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Does Certain Host and Food Deprivation Period Affect Host Feeding and Oviposition Behaviour of *Eretmocerus warrae* (Hymenoptera: Aphelinidae)

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ABSTRACT

Eretmocerus warrae (Naumann and Schimdt) is a thelytokous aphelinid parasitoid of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood). It was first detected in New Zealand in 1997 during a survey of greenhouses in Auckland. We investigated the effects of certain duration of food and host deprivation after emergence on feeding, oviposition behavior and egg resorption of *E. warrae* with seven treatments (0, 5, 10, 15, 20 and 25 hours of food deprivation, and 24 hours of honey feeding) before being used for the experiment. The experiments were conducted at Institute of Natural Resources, Massey University, Palmerston North, New Zealand. Under 22±1°C, 60±5% RH and 16:8 h light:dark. Results indicated that the parasitoids deprived of food and hosts for 5 hours laid significantly more eggs, lived significantly longer and fed on and parasitised significantly more hosts (P<0.0001). With the increase in food and host deprivation period from 5 to 25 hours the average daily host feeding rate significantly increased (P<0.0001). Significant egg resorption was detected after 3 days of food and host deprivation (P<0.0001). However, in honey fed parasitoids, significant egg resorption occurred after 6 days of host deprivation (P<0.0001). This study suggests that food and host deprivation for 5 h is the optimal period for host feeding, fecundity and longevity in *E. warrae*.

*Key words: Eretmocerus warrae***,** host density, fecundity, host feeding, parasitism.

INTRODUCTION

Greenhouse whitefly, *Trialeurodes vaporariorum*, Westwood (Homoptera: Aleyrodidae) is one of the most important pests of glasshouse crops in many countries causing millions of dollars of damage to agronomic, horticultural and ornamental crops in temperate regions of the world (Byrne and Bellows, 1991; Henneberry *et al.*, 1998). It was first recorded in UK in 1856 (Van Lenteren *et al*., 1996). Since then it has been reported as a major pest of greenhouse crops throughout the world (Van Lenteren *et al*., 1996). It damages the plant by direct feeding, honeydew (excreta) contamination, and transmission of viral pathogens which can cause even more serious economic

damages (Byrne and Bellows, 1991). It is well known that whitefly nymphs are sessile and susceptible to parasitism (Gerling *et al*., 1990); therefore, *T. vaporariorum* has been successfully managed in glasshouse systems with parasitoids (Vet *et al*., 1980).

Eretmocerus spp. are solitary and ecto-endoparasitoids of whiteflies with unique developmental strategy (Rose and Zolnerowich, 1997; Gelman *et al*., 2005; Zolnerowich and Rose, 2008). Eggs are deposited between the venter of the host nymph and leaf surface, the early developmental stages (1st instar) grow outside and the later larval stages within the whitefly (Gerling *et al*., 1998). The first instar larva, after hatching, penetrates the host, which involves the piercing of the host's epidermis with the mandibles, followed by resettlement of the larva into the host (Gerling, 1966; Gerling *et al*., 1990). The parasitoid penetration stimulates host epidermal cells to form a capsule around the parasitoid (Gerling *et al*., 1990, 1991) which prevents a confrontation between the parasitoid and the host's immunological systems (Gerling *et al*., 1990).

Many aphelinid genera, such as *Eretmocerus*, *Aphytis*, *Aphelinus*, *Coccophagus*, and *Encarsia*, are considered to be important biological control agents of whiteflies through their reproductive as well as host-feeding activities (Hoddle, 1998; Qiu *et al*. 2004; Zang and Liu, 2009). These parasitoids are non-concurrent destructive host feeders and superior biological control agents to non-host feeders (Jervis and Kidd, 1986; Jervis and Copland, 1996). In these parasitoids tendency to host feeding increases with (1) declining egg load (Heimpel and Rosenheim, 1995), (2) declining nutritional reserves and (3) increasing probability of survival (Chan and Godfray, 1993; Heimpel and Rosenheim, 1995). Apart from these factors, food and host deprivation for a certain period of time affects the host feeding and oviposition behaviour and egg resorption in many parasitoids (Legner and Gerling, 1967; Collier, 1995). For example, in *Coccophagus bartlettz* Annecke and Insley (Hymenoptera: Aphelinidae) egg resorption occurs after 10 days of host deprivation (Walter, 1988). In *Encarsia sophia* Girault and Dodd, host and food deprivation up to 6 hours increases the host feeding, parasitism and longevity of adults (Zang and Liu, 2009). However, in *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae), it reduces the parasitism but does not affect longevity (Fleury and Bouletreau, 1993).

