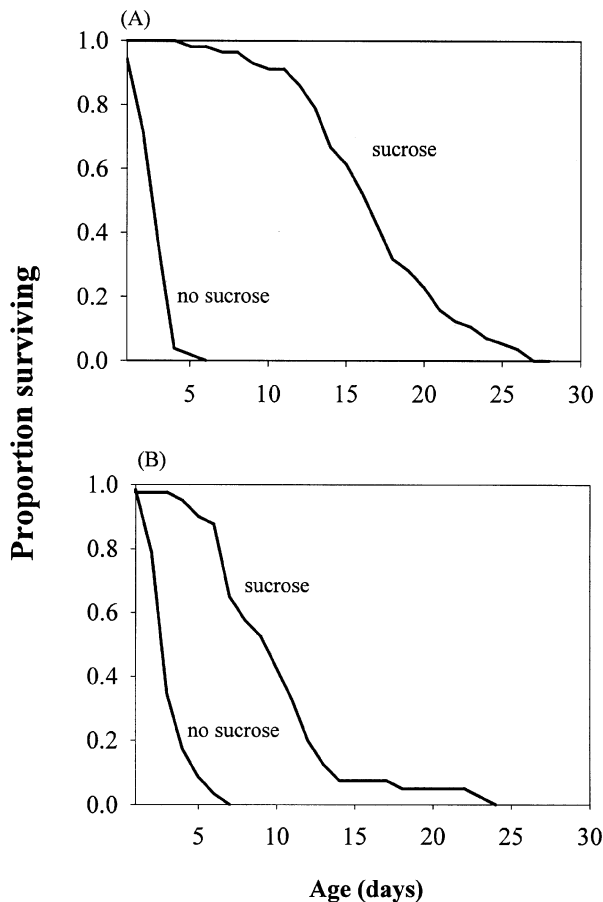

Fig. 2. Survivorship curves for (A) female and (B) male *Macrocentrus grandii* provided sucrose and water or water only. Median longevity = 17 and 3 days for 64 and 60 females in the sucrose and control treatments, respectively. Median longevity = 10 and 3 days for 45 and 60 males in the sucrose and water and control treatments, respectively.

Table 1. Proportional hazards model testing for effects of the diet treatment, sex, winglength and interactions of these variables on survival of *Macrocentrus grandii*.

Source of variation	d.f.	χ^2	P
Diet	1	1.107	0.293
Sex	1	7.29	0.007
Winglength	1	6.54	0.011
Diet × Winglength	1	7.01	0.008
Sex × Winglength	1	6.26	0.012
Diet × Sex	1	10.33	0.001
Diet × Winglength × Sex	1	8.83	0.003

Results

Survivorship

Sucrose meals increased the longevity of both male and female *M. grandii* (Fig. 2). A proportional hazards model including diet, sex and the interaction of these two variables revealed significant effects of both sex and diet on longevity (diet: $\chi^2 = 230$, $P < 0.0001$; sex: $\chi^2 = 14$, $P < 0.001$). While sucrose-fed females lived longer than sucrose-fed males, sex did not have a significant effect on the longevity of starved parasitoids, leading to a significant sex × diet interaction ($\chi^2 = 29$, $P < 0.0001$). Females were significantly larger than males in both treatments ($P < 0.0001$), leading to a significant interaction between sex and winglength in a more complete model (Table 1). Furthermore, the effect of parasitoid winglength on longevity was dependent upon both sex and diet, leading to a significant three-way interaction among diet, sex

and winglength, which obscured the direct effect of diet in the model (Table 1). Winglength had a significant positive effect on longevity of sucrose-fed males (longevity = 25.2 + 10.8 winglength, $r^2 = 0.244$, $P = 0.003$; $n = 45$), but no effect on longevity in starved males or females from both diet treatments ($P > 0.2$ for all analyses).

