Entomol. exp. appl. **62**: 147–154, 1992.

Flight initiation in the egg parasitoid *Trichogramma minutum*: Effects of ambient temperature, mates, food, and host eggs

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Accepted: September 12, 1991

Key words: Flight, emergence, egg parasitoid, *Trichogramma minutum*

Abstract

Emergence, preening, and flight initiation were studied in laboratory-reared *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae). Male parasitoids emerged first and flew before females. When both sexes were present in flight cylinders, female parasitoids flew before males. Flight propensity in males was negatively related to the number of emerging females, while flight propensity in females was independent of the number of males present. Ambient temperature significantly affected the propensity and timing of flight; between 70–80% of the parasitoids flew at 25 and 30 °C while less than 4%, mostly males, flew at 20 °C. No flights were observed at 15 °C. The presence of fresh host eggs caused a reduction in the proportion of female parasitoids that flew and a delay in the time to flight for the females that did fly. The presence of food increased the flight propensity of female parasitoids, but did not affect the timing of flight. The relationship between flight behaviour and the 'efficiency' of mass-reared *Trichogramma* is discussed in terms of its importance for inundative release programmes.

Introduction

Trichogramma species have been studied worldwide as candidates for inundative release against lepidopterous pests (Stinner, 1977). Much of the interest in parasitoid efficiency has been focused on factors such as the mechanism of host finding (Vinson, 1984). There have been intensive studies of the walking behaviour of *Trichogramma*, mostly for use as a quality index in mass rearing programmes (Bigler *et al.*, 1988). In contrast, observations on flight by *Trichogramma* are rare (reviewed in Keller *et al.*, 1985; