DIURNAL PERIODICITY OF ADULT ECLOSION, MATING AND OVIPOSITION OF THE EUROPEAN LEAFMINER *SCAPTOMYZA FLAVA* (FALLÉN) (DIPTERA: DROSOPHILIDAE)

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ABSTRACT

The European leafminer, *Scaptomyza flava*, is an important pest of brassicas, peas and gypsophila in New Zealand. The patterns of *S. flava* adult eclosion, mating and oviposition were determined in the laboratory. Most adults (97%) emerged in the photophase with maximum adult eclosion occurring between 2 and 3 h into the photophase. Mating took place exclusively in the photophase and peaked about 30 min after lights on. Both male and female *S. flava* mated more than once. Females started oviposition 3 days after emergence, and the fecundity (number of eggs laid) and fertility (number of eggs hatched) peaked between 5 and 10 days after emergence. The mean lifetime fecundity was 133.5 ± 4.1 eggs with the fertility rate being 57% during the 40-day oviposition period.

Keywords: *Scaptomyza flava*, eclosion, mating, oviposition, fecundity, fertility.

INTRODUCTION

Circadian clocks enable organisms to adapt to ambient environmental conditions by coupling behavioural and physiological events to cyclic factors in the environment (Kumar et al. 2006). Synchronising such events maximises an organism's potential to survive under fluctuating environmental conditions, suggesting a role of circadian clocks in the regulation of life history traits (Kumar et al. 2006). Circadian rhythms occur for most important processes of insect life, such as feeding, mating, oviposition, pheromone release, adult migration, adult emergence, etc. (Jonušatė & Būda 2002). It is well known that adult eclosion in insects occurs at a particular time in day–night cycles (Saunders 1982). In some dipteran insects including *Drosophila pseudoobscura* (Frolova & Astaurov), *D. littoralis* Meigen, *D. subobscura* Collin, *Dacus dorsalis* Hendel, *Sarcophaga argyrostoma* Robineau-Disvoidy and *Lucilia cuprina* (Wiedemann), eclosion peaks close to lights-on in light–dark cycles (Watari 2002). The timing of adult eclosion is an important biological/behavioural characteristic for a given species (Jonušatė & Būda 2002). The primary function of mating is the transfer of sperm to females, and single mating does not in general maximise female fitness in insects (Arnqvist & Nilsson 2000). Mating activity also has a circadian rhythm (Bauziene et al. 2004).

The European leafminer, *Scaptomyza flava* (Fallén, 1823) (Diptera: Drosophilidae), is polyphagous (Martin et al. 2006), and a pest of brassicas, peas and gypsophila in New Zealand (Martin 2004). It was first detected in New Zealand in 1964 and has spread rapidly in New Zealand (Martin 2004). Leafminer damage results mainly from larval feeding, which causes aesthetic damage, reduced yield and, at high densities, plant death (Bjorksten et al. 2005). Aesthetically unacceptable mines represent a direct yield loss to leafy vegetables (Spencer 1981). Potentially vulnerable crops include potatoes, celery,

tomato, onion, brassicas and ornamentals such as chrysanthemum and gerbera (Bjorksten et al. 2005). However, little is known about the biology of this pest. In the present study, the circadian rhythms of adult eclosion, mating, fecundity (number of eggs laid) and fertility (number of eggs hatched) of *S. flava* were investigated in the laboratory.