Nutrient analyses

Fructose. Fructose amounts were estimated with the cold anthrone test and represent one-half of the sugar present in the gut, owing to the fact that sucrose is composed of equal amounts of fructose and glucose. As expected, the cold anthrone tests failed to detect fructose within unfed *M. grandii* and both males and females that were fed sucrose showed consistently high fructose levels throughout their adult lives (Fig. 3). Fructose levels were more variable in male than female parasitoids, but no consistent trends in fructose levels could be detected in either females or males over time (linear regression of fructose levels over time for both sexes: $P > 0.5$).
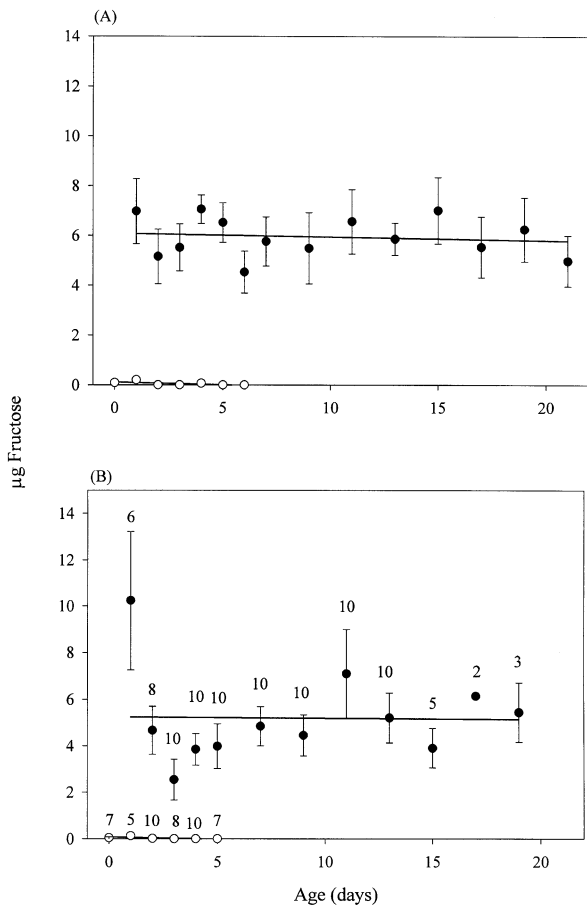

Fig. 3. Amounts of fructose (mean ± SEM) detected over the lifespans of (A) female and (B) male *Macrocentrus grandii* that were provided sucrose and water (●) and water only (○). Sample sizes are 10 for each of the time periods for sucrose-fed females, 10 for time periods 0–5 for starved females, and 8 and 4 for starved females aged 5 and 6, respectively. For males, sample sizes are above standard error bars.
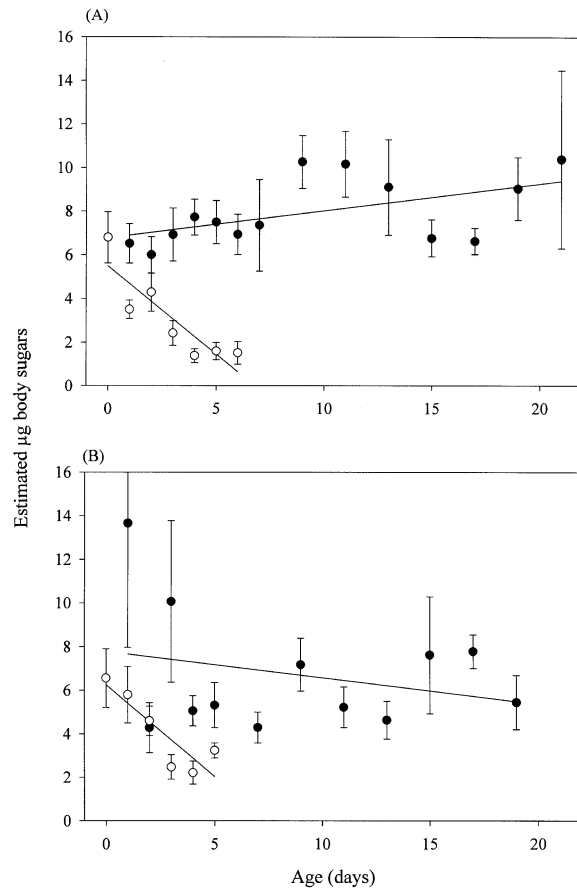

Fig. 4. Estimated amounts of body sugars (mean ± SEM) detected over the lifespans of (A) female and (B) male *Macrocentrus grandii* that were provided sucrose and water (●) and water only (○). Sample sizes are as in Fig. 3.

Table 2. Multiple regression analysis testing the effect of diet, age, the interaction between diet and age, and winglength on absorbance values for estimated body sugars, glycogen and lipids of male and female *Macrocentrus grandii*, as well as the egg load of females. All analyses were done on transformed values as described in the text and the diet treatment was coded as a binary variable.