Host shortage is likely to occur in the field and laboratory, which can affect the efficiency of a parasitoid as a biological control agent. Understanding an optimal duration of food and host deprivation of a parasitoid would help improve its host feeding and parasitising efficiency. So far, no information on the optimal duration of food and host deprivation of *E. warrae* is available. The objectives of the present study were to determine the effect of food and host deprivation after a certain period of time on host feeding and oviposition behaviour, and fecundity and longevity of *E. warrae*.

MATERIALS AND METHODS

Plants

Tomato*, Lycopersicon esculentum* Mill. cv. Money Maker was used as a host plant for *T. vaporariorum*. The seeds were sown in 60 cell plastic trays with each cell (4.5

cm diameter × 4.5 cm height) filled with commercial potting mix (15 % N, 8.4 % P and 10.8 % K). After 3-4 weeks, seedlings were transplanted into plastic pots (10 cm in diameter × 8 cm in height) filled with potting mix. One and a half to two month old plant or branches/leaves were used depending on the experiments. To avoid wilting a branch was placed in a water-filled transparent plastic container (3.5 cm in diameter × 5.5 cm in height) via a hole (0.5 cm in diameter) in the centre of the lid.

Insects

The colony of *T. vaporariorum* started with 500-600 pupae obtained from Bio Force Ltd, Auckland, New Zealand. A tomato plant was placed in an aluminum framed cage (60 × 45 × 40 cm) with fine metal screen (aperture diameter=0.2 mm) on the back and both sides and Perspex on the top and front for egg laying. After 24 hours, the plant was removed and kept in another rearing cage (90 × 60 × 60 cm) with a fine metal mesh from two sides and back. The top, bottom and front were made of steel with a 30 × 30 cm window of fine metal mesh. When whiteflies pupated, the infested branches were cut off from the plant and placed in above mentioned transparent plastic containers with water to avoid wilting. Those containers were kept in the above mentioned aluminum framed cages for maintenance of the colony.

The colony of *E. warrae* was initiated with 400-500 parasitised pupae obtained from Bio Force Limited, Auckland, New Zealand. These pupae were kept in plastic Petri dishes (5.5 cm in diameter × 1.3 cm in height) and transparent glass vials (1.5 cm diameter × 5 cm height) with a 0.5 cm mesh covered hole in lids. As *E. warrae* is thelytokous in nature (Hanan *et al*., 2009, 2010, 2012), no males are found in the colony. The emerging adult parasitoids were directly released onto the plants/branches infested with the second and third instar nymphs of *T. vaporariorum* kept in perplexed cages (30 × 30 × 30 cm) with fine metal screen (aperture diameter=0.2 mm) on top for ventilation for parasitisation. When parasitised nymphs reached the pupal stage, they were harvested and placed individually into transparent glass vials or plastic Petri dishes for emergence and used for experiments and colony maintenance.

Environmental Conditions

All experiments were carried out at 22 ± 1°C and 60 ± 5% RH, with a photoperiod of 16:8 h (light:dark) (lights on from 09:00 to 01:00 hours and off from 01:00 to 09:00 hours). Lighting was provided by high frequency broad-spectrum biolux tubes (Osram, Germany).

Effect of food and host deprivation on host feeding and oviposition behaviour and longevity

To determine whether and how certain duration of food and host deprivation after emergence affected host feeding and oviposition behaviour of *E. warrae*, seven treatments were set up: 0, 5, 10, 15, 20 and 25 hours of food deprivation, and 24 hours of honey feeding before being used for the experiment. In treatments 1 to 6, the parasitoids were reared individually in glass vials without food and host while in the treatment 7, the parasitoids were reared individually in Petri dishes and fed with 10%

honey solution. Honey solution was provided in a cotton wick inserted into the Petri dish through a 1 cm hole in the lid. Ten parasitoids (replicates) were used for each treatment. For each replicate, one parasitoid was released into a Petri dish with a fresh leaf infested by 100 2nd instar nymphs, allowed to stay for 24 h, and then moved into another Petri dish containing the same number of nymphs. This process was repeated until she died. The fecundity and parasitism of *E. warrae* were determined by turning over all the nymphs and counting the number of eggs laid in each Petri dish. Host feeding was recorded if the nymph body fluid was found to have effused as a result of penetration of the female ovipositor into the vasiform orifice of host nymphs. The longevity of adult parasitoids was also recorded.

Effect of food and host deprivation on egg resorption

To determine the effects of food and host deprivation on egg resorption of *E. warrae*, two experiments were set up. In the first experiment, nine treatments were set up: 0, 5, 10, 15, 20, 25, 48 and 72 hours of food deprivation, and 24 hours of honey feeding of parasitoids before being dissected for the experiment. In treatments 1 to 8, the parasitoids were reared individually in glass vials without food and host while in the treatment 9, the parasitoids were reared individually in Petri dishes and fed with 10% honey solution. Honey solution was provided in a cotton wick inserted into the Petri dish through a 1 cm hole in the lid. For each treatment, a newly emerged parasitoid (0 h after emergence) was released into a Petri dish and dissected after 0, 5, 10, 15, 20, 25, 48 and 72 hours of food and host deprivation, and 24 hours of honey feeding. They were dissected in 70% alcohol on a slide under the stereomicroscope. One drop of 1 % acetocarmine was added to the alcohol and allowed to stand for 3 to 5 minutes for staining. The chorion of mature eggs prevents the stain but immature eggs absorb the stain (Edwards, 1954). The number of mature eggs in the ovaries was then counted under a compound microscope to record the effects of food and host deprivation and 24 hours honey feeding on egg resorption in *E warrae*. Ten parasitoids were dissected for each treatment.

In the second experiment, 120 parasitoids (0 h after emergence) were reared individually in Petri dishes and fed with 10% honey solution. The honey solution was provided in a cotton wick as described above. Ten parasitoids were then selected randomly and dissected daily for up to ten days. They were dissected in 70% alcohol as described above. Those parasitoids which died naturally during the experiment were discarded from the experiment. The number of mature eggs in the ovaries was then counted under a compound microscope to record the effects of honey feeding on egg development and resorption in *E warrae*.

Statistical analyses

A goodness-of-fit test was used to test data normality. All data were normally distributed, and thus analysed using one way ANOVA followed by Tukey's studentised range (HSD) test.

RESULTS

Effect of food and host deprivation on host feeding, oviposition behaviour and longevity

Food and host deprivation for certain period of time significantly affected the longevity and host feeding behaviour of *E. warrae* (Figs. 1, 2). The results show that the parasitoids deprived of food and hosts for 5 hours laid significantly more eggs, lived significantly longer and fed on and parasitised significantly more hosts (F=33.69, 44.27, 32.95 and 33.86, for number of eggs laid, longevity, number of hosts fed on and parasitised, respectively; df=6,63; P<0.0001) (Fig. 1). However, no significant difference was found in the longevity between the parasitoids deprived of food and hosts for 5 hours and those fed with honey for 24 hours (Fig. 1d).

The average daily host feeding rate significantly increased with the increase in food and host deprivation period from 5 to 25 hours while it was significantly lower in 24-hour honey fed parasitoids (F=16.20; df=6,63; P<0.0001) (Fig. 2a). No significant difference in the parasitism rate was found in the parasitoids deprived of food and host for upto 15 hours and 24-hour honey fed parasitoids. Similarly, no significant difference in the parasitism rate was found in the parasitoids deprived of food and host for 0, 10 to 20 hours and 24-hour honey-fed parasitoids. However, the parasitism rate was significantly higher in the parasitoids deprived of food and host for 5 hours than the parasitoids deprived of food and host for 20 and 25 hours followed by the newly emerged parasitoids and 24-hour honey fed parasitoids (F=6.79; df=6,63; P<0.0001) (Fig. 2b).

