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Effects of a lipid-rich diet on adult parasitoid income resources and survival

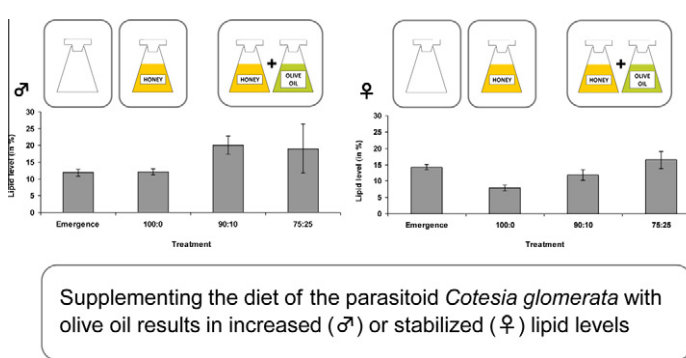
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HIGHLIGHTS

- ▶ Olive oil was added to the diet of the adult parasitoid *Cotesia glomerata*.
- ▶ Lipid levels stabilized and increased when olive oil was included in the diet.
- ▶ Longevity was unaffected after 1 day, but decreased during continuous feeding.
- ▶ An olive oil enriched diet increases income resources available for allocation.
- ▶ Dietary lipid supplementation could increase parasitoid effectiveness in biocontrol.

GRAPHICAL ABSTRACT



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ABSTRACT

Effectiveness of parasitoids as natural enemies in agro-ecosystems depends on key traits such as fecundity and longevity. Energy sources allocated into survival and reproduction can be mobilized from capital stores acquired during larval feeding, or from income resources through adult feeding. Adult parasitoids have a restricted diet consisting solely of carbohydrates and most species do not replenish lipid levels after emergence from their host. Here, we have adopted a novel approach that could improve pest elimination by parasitoids, in which we added different ratios of olive oil to the food of adults to reveal the potential of a lipid-rich diet to increase lipid levels and allocation into survival. Our results show that males of the parasitoid *Cotesia glomerata* had higher lipid levels when fed a diet containing a ratio of 90:10% honey to olive oil when compared to males at emergence and males fed honey-only. For females, lipid levels at emergence were similar to those of females fed a diet supplemented with a ratio of 75:25% honey to olive oil, yet in both of these treatments lipid levels were significantly higher when compared to females fed honey-only. This suggests that females on a lipid-containing diet economized on their lipid use. In contrast, the survival of males and females was negatively affected by the addition of olive oil and no differences in survival were found when wasps were fed 1 day on various ratios of honey to olive oil compared to honey-only. Our results show that the addition of a lipid source can increase or maintain nutrient availability and further research into lipid supplementation could minimize detrimental effects in order to increase the effectiveness of parasitoids in certain agro-ecosystems.

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1. Introduction

Parasitoids are widely used as natural enemies of pest species in agro-ecosystems, but their effectiveness depends on life history traits such as longevity and fecundity (Jervis, 2007). Resource allocation into these key traits depends on the nutritional content

of the host that the parasitoid larva developed on, as well as on the environmental conditions the adult parasitoid encounters (Jervis et al., 2008), particularly the availability of income food sources, such as nectar, fruit juices, honeydew, and hemolymph (Eijs et al., 1998; Godfray, 1994). Dietary concentrations of different types of carbohydrates, such as sugars (Wäckers, 2001), honeydew (Lee et al., 2004) and nectar (Winkler et al., 2006) have been shown to drastically increase parasitoid fecundity and longevity under laboratory conditions (Jervis et al., 1996; Wäckers et al., 2005).

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For example, in a laboratory study Witting-Bissinger and colleagues (2008) have shown that longevity of two parasitoid species *Trichogramma exiguum* and *Cotesia congregata* increased 8.5 fold when wasps had access to floral nectar. For *T. exiguum* fecundity was shown to increase 2.3–6.3 folds when provided with nectar, depending on plant species. However, carbohydrates are typically limited in agro-ecosystems, possibly putting constraints on pest elimination efficiency in agro-ecosystems (Heimpel and Jervis, 2005; Wäckers, 2003).

To increase parasitoid effectiveness in eliminating pests, research has focused on the provisioning of carbohydrates in the field. Approaches to increase carbohydrate availability in the field include spraying of sugar-rich food sources and the introduction of flowering plants (Landis et al., 2000). Indeed, increased carbohydrate availability positively affects parasitoid effectiveness under field conditions. A study by Cappuccino and colleagues (1999) showed that sugar provisioning by means of spraying resulted in higher levels of parasitism by the parasitoid *Elachertus cacoeciae* on its host *Choristoneura fumiferana* in treated plots, when compared to untreated control plots and plots where understory herbs had been removed. Similar findings have been obtained in other studies (Baggen and Gurr, 1998; Evans et al., 2010).

Although sugar-rich food sources positively affect survival and reproduction, the benefits of parasitoids are limited compared to those of other insect species. In general, insects exploit available sugar resources not only for acute energy needs, but also convert them into fatty acids and triglycerides to serve as a long-term energy source. Most parasitoid species, however, do not accumulate lipids as adults, even when they have access to carbohydrates (Giron and Casas, 2003; Visser and Ellers, 2008; Visser et al., 2010). This means that although the rate of lipid-use can be reduced through carbohydrate feeding, lipid reserves are not replenished during life (Ellers, 1996). As a consequence, parasitoids need continuous access to carbohydrates in order to preserve their irreplaceable lipid stores and to achieve fitness effects of carbohydrate feeding.

On the contrary, a diet that would increase parasitoid lipid reserves would only have to be accessed intermittently to have long-term fitness benefits, as is the case in other insect species (Jacome et al., 1995; Leahy and Andow, 1994). Many insects use dietary lipids during life and take up lipids from the gut for storage, egg production or catabolism to release energy (Turunen, 1979; Grillo et al., 2007). Even though the diet of parasitoids typically contains few lipids, uptake of more complex lipids through host feeding is essential for egg production in some species (Mondy et al., 2006). Here, we explored the potential of a lipid-rich food source to increase nutrient availability and associated life history traits in parasitoids. We fed adults of the parasitoid *Cotesia glomerata*, a species that does not accumulate lipids as an adult (Visser et al., 2010), with various mixtures of honey and olive oil. Compared to other oils, such as palm or sunflower oil, olive oil shows the highest similarity in the composition of fatty acids to Hymenoptera and Lepidoptera, especially in the ratio of the predominant fatty acids palmitic, oleic and linoleic acid (Belitz et al., 2009; Ramírez-Tortosa et al., 2006; Thompson, 1973). We tested if this novel approach of provisioning a lipid-rich dietary substrate increased overall lipid content and longevity of adult *C. glomerata*.

2. Materials and methods

2.1. Species

Parasitized cocoons containing *C. glomerata* were obtained from an existing laboratory culture at the University of Wageningen, The Netherlands. *C. glomerata* is a parasitoid that attacks caterpillars of *Pieris* sp., a pest species found on plants of the cabbage family

Brassicaceae. Cocoons were kept in small pots at 23 °C at a 12:12 light:dark regime and a relative humidity of 75%.

2.2. Experimental set-up

Pots were inspected daily for newly emerged individuals. After emergence 20 males and 20 females per treatment were isolated and either frozen directly at –20 °C or placed in a vial containing wet cotton wool. Twenty males and females per treatment were presented with different ratios of mixed honey and olive oil (v/v) on a piece of parafilm; 100% honey (100:0), 90% honey and 10% olive oil (90:10) or 75% honey and 25% olive oil (75:25). After 4 days of continuous exposure, surviving individuals were frozen and stored at –20 °C for lipid content analyses. Lipid levels were determined using the following method: Individuals were freeze-dried for 2 days, after which dry weight was determined to the nearest µg on a microbalance (Mettler Toledo UMT2). Individuals were subsequently placed in a vial containing 4 mL of ether to extract triglycerides. After 24 h, ether was removed and individuals were washed with fresh ether. Individuals were freeze-dried for 2 days after ether extraction and dry weight was determined again.

Twenty-five to 30 males and females were used per treatment in longevity experiments. After emergence from host pupae, individuals were isolated and allowed to feed for 1 day on a ratio of 100:0 or a ratio of 75:25 honey to olive oil. In addition, in three treatments parasitoids were allowed to feed continuously on a ratio of 100:0, 90:10 or 75:25 honey to olive oil. Individuals were monitored every morning for survival.

2.3. Statistics

Data on males and females were analyzed separately. Lipid content was determined by subtracting dry weight after ether extraction from dry weight before ether extraction, which was then converted to the percentage of lipids to correct for differences in body size. Normality of the data was inspected by looking at the error structures of the residuals of the data. Non-normal data were either log or cube root transformed to normality. Treatments were compared using an ANOVA followed by a Tukey test.

Longevity data of males and females were analyzed separately. Using survival analysis, various models were compared to describe survival data and a model assuming a Weibull distribution of the data was used as this model showed the best fit. All statistical analyses were performed using R project version 2.9 (R Development Core Team, 2009).

3. Results

3.1. Lipid levels

Adult diet had a significant effect on lipid levels in *C. glomerata* males ($F_{3,45} = 3.715$, $P = 0.018$). Dietary intake of a ratio of 90:10 honey to olive oil resulted in a significant increase in male lipid content compared to males that had recently emerged ($n = 26$, Tukey: $P = 0.025$) and males fed honey-only ($n = 24$, Tukey: $P = 0.047$), but a ratio of 90:10 did not differ from that of 75:25 honey to olive oil ($n = 11$, Tukey: $P = 0.856$) (Fig. 1A). No significant differences were found when comparing recently emerged males with those fed on a ratio of 75:25 ($n = 25$, Tukey: $P = 0.295$) and honey-only ($n = 38$, Tukey: $P = 0.989$) or a ratio of 75:25 with honey-only ($n = 23$, Tukey: $P = 0.412$). For females, dietary treatment also had a significant effect on lipid content ($F_{3,40} = 8.336$, $P < 0.001$). Dietary intake of honey-only resulted in a significant decrease in lipid levels when compared to females fed on a ratio of 75:25 ($n = 23$, Tukey: $P < 0.001$) and freshly emerged females

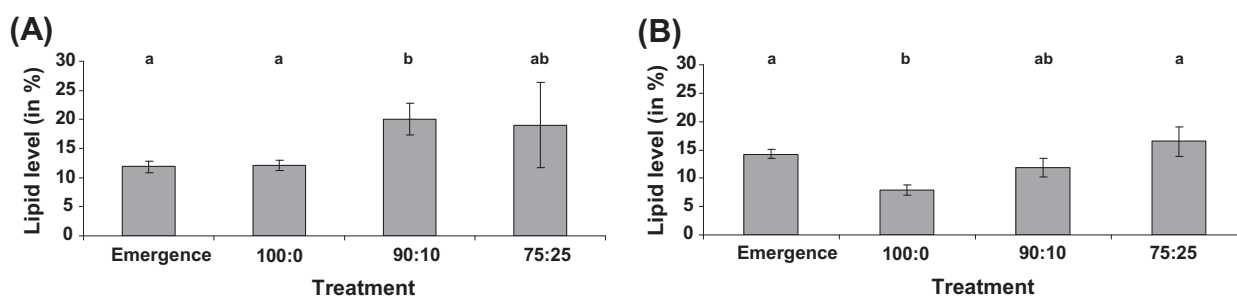


Fig. 1. Percentage of lipids for male (A) and female (B) *C. glomerata* at emergence and after feeding for 4 days on different ratios of honey to olive oil. Different letters represent significant differences at $\alpha = 0.05$.

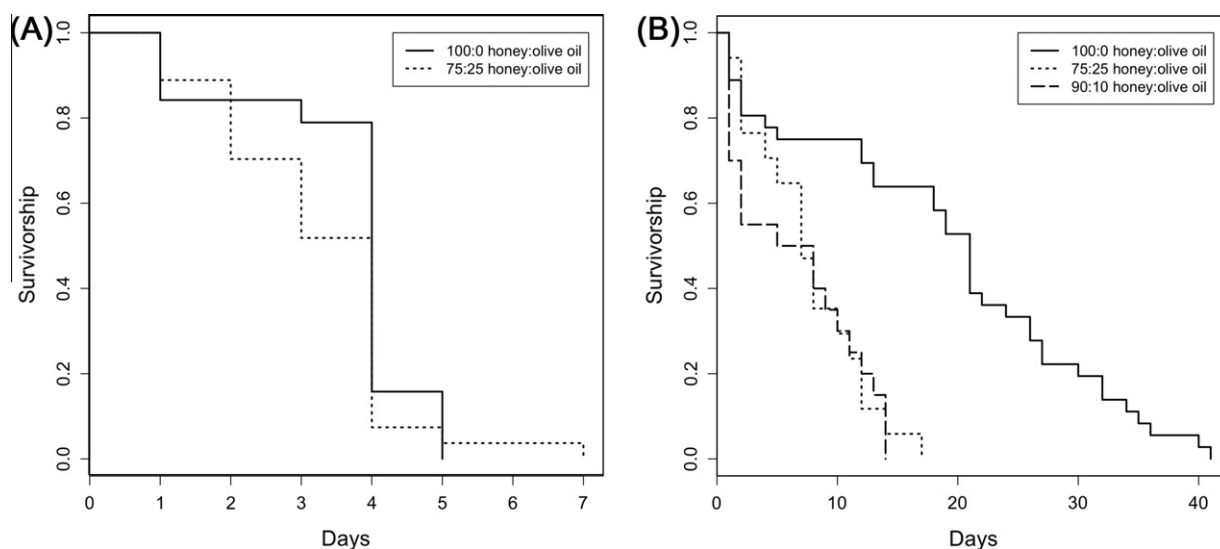


Fig. 2. Kaplan–Meier curves of survivorship in days for *C. glomerata* females fed for 1 day (A) and fed continuously (B) on different ratios of honey to olive oil (Kaplan–Meier curves for males were similar to those for females).

($n = 23$, Tukey: $P = 0.001$) (Fig. 1B). Lipid levels of females fed 90:10 honey to olive oil did not differ significantly from recently emerged females ($n = 21$, Tukey: $P = 0.438$) and those fed 75:25 honey to olive oil ($n = 21$, Tukey, $P = 0.272$) and honey-only ($n = 22$, Tukey: $P = 0.081$).

