DIEL ASYNCHRONY IN REPRODUCTIVE BEHAVIOUR OF *DIAERETIELLA RAPAE* (M'INTOSH) (HYMENOPTERA: APHIDIIDAE)

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ABSTRACT

Diaeretiella rapae is an important parasitoid of cabbage aphid. Diel variation in emergence, mating and oviposition of *D. rapae* was studied in the laboratory to understand the biology and behaviour of the parasitoid. The emergence of the parasitoid was recorded hourly in two bioassay rooms set up at 22 ± 2°C with 16 h photoperiod from 0800-2400 hours or from 1800-1000 hours. Greatest emergence was found during the early photophase. The parasitoids that emerged during the scotophase did not mate until the following photophase. Unmated females that emerged during the scotophase had a lower incidence of host attack and oviposition during the dark. However, the parasitoids became active and had a greater incidence of mating and oviposition when they were brought into the light even during the scotophase. This research suggests that light triggers parasitoid activity and that the parasitoids lose their reproductive fitness if they emerge in the scotophase.
Keywords: *Diaeretiella rapae*, *Brevicoryne brassicae*, biological control, diel variation, emergence, mating, oviposition.

INTRODUCTION

Diaeretiella rapae (M'Intosh) (Hymenoptera: Aphidiidae) is an important parasitoid of cabbage aphid (*Brevicoryne brassicae* (L) (Homoptera: Aphididae)) (George 1957), which causes severe damage to most cruciferous crops (Hughes 1963). Beside parasitising aphids of cruciferous plants, *D. rapae* also attacks aphids that infest other plants, such as Russian wheat aphid *Diurpahis noxia*. *Diaeretiella rapae* is a solitary endoparasitoid (Ayal 1987; Kant et al. 2008), that lays one or more eggs in its host but only one egg develops into an adult (Godfray 1994). The diel pattern of parasitoid activity has not been documented.

Reproductive activities, such as mating and oviposition, in insects vary greatly during day and night. Some insects are active in light while others are active in dark, and their activities are very rhythmic (Saunders 1982). Behaviour of the parasitoids changes during day and night as well as during different times of photophase and/or scotophase (Saunders 1982; England 1995). Some parasitoids are active during early photophase (England 1995; Nakamura 1997) while others are active in late photophase (Allen 1998). To understand the reproductive strategies of parasitoids, it is important to know their emergence, mating and oviposition patterns (He et al. 2004). Parasitoids may also synchronise the timing of their emergence in order to increase their chances of mating and oviposition, which will ultimately maximise reproductive fitness.

In the current study, the diel pattern of emergence, mating and oviposition was investigated in *D. rapae*.

MATERIALS AND METHODS

Insect colonies

Colonies of cabbage aphid and its parasitoid *D. rapae* were established from a commercial cauliflower field near Palmerston North. Insects were reared in the laboratory on cabbage seedlings ('Autumn Pride') in plexiglass cages (30×30×30 cm). The colonies were maintained at 22 ± 2°C, 60-70% RH and 16 h photoperiod. Parasitoids used in the experiments emerged from 4-5 day old parasitised aphids.

Parasitoid emergence

Two controlled temperature rooms (22 ± 2°C) were set up at 16 h photoperiod to observe the emergence of the parasitoids during day and night. In room one, the photoperiod started at 0800 hours and ended at 2400 hours. In room two, the photoperiod started at 1800 hours and ended at 1000 hours. Five pairs of male and female parasitoids were released into an above mentioned cage that contained cabbage seedlings infested with about 100 aphids aged 4-5 days. Five sets of cages were used in each room. The parasitoids were allowed to parasitise aphids for 24 h and then transferred to another cage with fresh aphids. The process was followed until the death of the parasitoids. The aphids were allowed to feed and develop on the plants for 10 days. The mummified aphids were individually collected into 2-ml transparent Eppendorf tubes. The emergence of the parasitoids was recorded hourly during the light period in the room one and during the dark period in room two. The percentage emergence of males and females was estimated on the basis of total numbers of males and females that emerged during the entire period. The emerged parasitoids were used for the mating and oviposition experiments.