		Females			Males		
	d.f.	MS	F	*P*	MS	F	*P*
Body sugars							
Diet	1	0.10	3.7	<0.055	0.02	0.7	0.41
Age	1	0.58	21.9	<0.0001	0.17	5.6	0.02
Diet × Age	1	0.73	27.4	<0.0001	0.14	4.7	0.03
Winglength	1	0.17	6.5	<0.012	0.31	10.3	0.002
Glycogen							
Diet	1	19.0	67.3	<0.0001	3.1	7.2	0.008
Age	1	19.5	69.2	<0.0001	4.6	10.5	0.002
Diet × Age	1	12.2	43.1	<0.0001	6.4	14.7	<0.001
Winglength	1	7.7	27.1	<0.0001	7.0	16.0	<0.001
Lipid							
Diet	1	0.001	0.01	<0.9	0.76	2.1	0.15
Age	1	0.74	4.9	<0.03	3.7	10.3	<0.01
Diet × Age	1	0.08	0.5	<0.47	0.81	2.3	0.13
Winglength	1	2.14	14.3	<0.001	2.2	6.2	0.01
Number of Eggs							
Diet	1	0.02	29.4	<0.0001	–	–	–
Age	1	0.04	46.8	<0.0001	–	–	–
Diet × Age	1	0.05	58.6	<0.0001	–	–	–
Winglength	1	0.01	15.2	0.0001	–	–	–

Table 3. Diet-specific results of linear regression analyses testing for the effect of *Macrocentrus grandii* winglength on untransformed values of fructose, body sugars, glycogen and lipids for both males and females as well as egg loads for females. Regression parameters are only reported for *P* < 0.10.

		Females				Males			
Variable	Diet	r^2	*P*	β_o	β_1	r^2	*P*	β_o	β_1
Fructose	Sucrose	0.03	0.04	−3.7	2.7	0.004	0.56	–	–
	Control	–	–	–	–	–	–	–	–
Body sugars	Sucrose	0.02	0.13	–	–	0.06	0.01	−12.2	5.5
	Control	0.05	0.08	−8.5	3.3	0.08	0.05	−5.4	2.8
Glycogen	Sucrose	0.12	<0.0001	−68	35.4	0.11	<0.001	−72.1	36.2
	Control	0.01	0.41	–	–	0.17	<0.01	−66.5	23.7
Lipids	Sucrose	0.08	<0.001	−18.2	9.2	0.08	<0.01	−13.4	7.4
	Control	0.08	0.03	−33	14.3	0.16	<0.01	−42.8	18.2
Eggs	Sucrose	0.12	<0.0001	−73.7	40.1	–	–	–	–
	Control	0.06	0.05	−99.0	50.4	–	–	–	–

Sucrose-fed female and male *M. grandii* carried an average (± SEM) of 5.9 ± 0.3 and 4.6 ± 0.3 μg of fructose, respectively (ages pooled; $t_{233} = 2.5$, $P = 0.01$). A very weak but significant positive relationship between winglength and fructose levels was detected in females, but not in males (Table 3).

Body sugars. The mean estimated amount of body sugars present in female and male *M. grandii* upon emergence was 6.8 ± 1.2 μg (*n* = 10) and 6.6 ± 1.3 μg (*n* = 7), respectively (Fig. 4). Body sugar levels then dropped rapidly over the lifespan of starved adults of both sexes, whereas they increased over the lifespan of sucrose-fed females and declined very slightly over the lifespan of sucrose-fed males (Fig. 4, Table 2). There was no significant relationship between the age of either females or males and their winglengths in either of the diet treatments (*P* > 0.1 for all sex/diet combinations). This suggests that there was little, if any, effect of parasitoid size on survivorship during the course of the experiment and makes
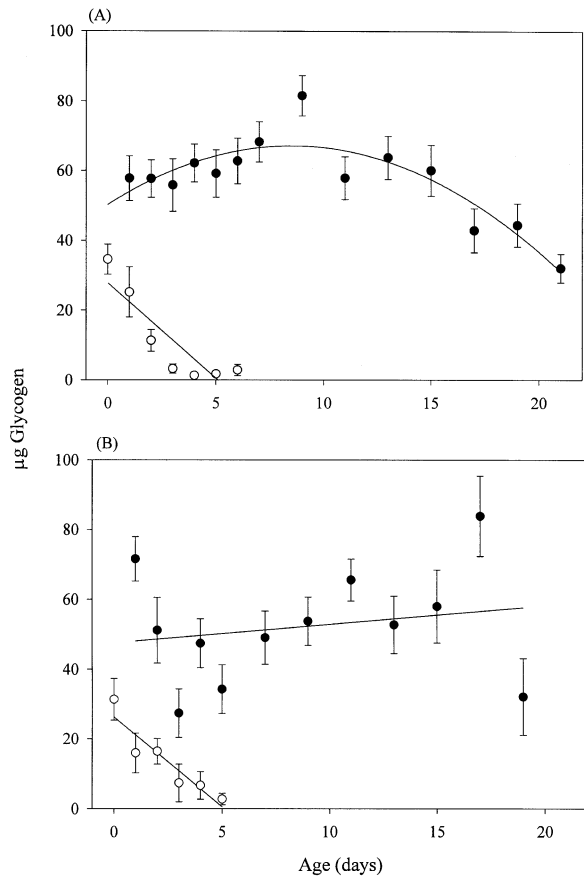
Fig. 5. Amounts of glycogen (mean ± SEM) detected over the lifespans of (A) female and (B) male *M. grandii* that were provided sucrose and water (●) and water only (○). Sample sizes are as in Fig. 3.
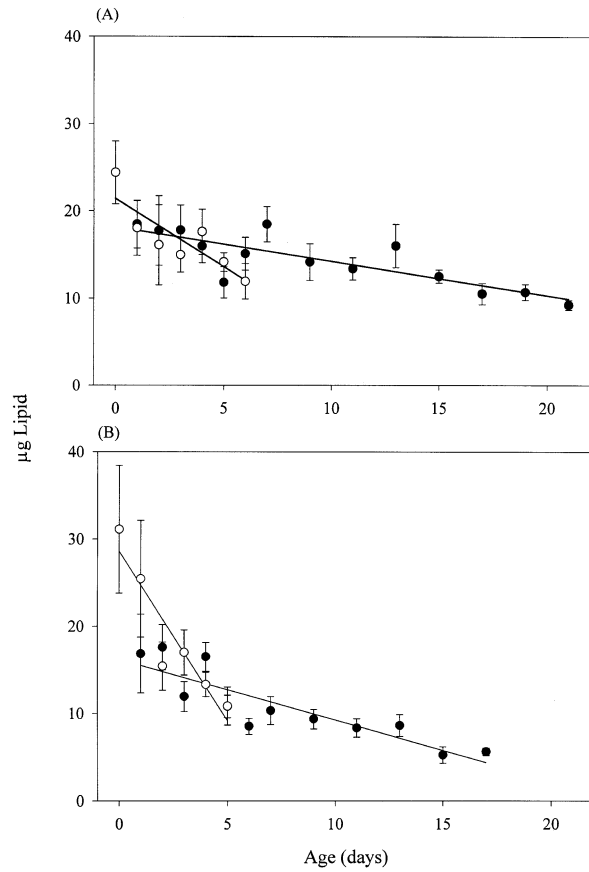

Fig. 6. Amounts of lipids (mean ± SEM) detected over the lifespans of (A) female and (B) male *M. grandii* that were provided sucrose and water (●) and water only (○). Sample sizes are as in Fig. 3.
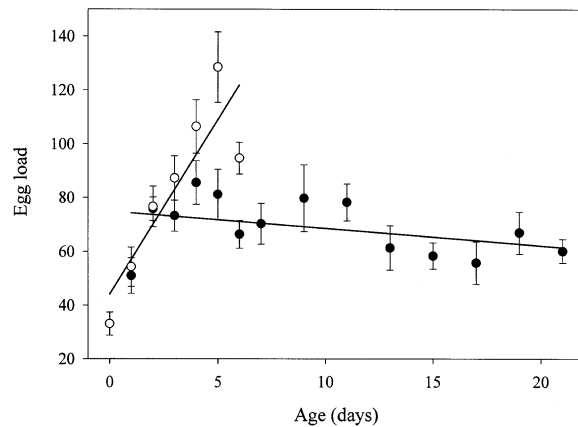
it unlikely that the effects of parasitoid age on sugar levels are due to differences in the size distributions of individuals in the different age groups. The relationship between winglength and body sugars was significant and weakly positive for starved parasitoids of both sexes as well as for sucrose-fed males and non-significant for sucrose-fed females (Table 3).

Glycogen. Glycogen levels of emerging female and male *M. grandii* were 34.6 ± 4.3 µg (*n* = 10) and 31.3 ± 6.0 µg (*n* = 7), respectively (Fig. 5). They then dropped rapidly over the lifespan of both sexes in the starvation treatment and increased to levels approximately twice the emergence levels in sucrose-fed females and males (Fig. 5). In sugar-fed females, glycogen levels declined to emergence levels by day 21, and second-order polynomial (quadratic) regression showed that the relationship between age and glycogen levels in sugar-fed females was significantly domed (Fig. 5). Glycogen reserves of starved *M. grandii* were depleted by day 5, which coincides approximately to maximum longevity in the starvation treatment (see Fig. 2) and remained uniformly high over the lifespan of sucrose-fed parasitoids. The multiple regression analysis showed that diet, age, diet × age, and winglength all significantly affected glycogen levels of both sexes (Table 2).

Fig. 7. Numbers of mature eggs (mean ± SEM) dissected from female *M. grandii* that were provided sucrose and water (●) and water only (○) over their lifetimes. Sample sizes are as in Fig. 3(A).

The relationship between winglength and glycogen levels was significantly positive for sucrose-fed parasitoids of both sexes

and for starved males, but non-significant for starved females (Table 3).

Lipids. The highest lipid levels in both male and female *M. grandii* were detected in individuals that had just eclosed (Fig. 6). Lipid levels then declined significantly over the lifespan of both sexes in both diet treatments (Fig. 6, Table 2). There was no statistically significant difference in lipid levels due to the diet treatment in either sex (Table 2) and although the rate of lipid decline appeared to be greater in starved individuals of both sexes (Fig. 6), the diet–age interaction was insignificant for both males and females (Table 2). The relationship between winglength and lipids was significant and positive for parasitoids of both sexes in both diet treatments (Table 3).

Egg load. At emergence, *M. grandii* females carried an average of 33.1 ± 4.4 ($n = 10$) mature eggs (Fig. 7). The maximum egg load for sucrose-fed females was 85.5 ± 8.0 ($n = 10$) and occurred on day 4, and the maximum egg load for starved females was 128.5 ± 13.1 ($n = 8$) and occurred on day 5. Overall, egg loads of sucrose-fed females were significantly lower than egg loads of starved females, and there was a significant interaction between diet and age (Fig. 7, Table 2). Winglength had a significant positive effect on the number of mature eggs of females that were fed sugar, but only a marginally significant effect on the egg load of starved females (Table 3). There was some evidence for modest but highly variable egg resorption in the sugar-fed group between the day of maximum egg load and the last day of the study. Egg loads dropped significantly between days 4 and 21 in the sucrose-fed females ($r^2 = 0.08$, $P < 0.01$, slope $= -1.3$). The significance of this relationship was increased slightly when winglength was added to the analysis. Because of the small number of females in the starvation treatment that survived past 5 days, a similar analysis could not be done for this treatment.

Discussion

One goal of this study was to develop analytical tools that could be used with field-collected parasitoids to determine whether individuals had recently fed on sugar and to distinguish between individuals that are starving and individuals that are relatively well-fed. We have demonstrated here that the techniques used for various dipteran species are applicable to the parasitoid *M. grandii*. First, we showed that the cold anthrone test, which detects the nectar sugar fructose, does not yield positive results with starved parasitoids. Second, we determined that substantial and significant differences exist between the body sugar levels and glycogen reserves of sugar-fed compared with starved *M. grandii*.

Macrocentrus grandii males and females emerged with relatively high reserves of body sugars, glycogen and lipids. Body sugars and glycogen reserves declined rapidly in starved individuals and were nearly depleted by the age of 4 days in both males and females. Maximum longevity in the starvation treatment was approximately 6 days for both sexes, suggesting that death may be attributable to the depletion of either or both of these nutrients. Adult *M. grandii* that were fed a 50%

sucrose solution maintained body sugar levels and glycogen reserves that were at least as high as emergence levels and lived significantly longer than starved individuals. Lipid levels declined post-emergence in both sucrose-fed and starved individuals and the lowest lipid levels were recorded from the oldest individuals that were fed sucrose. Thus, it is possible that lipid depletion contributes to mortality in sugar-fed, but not starved, individuals.

Although both male and female *M. grandii* adults in our study were able to synthesize glycogen from sucrose, we found no evidence for lipogenesis in *M. grandii* adults. This is in contrast to *de novo* lipid synthesis in a number of other insect species fed only sugar (e.g. Nayar & van Handel, 1971; Brown & Chippendale, 1974; Downer & Matthews, 1976; van Handel, 1984; Warburg & Yuval, 1996). In our study, lipid levels declined monotonically post-emergence, whether or not sugar was provided, as has been observed for the braconid parasitoid *Asobara tabida* (Nees) (Ellers, 1996), the house fly, *Musca domestica* (van Handel, 1984), and the tephritid *Anastrepha serpentina* (Wiedmann) (Jacome *et al.*, 1995). A more rapid decline in lipid reserves in starved compared with sugar-fed individuals has also been reported for some insects (Nestel *et al.*, 1985; Jacome *et al.*, 1995; Ellers, 1996) and although our data do not bear out this trend statistically (i.e. see the lack of a significant diet–age interaction in Table 2), the general direction of our data is consistent with such a pattern.

Our egg load data are somewhat difficult to interpret. Upon emergence, females carried on average 33 mature eggs, a number similar to that reported by Parker (1931). Starved females then went on to develop more eggs than sugar-fed females. Our data are consistent with modest but highly variable egg resorption over a 17-day period in the sugar-fed females and we have no evidence for egg resorption in the starved group. This is in contrast to a relatively large literature demonstrating higher rates of egg resorption in starved rather than sugar-fed insects that includes many parasitoid species (Bell & Bohm, 1975; Jervis & Kidd, 1986; van Lenteren *et al.*, 1987; Antolin & Williams, 1989; Heimpel & Rosenheim, 1995; Heimpel *et al.*, 1997; Quicke, 1997). Egg resorption is presumed to increase life expectancy (Bell & Bohm, 1975; Collier, 1995; Heimpel *et al.*, 1997) and could therefore increase the possibility that sugar meals are located in the field. Also, increased egg maturation is presumably metabolically costly and is likely to lead to decreased life expectancy (e.g. Roitberg, 1989; Tatar & Carey, 1995). How then can we explain our finding that starved females produced more eggs than did sugar-fed females? First, it is conceivable that this result is attributable to an experimental artefact, namely that females with lower egg loads died disproportionately earlier in the starvation than in the sugar-fed treatment. Such an outcome is not necessarily unexpected, as smaller individuals of many parasitoid species have lower survivorship than do larger individuals (Godfray, 1994) and we have shown that egg load increases with size in *M. grandii*. However, our survivorship data indicated no effect of size on survivorship of female *M. grandii*. Neither did we observe increased representation of larger wasps with age for any treatment/sex combination in the nutrient levels study. Because of these considerations, we are

reluctant to attribute the higher egg loads in the starved group to changes in the composition of females as they aged.

An alternative explanation is that starved females may invest more energy into reproduction precisely because of the perception of lower life expectancy. Similar behavioural responses, in which the oviposition rate is increased under conditions of lowered life expectancy, have been documented for a number of parasitoid species (Roitberg *et al.*, 1992, 1993; Visser *et al.*, 1992; Fletcher *et al.*, 1994; but see Heimpel & Rosenheim, 1995), despite the fact that increased reproductive effort has been shown to lower life expectancy in parasitoids and other taxa (Bell & Koufopanou, 1986; Ellers & van Alphen, 1996). Increased egg maturation by starved females may be a viable strategy to increase lifetime reproductive success if insects are either incapable of resorbing eggs, or egg resorption does not substantially increase lifespan, and if the metabolic costs of increased egg maturation are slight or not incurred early in life.

Our data suggest that sugar-starved females may be more limited by sugar than lipid reserves. If egg production is cheap in terms of sugar but costly in terms of lipids, then sugar-starved females that are not limited by lipids may be able to use those lipids to mature more eggs while incurring only minimal costs in terms of survivorship. Such a strategy may enhance short-term opportunities for reproduction, especially if a rich patch of hosts is encountered by a female with a low life expectancy.

Acknowledgements

References

Andreadis, T.G. (1980) *Nosema pyrausta* infection in *Macrocentrus grandii*, a braconid parasite of the European corn borer, *Ostrinia nubilalis*. *Journal of Invertebrate Pathology*, **35**, 229–233.

Antolin, M.F. & Williams, R.L. (1989) Host feeding and egg production in *Muscidifurax zaraptor* (Hymenoptera: Pteromalidae). *Florida Entomologist*, **72**, 129–134.

Baker, W.H., Bradley, W.G. & Clark, C.A. (1949) *Biological Control of the European Corn Borer in the United States*. United States Department of Agriculture Technical Bulletin no. 983, Washington, DC.

Bell, G. & Koufopanou, V. (1986) The cost of reproduction. *Oxford Surveys in Evolutionary Biology* (ed. by R. Dawkins and M. Ridley), pp. 83–131. Oxford University Press, Oxford.

Bell, W.J. & Bohm, M.K. (1975) Oosorption in insects. *Biological Reviews*, **50**, 373–396.

Bidlingmayer, W.L. & Hem, D.G. (1973) Sugar feeding by Florida mosquitoes. *Mosquito News*, **33**, 535–538.

Brown, J.J. & Chippendale, G.M. (1974) Migration of the monarch butterfly, *Danaus plexippus*: energy sources. *Journal of Insect Physiology*, **20**, 1117–1130.

Chapman, R.F. (1982) *The Insects: Structure and Function*. Harvard University Press, Cambridge, MA.

Collier, T.R. (1995) Host feeding, egg maturation, resorption and longevity in the parasitoid *Aphytis melinus* (Hymenoptera: aphelinidae). *Annals of the Entomological Society of America*, **88**, 206–214.

Downer, R.G.H. & Matthews, J.R. (1976) Patterns of lipid distribution in insects. *American Zoologist*, **16**, 733–745.

Edwards, O.R. & Hopper, K.R. (1998) Using superparasitism by a stem borer parasitoid to infer a host range. *Ecological Entomology*, **24**, 7–12.

Ellers, J. (1996) Fat and eggs: an alternative method to measure the trade-off between survival and reproduction in insect parasitoids. *Netherlands Journal of Zoology*, **46**, 227–235.

Ellers, J. & van Alphen, J.J.M. (1996) Life history evolution in *Asobara tabida*: plasticity in allocation of fat reserves to survival and reproduction. *Journal of Evolutionary Biology*, **10**, 771–785.

Evans. E.W. (1993) Indirect interactions among phytophagous insects: aphids, honeydew and natural enemies. *Individuals, Populations and Patterns in Ecology* (ed. by A. D. Watt, S. R. Leather, K. E. F. Walters and N. J. Mills), pp. 63–80. Intercept Press, Andover.

Fletcher, J.P., Hughes, J.P. & Harvey, I.F. (1994) Life expectancy and egg load affect oviposition decisions of a solitary parasitoid. *Proceedings of the Royal Society of London Series B*, **258**, 163–167.

Friedman, S. (1985) Intermediary metabolism. *Fundamentals of Insect Physiology* (ed. by M. S. Blum), pp. 467–506. John Wiley & Sons, New York.

Godfray, H.C.J. (1994) *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press, Princeton NJ.

Hagen, K.S. (1986) Ecosystem analysis: plant cultivars (HPR), entomophagous species and food supplements. *Interactions of Plant Resistance and Parasitoids and Predators of Insects* (ed. by D. J. Boethel and R. D. Eikenbary), pp. 151–197. Ellis Horwood Limited Press, West Sussex, U.K.

Harborne, J.B. (1988) *Introduction to Ecological Biochemistry*. Academic Press. London.

Heimpel, G.E. & Rosenheim, J.A. (1995) Dynamic host feeding by the parasitoid *Aphytis melinus*: the balance between current and future reproduction. *Journal of Animal Ecology*, **64**, 153–167.

Heimpel, G.E., Rosenheim, J.A. & Kattari, D. (1997) Adult feeding and lifetime reproductive success in the parasitoid *Aphytis melinus*. *Entomologia Experimentalis et Applicata*, **83**, 305–315.

House, H.L. (1974) Nutrition. *The Physiology of Insecta*, Vol. V (ed. by M. Rockstein), pp. 1–62. Academic Press, New York.

Jacome, I., Aluja, M., Liedo, P. & Nestel, D. (1995) The influence of adult diet and age on lipid reserves in the tropical fruit fly *Anastrepha serpentina* (Diptera: Tephritidae). *Journal of Insect Physiology*, **41**, 1079–1086.

Jervis, M.A. & Kidd, N.A.C. (1986) Host-feeding strategies in hymenopteran parasitoids. *Biological Reviews*, **61**, 395–434.

Jervis, M.A. & Kidd, N.A.C. (1996) Phytophagy. *Insect Natural Enemies. Practical Approaches to Their Study and Evaluation* (ed. by M. Jervis and N. Kidd), pp. 375–394. Chapman & Hall, London.

Jervis, M.A., Kidd, N.A.C. & Walton, M. (1992) A review of methods for determining dietary range in adult parasitoids. *Entomophaga*, **37**, 565–574.

Jervis, M.A., Kidd, N.A.C., Fitton, M.G., Huddleston, T. & Dawah, H.A. (1993) Flower-visiting by hymenopteran parasitoids. *Journal of Natural History*, **27**, 67–105.

Jervis, M.A., Kidd, N.A.C. & Heimpel, G.E. (1996) Parasitoid adult feeding behaviour and biological control – a review. *Biocontrol News and Information*, **17**, 11N–26N.

Leahy, T.C. & Andow, D.A. (1994) Egg weight, fecundity, and longevity are increased by adult feeding in *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Annals of the Entomological Society of America*, **87**, 342–349.

Magnarelli, L.A. (1979) Diurnal nectar-feeding of *Aedes cantator* and *A. sollicitans* (Diptera: Culicidae). *Environmental Entomology*, **8**, 949–955.

Magnarelli, L.A. & Anderson, J.F. (1981) Sugar feeding by female tabanids (Diptera: Tabanidae) and its relation to gonotrophic activity. *Journal of Medical Entomology*, **18**, 429–433.

Nayar, J.K. & van Handel, E. (1971) The fuel for sustained mosquito flight. *Journal of Insect Physiology*, **17**, 471–481.

Nestel, D., Galun, R. & Friedman, S. (1985) Long-term regulation of sucrose intake by the adult Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). *Journal of Insect Physiology*, **31**, 533–536.

Neter, J., Wasserman, W. & Kutner, M.H. (1990) *Applied Linear Statistical Models*. 3rd edn. Richard D. Irwin Press. Homewood, IL.

Olson, D.M. & Andow, D.A. (1998) Larval crowding and adult nutrition effects on longevity and fecundity of female *Trichogramma nubilale* Ertle & Davis (Hymenoptera: Trichogrammatidae). *Environmental Entomology*, **27**, 508–514.

Orr, D.B. & Pleasants, J.M. (1996) The potential of native plant species to enhance the effectiveness of the *Ostrinia nubilalis* parasitoid *Macrocentrus grandii*. *Journal of the Kansas Entomological Society*, **69**, 133–143.

Parker, H.L. (1931) *Macrocentrus gifuensis Ashmead, a Polyembryonic Braconid Parasite in the European Corn Borer*. USDA Technical Bulletin no. 230.

Quicke, D.L.J. (1997) *Parasitic Wasps*. Chapman & Hall. London.

Rivero, A. & Casas, J. (1999) Incorporating physiology into parasitoid behavioural ecology: the allocation of nutritional resources. *Researches on Population Ecology*, **41**, 39–45.

Roitberg, B.D. (1989) The cost of reproduction in rosehip flies, *Rhagoletis basiola*: eggs are time. *Evolutionary Ecology*, **3**, 183–188.

Roitberg, B.D., Mangel, M., Lalonde, R.G., Roitberg, C.A., van Alphen, J.J.M. & Vet, L.E.M. (1992) Seasonal dynamics shifts in patch exploitation by parasitic wasps. *Behavioral Ecology*, **3**, 156–165.

Roitberg, B.D., Sircom, J., Roitberg, C.A., van Alphen, J.J.M. & Mangel, M. (1993) Life expectancy and reproduction. *Nature*, **364**, 108.

Rogers, C.E. (1985) Extrafloral nectar: entomological implications. *Bulletin of the Entomological Society of America*, **31**, 15–20.

SAS Institute (1995) *JMP Statistics and Graphics Guide*, Version 3.1. SAS Institute, Cary, NC.

Siegel, J.P., Maddox, J.V. & Ruesink, G.W. (1986) Impact of *Nosema pyrausta* on a braconid, *Macrocentrus grandii*, in central Illinois. *Journal of Invertebrate Pathology*, **47**, 271–276.

Tatar, M. & Carey, J.R. (1995) Nutrition mediates reproductive trade-off with age-specific mortality in the beetle *Callosobruchus maculatus*. *Ecology*, **76**, 2066–2073.

van Achterberg, C. & Haeselbarth, E. (1983) Revisionary notes on the European species of *Macrocentrus* Curtis *sensu stricto*. *Entomofauna*, **4**, 37–60.

van Handel, E. (1984) Metabolism of nutrients in the adult mosquito. *Mosquito News*, **44**, 573–579.

van Handel, E. (1985a) Rapid determination of glycogen and sugars in mosquitoes. *Journal of the American Mosquito Control Association*, **1**, 299–301.

van Handel, E. (1985b) Rapid determination of total lipids in mosquitoes. *Journal of the American Mosquito Control Association*, **1**, 302–304.

van Handel, E. & Day, J.F. (1988) Assay of lipids, glycogen and sugars in individual mosquitoes: correlations with wing length in filed-collected *Aedes vexans*. *Journal of the American Mosquito Control Association*, **4**, 549–550.

van Handel, E., Haeger, J.S. & Hansen, C.W. (1972) The sugars of some Florida nectars. *American Journal of Botany*, **59**, 1030–1032.

van Lenteren, J.C., van Vianen, A., Gast, H.F. & Kortenhoff, A. (1987) The parasite–host relationship between *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) and *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae). XVI: food effects on oogenesis, life span and fecundity of *Encarsia formosa* and other hymenopterous parasites. *Zeitschrift für Angewandte Entomologie*, **103**, 69–84.

Visser, M.E., van Alphen, J.J.M. & Hemerik, L. (1992) Adaptive superparasitism and patch time allocation in solitary parasitoids: an ESS model. *Journal of Animal Ecology*, **61**, 93–101.

Warburg, M.S. & Yuval, B. (1996) Effects of diet and activity on lipid levels of adult Mediterranean fruit flies. *Physiological Entomology*, **21**, 151–158.

Yuval, B. & Schlein, Y. (1986) Leischmaniasis in the Jordan Valley III. Nocturnal activity of *Phlebotomus papatasi* (Diptera: Psychodidae) in relation to nutrition and ovarian development. *Journal of Medical Entomology*, **23**, 411–415.

Yuval, B., Holliday-Hansen, M.L. & Washino, R.K. (1994) Energy budget of swarming mosquitoes. *Ecological Entomology*, **19**, 74–78.