3.2. Longevity

Males fed 1 day on a ratio of 75:25 honey to olive oil and honey-only lived on average 2.3 and 2.7 days, whereas females lived on average 3.3 and 3.6 days on those dietary treatments, respectively. Males and females that were fed 1 day with a ratio of 75:25 honey to olive oil showed no extended longevity compared to those fed on honey-only (males: $n = 58$, $P = 0.350$; females: $n = 46$, $P = 0.577$, Fig. 2A). Continuous feeding on a ratio of 75:25, 90:10 and honey-only resulted in an average lifespan of 2.9, 2.4 and 12.6 days respectively for males and 7.6, 6.5 and 18.9 for females. When fed continuously on honey-only, males and females lived significantly longer compared to those fed on 75:25 and 90:10 honey to olive oil (males: $n = 61$, $P < 0.001$; females: $n = 73$, $P < 0.001$, Fig. 2B).

4. Discussion

Our results show that parasitoids can ingest lipids when allowed to feed on a food source containing honey supplemented with olive oil. The uptake of lipids in adult males resulted in an increase in lipid levels; in females the inclusion of olive oil in the

diet resulted in a lower rate of decrease of lipid reserves compared to females that were fed on honey-only. If fed on a diet containing only carbohydrates, females need to utilize their capital lipid stores, obtained during larval feeding, for allocation into survival and reproduction. Females fed on honey that was supplemented with olive oil can economize on their lipid use, because a source of lipids is provided in the food. Therefore, their lipid levels may remain stable or decrease at a slower rate.

We found no evidence that dietary lipids can be used to extend longevity. Our data show that 1 day of feeding on honey supplemented with olive oil did not result in increased lifespan. Moreover, longevity was negatively affected by the addition of olive oil when parasitoids were allowed to feed on the mixture *ad libitum* during their lives. This would suggest that olive oil becomes toxic to *C. glomerata*, even after only a few days of feeding. We expected olive oil to be a suitable lipid substrate, because the main component consists of triglycerides with a total fatty acid composition that shows most similarity to that of insects when compared to other oils (Belitz et al., 2009; Ramírez-Tortosa et al., 2006; Thompson, 1973). Although lipid compositions are comparable, relatively large quantities of oleic acid are present in olive oil (between 68% and 81.5%) (Belitz et al., 2009; Ramírez-Tortosa et al., 2006), while Hymenoptera, in particular, have on average 45.8% oleic acid (Thompson, 1973). Aside from the relative large quantity of oleic acid, olive oil contains several other compounds, such as hydrocarbons, sterols, non-glyceride esters, and alcohols (Ramírez-Tortosa et al., 2006). Mismatch in lipid ratios or the presence of other compounds in olive oil could be

responsible for the observed toxicity in parasitoids when fed a lipid-rich diet continuously.

An important question that remains to be answered is whether parasitoids are capable of using lipids provided in their diet for allocation of resources into reproduction. Other insects have been shown to readily incorporate dietary lipids in their fat body (McGuire and Gussin, 1967) and to subsequently use these lipid stores for allocation into eggs (Ziegler and Ibrahim, 2001). Inclusion of lipids in the diet of *C. glomerata* could increase egg size or number; however, *C. glomerata* fecundity is very high compared to most other parasitoids. Newly emerged females usually contain over 450 eggs, and those fed on honey for a week contain more than 950 eggs (Sato, 1975). It is unclear if capital lipid reserves are limiting lifetime fecundity, or if fecundity is limited by other factors such as egg maturation rate.

Our novel approach to increase lipid reserves in parasitoids was only partly successful. We have shown that *C. glomerata* can successfully ingest lipids during adult life, and employ these to increase lipid reserves or economize on the rate of lipid use. One possibility to avoid toxicity of ingested lipids would be to solely add palmitic acid to the diet. Palmitic acid is the end-product of the lipogenesis pathway, which can be modified into other fatty acids by elongation or desaturation (Garrett and Grisham, 1999). In a study using the cockroach *Periplaneta americana* labeled palmitic acid was readily ingested and subsequently absorbed and redistributed as diglycerides in the hemolymph of the insect (Chino and Downer, 1979). Even though parasitoids do not increase their lipid reserves, they are likely capable of modifying palmitic acid, for instance for the formation of phospholipids that are an important part of the cell membrane and as precursors for the production of pheromones, sterols and eicosanoids (Stanley-Samuelson et al., 1988). Experiments in which more complex fatty acids are added, such as certain sterols or triglycerides, could also prove valuable as sterols are important for oogenesis (Mondy et al., 2006) and triglycerides are the bulk form in which lipids are stored in the insect fat body.

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