Mating behaviour

Upon emergence, the parasitoids were kept individually in a glass vial (2.5 cm in diameter and 7.5 cm in height), which contained a cotton wick soaked in a 10% honey solution, for at least 24 h before being paired for mating. The parasitoids were paired (n=67) in another glass vial during different times (early, middle and late) of photophase and scotophase without honey solution. The mating behaviour of parasitoids was observed and recorded for 1 h. Mating behaviour of parasitoid pairs (n=48) in scotophase was observed in the presence of red light. The pre-mating period (time between the pairing and mating) and the copulation time (duration between start and termination of mating) were recorded. The parasitoids that did not mate during the 1 h observation period were considered unsuccessful. The parasitoids that mated within the 1 h observation period were used for the oviposition experiment. Parasitoids that had been unsuccessfully paired during photophase were discarded. However, parasitoids that had been unsuccessfully paired during the scotophase were immediately brought into light and were observed for mating behaviour for another 1 h.

Oviposition behaviour

Oviposition behaviour of *D. rapae* was tested in scotophase and photophase. Thirty aphids (4-5 days old) were transferred to a cabbage seedling in a transparent plastic container (16 cm in diameter and 24 cm in height). A mated female was released into the container at the beginning of scotophase and then the female was removed from the container at the end of scotophase. The same female was released into another plastic container with a fresh set of 30 aphids at the beginning of photophase and her oviposition behaviour was observed during the photophase. Thirteen mated females were tested in this experiment. The aphids were allowed to feed and develop on the plants for 10 days and the number of mummies per container was recorded.

In order to test the effect of light on oviposition behaviour, a mated female *D. rapae* was released into a Petri dish (9 cm in diameter and 1.5 cm in height) with ten 5-day-old aphids during the middle of scotophase and her oviposition behaviour was observed in the presence of red light for 30 min. At the end of this 30-min period the same female was immediately released into a Petri dish with a similar set of aphids in light and the observations on oviposition behaviour were continued for another 30 min. Oviposition was considered successful even if the female oviposited only one host during the 30-min period. The oviposition behaviours of unmated females (n =23) were also tested using the same procedure.

Statistical analyses

A goodness-of-fit test was used to test the distribution of the data. ANOVA was used for analysing pre-mating duration and copulation time of different mating treatments, and for analysing parasitism during photophase and scotophase. The means were separated using a Tukey's studentised range (HSD) test. Analysis of regression (AOR) was used to determine the slope for hourly decline in male and female emergence. A chi-square test was used to determine the difference in parasitoid emergence during photophase and scotophase. Mating and oviposition success of parasitoids during photophase and scotophase was estimated by Mann-Whitney U Test (MWT). All analyses were done at the P=0.05 level of significance.

RESULTS

Parasitoid emergence

A total of 293 adults emerged during hourly observations and more than 95% of them emerged during photophase. The number of adults that emerged during the 16 h photophase (3.5 adults per hour) was significantly higher than those emerged during the 8 h scotophase (0.25 adults per hour) (P<0.0001). The diel pattern of their emergence is shown in Figure 1.

The emergence pattern was similar in males and females, with emergence peaking during the first 1-2 h of photoperiod. The emergence of males declined quickly after the second hour of photoperiod, while female emergence declined gradually over the photoperiod. The equations for the decline in male and female emergence over time (for 5 hours) were: $y = -4x + 22.2$ ($R^2 = 0.88$) and $y = -0.857x + 12.66$ ($R^2 = 0.47$), respectively, where y=percentage male or female emergence and x=time (hours) after the start of photoperiod.

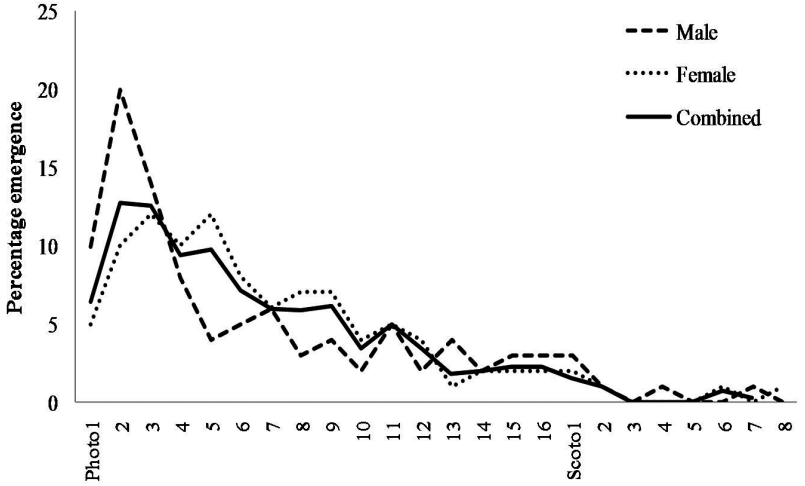

FIGURE 1: Percentage emergence of male and female *Diaeretiella rapae* during photophase and scotophase.

Mating behaviour

The parasitoids paired during the photophase were highly successful in mating compared to those paired in scotophase (P<0.0001) (Fig. 2). Mating success in *D. rapae* was not significantly affected by the time of the photophase at which they were paired (P>0.05) (Fig. 2). However, the parasitoids were more successful in mating during early scotophase than the middle and the late scotophase (Fig. 2).

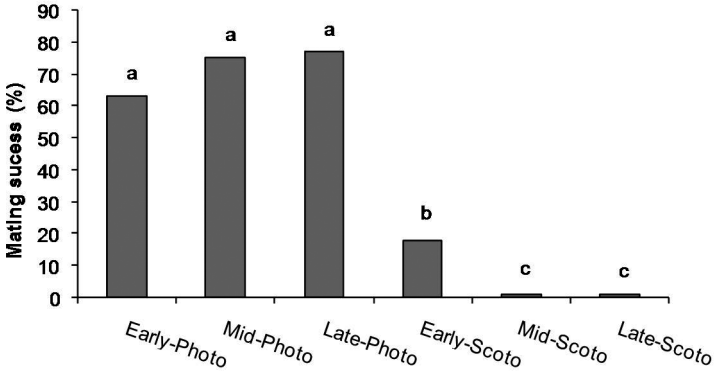

FIGURE 2: Mating success (% of pairs that mated within 60 min) in *Diaeretiella rapae* during different times of photophase and scotophase. Bars with the same letter are not significantly different (P>0.05).

The pre-mating periods of the parasitoid pairs that mated during different times of the photoperiod were not significantly different (P>0.05) (Table 1). A similar result was also found for their copulation periods (P>0.05) (Table 1).

TABLE 1: Pre-mating period (min) and copulation time (s) of the parasitoids that emerged during different time of photophase.

Time of mating	Pre-mating time	Copulation time
Early photophase	15 ± 4	58 ± 4
Middle photophase	21 ± 6	61 ± 7
Late photophase	10 ± 5	60 ± 3
ANOVA	not significant	not significant

It was observed that light had a significant effect on the mating behaviour of *D. rapae*. Success in mating was not only higher in photophase but the pairs that were unsuccessful in mating in scotophase also mated when they were brought into light (P<0.0001). The highest mating success (69%) was observed in the pairs that were brought into light after being unsuccessful in mating during the late scotophase. However, mating success was not significantly different when early (49%) and middle (38%) scotophase parasitoids were brought into light (P<0.0001).

The parasitoids that were unsuccessful in mating during the late scotophase took a significantly longer time (53±3 min) to mate when they were brought into light, when compared to the parasitoids of early (12±3 min) and middle (24±9 min) scotophase (P<0.001). However, the copulation times of early (58±1 s), middle (56±3 s) and late (59±2 s) scotophase parasitoid pairs who mated when brought into light, were not significantly different (P>0.05).

Oviposition behaviour

Oviposition ability of females in scotophase and photophase was different. The mean number of aphids parasitised by a female was significantly higher in photophase (16±1.5) than in scotophase (1.5±0.5; P<0.0001). Females oviposited 96% of their total eggs during photophase.

Oviposition was quite low during scotophase, but was triggered ($P<0.0001$) when the females moved into the light, for both mated and unmated females (Fig. 3).There was no difference in the number of females ovipositing between mated and unmated females either during scotophase or after they were brought into the light ($P>0.05$) (Fig. 3).