MATERIALS AND METHODS

Breeding colony and experimental conditions

A breeding colony of *S. flava* was established at Massey University, Palmerston North, in February 2008. A 1-month-old Chinese cabbage plant was exposed to about 25 *S. flava* adult females in Perspex rearing cages (30 cm × 30 cm × 30 cm) with a steel mesh top for ventilation for 24 h to allow oviposition. Honey solution (10%, 4 ml) was provided in a Perspex tube (7.5 cm in height × 1.1 cm in diameter) open at one end, as food for adults. The open end of the tube was covered with cotton wool, and the tube was attached vertically to the top of the inner side of the rearing cage. The egg infested plant was removed from the cage and held for 10 days, when the larvae hatched from the eggs had made blotch mines. The mined leaves were then cut and put on sand-filled plastic trays (45 cm long × 30 cm wide × 8 cm deep). When mature, larvae left the leaves and pupated in the sand. The pupae were then collected and maintained in Petri dishes (5.5 cm in diameter × 1.3 cm in height). When adult flies emerged from these pupae, they were transferred to the above mentioned *S. flava* rearing cages. The breeding colony and all experiments were carried out in conditions of 20±1°C, with 60%±5 R.H. and 16:8 h light:dark.

Adult eclosion

To observe the circadian emergence pattern of *S. flava* from pupae to adults, two bioassay rooms were set up. The photophase was set during 0900-0100 h in one room (normal-light regime) and during 1300-2100 h in the other room (reverse-light regime). A 1-month-old Chinese cabbage plant was exposed to about 50 adult flies for 24 h in an above-mentioned Perspex rearing cage in each bioassay room. After the completion of egg and larval development in each light regime, respectively, pupae were collected and kept singly in glass vials (5 cm in height × 1.5 cm in diameter) containing moist sand, with a 0.5 cm mesh covered hole in lids in the same light regime. Two hundred and fifty pupae were collected and observed for adult emergence in each bioassay room. Observations on adult emergence were made hourly from 0800 to 0100h in the normal-light regime room, and from 1300 to 2100h in the reverse-light regime room.

Mating

More than 80% of *S. flava* male and female adults are sexually mature when they have reached 24 h old, and almost all of them have mated after they are 48 h old (M. Shakeel, unpubl. data). Thus, 48-h-old adults were used for this experiment. One virgin male was paired with one virgin female in a glass vial 2 h before the lights went on. There were twenty pairs during 0700-0100 h in the normal-light regime room. The pairs were observed once every 1 min during the pairing period. There were two observers for this experiment. Mating during the 2-h dark period was observed under red-light conditions. The number of pairs mating at a specific time, frequency of mating for each pair and mating durations were recorded.

Oviposition

The pupae from the breeding colony were maintained singly in glass vials with moist sand. When adult flies emerged, they were kept individually in the same vial for 1 day, and females were provided with a Chinese cabbage leaf for feeding. Males were provided with 3 drops of 10% honey solution dropped onto the inner wall of the glass vial. When male and female flies were 24 h old, they were paired for mating in glass vials. Flies were observed for 30 min after pairing, and only those that mated in that time were used in the experiment. Twenty mated females were individually transferred together with the male to a transparent plastic cylinder (8.5 cm in diameter × 10.5 cm in height) with a Chinese cabbage leaf of at least 5 cm long with the leaf pedicel immersed in tap water in a clear glass vial. The top of the vials were covered with cotton wool so that the flies

would not drown. Honey solution was provided in each cylinder as mentioned above. Twenty-four hours later, the couple was moved to another plastic cylinder with a fresh leaf, and this process was repeated until the female died. The numbers of eggs laid by each female in the feeding punctures on the leaf per day was counted under the stereomicroscope (Leica MZ12, Germany) to estimate the daily fecundity. As the larvae form the mines by feeding, the number of mines on each leaf created by the neonate larvae was counted to estimate the number of eggs hatched. Observations were made up until the 3rd day as the incubation period of *S. flava* eggs has been estimated at 69.9 ± 0.3 h (M. Shakeel unpubl. data). Pre-oviposition (days from adult eclosion to oviposition), oviposition (days from initiation to termination of oviposition) and fertility (days of production of viable eggs) periods were also recorded.

Statistical analysis

Data on the number of pairs mating for once, twice and thrice were analysed using Marascuilo's non-parametric procedure (i.e. U test) (Daniel 1990). ANOVA was used to analyse the difference in mating duration between the first and second matings.