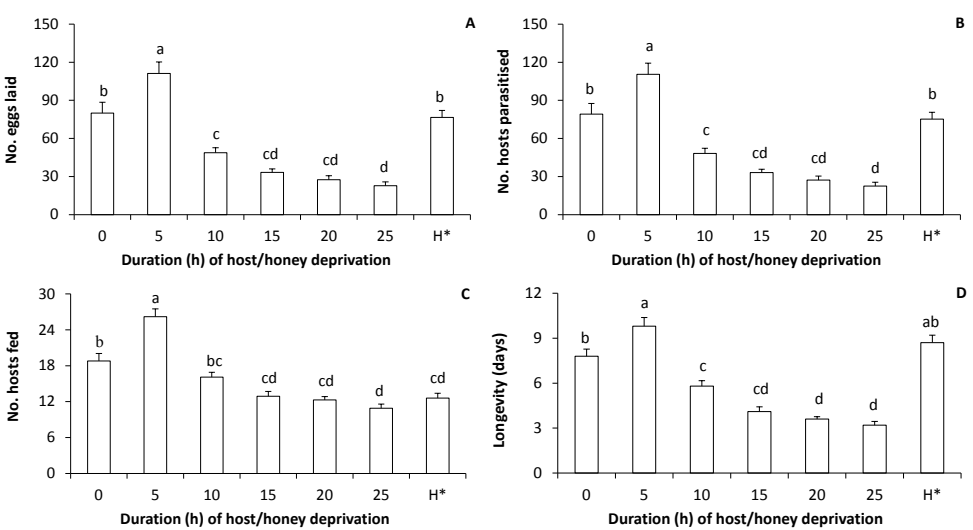

Fig. 1. Effect of host and food deprivation on the total number of eggs laid (A), hosts para itised (B), hosts fed (C), and longevity (D) in *E. warrae*. Columns with the same letters are not significantly different (P>0.05). H*=10% honey feeding for 24 hours.

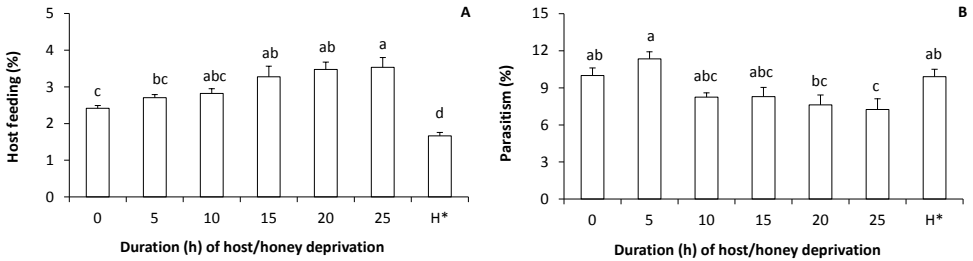

Fig. 2. Effect of host and food deprivation on the average daily host feeding (A) and parasitism (B) in *E. warrae.* Columns with the same letters are not significantly different (P>0.05). H*=10% honey feeding for 24 hours.

Effect of food and host deprivation on egg resorption

No significant egg resorption was detected after up to 2 days of food and host deprivation but the number of mature eggs significantly decreased after 3 days of food and host deprivation in *E. warrae* (F=4.75; df=8,81; P<0.0001) (Fig. 3). However, in honey fed parasitoids, the number of mature eggs significantly decreased after 6 days of host deprivation (F=15.55; df=9,89; P<0.0001) (Fig. 4).

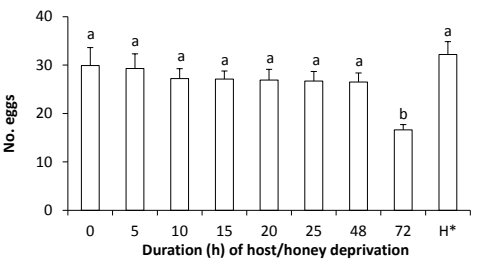

Fig. 3. Effect of host and food deprivation and 24 hours honey feeding on egg load in *E. warrae*. Columns with the same letters are not significantly different (P>0.05). H*=10% honey feeding for 24 hours.

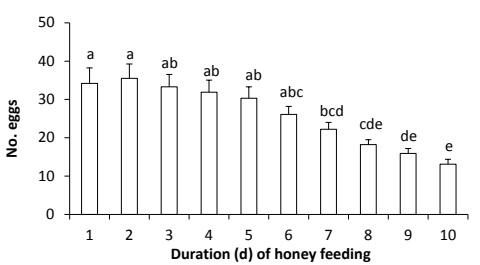

Fig. 4. Effect of honey feeding on egg resorption in *E. warrae*. Columns with the same letters are not significantly different (P>0.05).

DISCUSSION AND CONCLUSIONS

Many insects modify their feeding behaviour in response to food and host deprivation for a certain period of time (Legner and Gerling, 1967; Hickman *et al*.,

2001). The present study indicates that *E. warrae* fed on significantly more hosts when deprived of food and hosts for 5 hours. These results suggest that food and host deprivation for 5 hours is the optimum period for *E. warrae* host feeding. Similar results are reported for *E. melanoscutus* (Zang and Liu, 2010).

Numerous studies have demonstrated that host feeding has a linear relationship with the fecundity and longevity of parasitoids (Jervis and Kidd, 1986; Heimpel and Collier, 1996; Giron *et al*., 2004). In the present study, fecundity, parasitism, and longevity of *E. warrae* also significantly increased if it was deprived of food and hosts for 5 hours, suggesting that the increased host feeding may have a positive impact on the fecundity and longevity. Feeding on host haemolymph by the parasitoids provides lipids and carbohydrates necessary for higher fecundity and greater longevity of adult parasitoids, such as in *Eupelmus vuilletti* (Crowford) (Hymenoptera: Eupelmidae) (Giron *et al*., 2004).

The present study also indicates that although after >5h of food and host deprivation the daily host feeding rates significantly increased (Fig. 2), the longevity and consequently fecundity and parasitism significantly decreased in *E*. *warrae* (Figs. 1a, c, d). Similarly, *T. brassicae* reduces parasitism when starved for 4 days (Fleury and Bouletreau, 1993). These results imply that more than 5 hour starvation period is not favourable for *E*. *warrae* longevity, resulting in overall reduced fecundity. Sahragard *et al*. (1991) also demonstrated that decreased fecundity of *Dicondylus indianus* Olmi (Hymenoptera: Dryinidinae) is a positive function of decreased longevity.

In pro-ovigenic or pro-synovigenic species, certain time of food and host deprivation has serious consequences because egg resorption may occur during that period of deprivation (Jervis and Kidd, 1986). However, in the present study, no significant differences were found in egg load of *E. warrae* after up to two days of host deprivation and 24 hours of honey feeding (Fig. 3). These results suggest that *E. warrae* can maintain its egg load for up to this period of deprivation, and honey feeding does not increase its egg production. However, when fed with 10% honey solution with no hosts, egg resorption was detected after 5-7 days, suggesting that in the presence of honey *E warrae* maintains its egg load up to 5-7 days. Likewise, Walter (1988) found that *C. Bartlettz* retains its egg load for up to 10 days when fed with honey.

In conclusion, host feeding behaviour of *E*. *warrae* is influenced by initial food/host deprivation period after emergence. Food and host deprivation for 5 h is found to be the optimal period for host feeding, fecundity and longevity in *E*. *warrae*. To enhance biological control efficiency of *E*. *warrae*, emerging parasitoids may be kept without food and hosts for 5 hours and then released in the field or laboratory. Moreover, when hosts are rare or temporarily absent in a mass-rearing or biological control programme, *E*. *warrae* can be reared on honey solution for 5-7 days without egg depletion.

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