DIURNAL RHYTHMS OF EMERGENCE, HOST FEEDING AND OVIPOSITION OF *ERETMOCERUS WARRAE* (HYMENOPTERA: APHELINIDAE)

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ABSTRACT

Eretmocerus warrae is a parasitoid of greenhouse whitefly, *Trialeurodes vaporariorum*. It was first detected in New Zealand in 1997 during a survey of greenhouses in Auckland. In the laboratory at $22\pm1^{\circ}\text{C}$, $60\pm5\%$ RH and 16:8 h light:dark, significantly higher adult emergence occurred after 2–3 h of light. No emergence was observed during the scotophase. Host feeding and oviposition occurred in both the photophase and scotophase. In the photophase, host feeding by *E. warrae* tended to be higher after 4–6 h of light than at other stages of photophase. In the scotophase, the number of hosts fed on by *E. warrae* was significantly higher 2 h before lights came on. The number of eggs laid was significantly higher 10–14 h into the photophase than at other stages. There tended to be higher oviposition in the first 2 h of darkness.

Keywords: *Eretmocerus warrae*, whitefly, emergence, host feeding, eggs laid.

INTRODUCTION

Whiteflies (Homoptera: Aleyrodidae) are well known highly polyphagous insect pests and feed on almost any terrestrial plant (van Lenteren et al. 1996). The most important species are the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae), and sweet potato whitefly, *Bemisia tabaci* (Gennadius), which cause serious economic damage to agronomic, horticultural and ornamental crops throughout warm regions and glasshouses in temperate regions of the world (Byrne et al. 1990). *Trialeurodes vaporariorum* was first found in greenhouses in UK in 1856 (Van Lenteren et al. 1996). It causes billions of dollars of damage worldwide in crop losses each year (Henneberry et al. 1997; Chu & Henneberry 1998). It is well known that whitefly nymphs are sessile and susceptible to parasitism (Gerling 1990) and *T. vaporariorum* has been successfully managed in glasshouse systems with parasitoids (Vet et al. 1980).

Among the six species of *Eretmocerus* that have been reared from *T. vaporariorum* (Zolnerowich & Rose 2008), *E. warrae* (Nauman & Schmidt) (Hymenoptera: Aphelinidae) is a newly described thelytokous (no males) species (Workman et al. 2008). *Eretmocerus* sp. was observed to parasitise *T. vaporariorum* during a survey of greenhouses in Auckland, New Zealand, in 1997 (P.J. Workman, Plant & Food Research, pers. comm.). Ten years later, this species was identified as *E. warrae* using DNA sequencing (Workman et al. 2008). During the present study, adult wasps were sent to the Natural History Museum, London, for identification, and were confirmed as *E. warrae* (A. Polaszek, pers. comm.). However, little is known about the biology of this wasp.

Many behavioural, developmental and physiological events displayed by insects are controlled by endogenous circadian rhythms, which, in many cases, are modulated by external factors (Saunders 1982). The knowledge of a parasitoid's emergence, oviposition and feeding rhythms is fundamental for understanding the ecology and evolution of their reproductive strategies, which in turn contributes to the development and implementation

of biological control programs (He et al. 2004). Therefore, for better understanding of biological control ecology of *E. warrae*, experiments on the circadian patterns of emergence, oviposition and feeding were undertaken.

MATERIALS AND METHODS

Breeding colony and experimental conditions

The colonies of *T. vaporariorum* and *E. warrae* were initiated with parasitised and unparasitised pupae of the whitefly obtained from BioForce Limited, Auckland, New Zealand. 'Moneymaker' tomato plants were used for rearing whitefly. The colonies of *T. vaporariorum* and *E. warrae* were maintained and experiments were carried out at $22 \pm 1^\circ\text{C}$ with $60 \pm 5\%$ RH and 16:8 h light:dark, in the Entomology and IPM Laboratory, Massey University, Palmerston North, New Zealand. All parasitoids used for experiments emerged from pupae parasitised at the stage of 2nd and 3rd instar nymphs; and 2nd instar nymphs were used as hosts of parasitoids in all experiments.

Emergence

To observe the circadian emergence rhythm of *E. warrae*, two bioassay rooms were set up: a normal light regime in which photophase was set from 0800 h to 2400 h and a reverse-light regime in which scotophase was set from 1000 h to 1800 h. High-frequency, broad-spectrum biolux tubes (Osram, Germany) were used as light source. Observations in the scotophase were made under red photographic safe lamps (Phillips, Greensboro, NC).

To obtain parasitised whitefly nymphs, a tomato leaf infested with about 80-100 2nd instar nymphs were placed onto a Petri dish with a 0.5 cm layer of 1% agar solution for keeping the tomato leaf fresh. One newly emerged female parasitoid was released into the Petri dish for 24 h, and then moved each day into further Petri dishes containing the same number of whitefly nymphs until the parasitoid died. When nymphs had developed to pupal stage, they were collected and kept singly in glass vials (5 cm in height $\times$ 1.5 cm in diameter, with a 0.5 cm mesh covered hole in the lid) in the same bioassay room. Twenty female parasitoids were used in each room. Adult emergence was observed hourly in the entire photophase in the normal-light regime room, and in the entire scotophase in the reverse-light regime room.