RESULTS

Adult eclosion

Adult eclosion peaked during 2 and 3 h into the photophase with 57% adults emerging during this period, after which adult eclosion sharply decreased and no fly emerged after 12 h after lights on (Fig. 1). Only 2.7% of adults emerged in the last hour of scotophase.

FIGURE 1: Mean ($\pm$ SE) hourly (number/h) and cumulative (%) adult eclosion of *S. flava* during the photophase.

Mating

No mating was observed during the scotophase. The first mating was observed 10 min after lights on, and then mating incidence quickly increased and peaked about 30 min after lights on; 90% of pairs were observed to have mated once in the 60 min after lights went on (Fig. 2). Multiple mating occurred in this species, with significantly more adults mating once or twice than those mating thrice (U test: $U=24.32 > \chi^2_{2,0.05}=5.99$, $P<0.0001$). No mating was observed after 4 h after lights on.

When insects mated twice on the same day, the mating duration was significantly longer in the first mating (mean $\pm$ SE, 19 ± 2 min) than in the second mating (12 ± 1 min) ($P=0.03$).

FIGURE 2: The number of *S. flava* pairs that had mated once, twice or thrice during the photophase. Observations were made at 1-min intervals. The cumulative proportion of pairs that had mated once is also indicated.

Oviposition

Females started oviposition 2.7 ± 0.1 days after emergence. Oviposition peaked between 5 and 10 days after emergence and 50% of all eggs were laid in the first 14 days of oviposition (Fig. 3). Fertility also peaked between 5 and 10 days of oviposition compared to other days, with 50% of all viable eggs laid in the first 12 days of oviposition (Fig. 4). The mean lifetime fecundity and fertility were 133.5 ± 4.1 and 71.6 ± 1.6 eggs, respectively, with an overall fertility rate of 53.6%. The mean lifetime oviposition and fertility period was 37.9 ± 0.6 and 22.1 ± 0.3 days, respectively.

FIGURE 3: Mean ($\pm$ SE) daily (number of eggs laid/day) and cumulative (%) fecundity during the lifetime of *S. flava* females.

FIGURE 4. Mean ($\pm$ SE) daily (number of *S. flava* leaf mines/day) and cumulative (%) fertility during the lifetime of *S. flava* females.

DISCUSSION

Diurnal rhythm is characteristic of the majority of important processes of insect life, such as adult eclosion and migration, feeding, pheromone release, mating and oviposition (Jonušatte & Būda 2002). Wicker-Thomas (2007) indicated that in the Drosophilidae, visual, acoustic and chemical cues are required for courtship. The present study shows that *S. flava* adult eclosion occurred during the day time and peaked in the early morning. The pattern detected in this study is similar to that in many black fly species with adults emerging from pupae just before sunrise (Jonušatte & Būda 2002). It is assumed that emergence during the favoured period has advantages for group activities of flies, such as feeding and mating (Jonušatte & Būda 2002).

In the alfalfa blotch leafminer, *Agromyza frontella* (Rondani), adults are able to mate on the day of eclosion (Carrière & McNeil 1988). In the present study, both sexes of *S. flava* needed about 24 h to become sexually mature (M. Shakeel, unpubl. data), which is shorter than the pre-oviposition period (about 3 days). The shorter pre-mating period enables *S. flava* females to mate before oviposition starts. Moreover, mating mostly occurs in the early morning soon after lights on. This pattern may favour *S. flava* females in having sufficient time for food location and oviposition. The present study also indicates that *S. flava* is a polygamous species, suggesting that one mating may not be sufficient to maximise their reproductive success.

About half of the total number of eggs and fertile eggs were laid in the first 14 and 12 days after emergence, respectively, during the female's 40-day oviposition period. This information together with results of adult eclosion and mating of *S. flava* will provide vital information for further investigations on reproductive behaviour of this species.

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