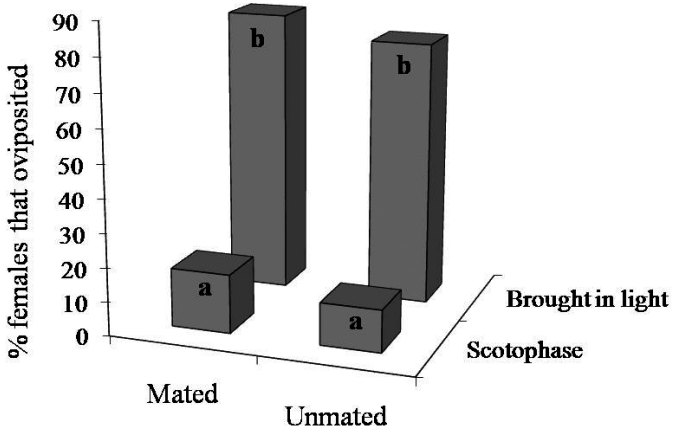

FIGURE 3: The percentage of mated and unmated *Diaeretiella rapae* females that oviposited at least one host (within 30 min) during scotophase or when brought from scotophase into light. Bars with the same letter are not significantly different ($P>0.05$).

DISCUSSION

Reproductive behaviours like mating and oviposition are greatly influenced by light in many insects. Emergence of *D. rapae* was triggered by light and prevented by darkness. Emergence of parasitoids at the start of photophase has also been observed in other species, such as *Bathyplectes curculionis*, *Exorista japonica* and *Aphidius ervi* (England 1995; Nakamura 1997; He et al. 2004). The emergence of *D. rapae* during early photophase gives them more time and opportunity for finding suitable mates and hosts. *Eriborus terebrans*, a parasitoid of European corn borer, was found to be more active in the morning than the afternoon and activity almost stopped in the dark (Dyer & Landis 1997). In most parasitic hymenopterans, males emerge earlier than females (Doutt 1964) and wait near the female pupae for females to emerge (Werren 1980) or leave the emergence site and mate when they find females (Myint & Walter 1990). Similarly, most *D. rapae* males also emerged earlier than females. Males get a better chance of encountering and mating with virgin females by emerging earlier than females (Nadel & Luck 1985). Those males that emerge later than females may get poorer quality females for mating and the mating frequency may also be reduced due to a lack of virgin females.

The emergence of males and females in the dark was negligible when compared to that in the light. By emerging in the dark, parasitoids would have to wait until the following photophase for mating and oviposition. This could be a mechanism used by parasitoids to maximise their reproductive fitness. On the other hand, emergence during photophase could merely be a result of evolutionary processes, since mating and oviposition do not occur in the dark. It has been suggested that parasitoids might also utilise scotophase as a resting period, which is also termed "tucking" (Vogt & Nechols 1991).

In general, mating in hymenopterans is synchronised with day and night changes (Nadel & Luck 1985). Mating in *D. rapae* occurred only during photophase. It is thought that males may not be attracted to females in the absence of light as females gradually stop releasing pheromone at the start of scotophase (McNeil & Brodeur 1995). However, a few *D. rapae* individuals had mated during early scotophase, which could be explained by the possibility that some females continued producing pheromone after the end of photophase. The mating success of *D. rapae* was not affected by the time of day. It is therefore suggested that *D. rapae* females continued to release their sex pheromones throughout the light period. However, other work has found that the mating activity of *A. ervi* was affected by the time of photophase (McClure et al. 2007). The parasitoid pairs that were unsuccessful in mating during scotophase did mate when they were brought into light, although they took almost 1 h to mate after the pairing. It could therefore be suggested that the effective release of pheromone by the females did not start immediately after the start of photophase.

In hymenopterans, visual cues play an important role in host searching and oviposition (Michaud & Mackauer 1994). The low parasitism rate of *D. rapae* duing scotophase and high parasitism rate during photophase would suggest that these females may also require light to select their host. It has been proposed that a female increases her reproductive fitness by laying the majority of her eggs in good quality hosts (Kant et al. 2008). Similar diel oviposition behaviour was observed by Armstrong et al. (1996) in the parasitoid *Microctonus aethiopoides* and by Mityakina et al. (1993) in the egg parasitoid *Edovum puttleri*. However, in the case of *M. hyperodae* more parasitism occurred in dark (Armstrong et al. 1996).

It can be concluded that light plays an important role in instigating reproductive behaviour of *D. rapae*. The parasitoid synchronises its emergence with photoperiod to maximise opportunities for mating and oviposition.

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