Oviposition and feeding

To determine circadian oviposition and feeding rhythms of *E. warrae*, the light regimes in the two bioassay rooms were set up as above. One newly emerged parasitoid was released into an agar-based Petri dish (as above) containing a tomato leaf infested with 20 2nd instar whitefly nymphs. The female was allowed to oviposit for 2 h (first oviposition and feeding period), then moved into another agar based Petri dish containing the same number of nymphs (second oviposition and feeding period). This procedure was repeated until eight oviposition and feeding periods in the photophase and four oviposition and feeding periods in the scotophase were completed. Ten replicate females were tested in each light regime. As *E. warrae* place their eggs between the venter of whitefly nymphs and leaf surface (Qiu et al. 2007), all nymphs were turned over to determine the presence or absence of eggs under the stereomicroscope (Leica MZ12, German) after each oviposition period. The oviposition and host feeding patterns were determined by counting the number of eggs laid and host feeding by the parasitoid in each oviposition and host feeding period. Host feeding was recorded if the nymph body fluid was found to have escaped as a result of penetration of the female ovipositor into the vasiform orifice of host nymphs (Vet et al. 1980; Viggiani 1984). The period between emergence and first oviposition was recorded as the pre-oviposition period.

Statistical analysis

Data on hourly emergence and number of eggs laid and hosts fed per period were not normally distributed even after transformation and thus were analysed using the non-parametric Kruskal-Wallis test (KWT), followed by Dunn's procedure for multiple comparisons (Zar 1999). ANOVA was used to examine the difference in the mean number of eggs laid and hosts fed per 2 h period between the photophase and scotophase.

RESULTS

Emergence

No emergence was observed in the scotophase. In the photophase, adult emergence was significantly higher between 2 and 3 h into the photophase and then significantly decreased (KWT: $\chi^2=80.00 > \chi^2_{5,0.05}=11.07$, $P<0.0001$) (Fig. 1). No adults emerged after 7 h into the photophase.

FIGURE 1: Emergence of *E. warrae* in the photophase. Bars with the same letters are not significantly different ($P>0.05$).

Oviposition and feeding

In the photophase, host feeding by *E. warrae* tended to be higher after 4–6 h of light than at other stages of photophase, but no significant difference was detected between feeding periods (KWT: $\chi^2=6.84 < \chi^2_{7,0.05}=14.07$, $P>0.05$) (Fig. 2). In the scotophase, the number of hosts fed by *E. warrae* was significantly greater after 6 h of the scotophase (KWT: $\chi^2=13.16 > \chi^2_{3,0.05}=7.82$, $P<0.01$) (Fig. 2).

Females laid significantly more eggs between 8 and 12 h after lights on than in other periods of the photophase (KWT: $\chi^2=19.30 > \chi^2_{7,0.05}=14.07$, $P<0.01$) (Fig. 2). In the scotophase, although higher oviposition was detected in the first 2 h after lights off, no significant difference was found between oviposition periods (KWT: $\chi^2=5.74 < \chi^2_{3,0.05}=7.82$, $P>0.05$) (Fig. 2). There was no difference in the mean number of eggs laid (0.75 ± 0.23 and 0.50 ± 0.11 , respectively) and hosts fed (1.28 ± 0.39 and 0.33 ± 0.17 , respectively) per period between the photophase and scotophase ($P>0.05$). The pre-oviposition period of *E. warrae* was 7.20 ± 1.27 h.

DISCUSSION

Results of this study indicate that emergence occurred exclusively during the photophase, peaking during the first few hours of the photophase and then decreasing rapidly afterwards (Fig. 1). It is suggested that the onset of light may act as a signal for adult emergence. Fantinou et al. (1998) suggested that in *Telenomus busseolae* Gahan, a solitary egg parasitoid of various Lepidoptera, emergence during early photophase probably coincides with more favourable conditions for their survival, as in the morning field temperature is lower and humidity is higher than the rest of the day. Furthermore, *E. warrae* emergence early in the morning (Fig. 1) may facilitate maximum oviposition in the afternoon (Fig. 2).

The present results also show that *E. warrae* is active throughout a 24 hour period, suggesting that oviposition and host feeding by *E. warrae* is not controlled by

FIGURE 2: The number of eggs laid and hosts fed on by *E. warrae* throughout the photophase and scotophase. Means ($\pm$ SE) followed by the same English letters within the oviposition line and the same Greek letters within host feeding line are not significantly different ($P>0.05$). Data from the photophase and scotophase were analysed separately.

endogenous oscillator or exogenous factor (i.e. the light), but rather the parasitoid may respond to cues from the host (Couch 1997). These properties may enable *E. warrae* to act successfully as an agent in the biological control of greenhouse whitefly.

Jervis & Kidd (1986) suggested that the primary role of host feeding is to secure nutrients necessary for egg maturation and studies have demonstrated that host feeding can promote parasitoid egg production (Giron et al. 2004; Burger et al. 2005). The main host feeding of *E. warrae* occurred before the oviposition peak in the photophase, suggesting that host feeding supplied nutrients for egg maturation.

The findings of this study have implications for laboratory mass rearing and field release of *E. warrae*. For example, pre-emerged *E. warrae* (i.e. beige pupal colour) should be placed in the greenhouse early in the morning so that with the light signal, parasitoids emerge and begin to feed upon whiteflies to promote oviposition.

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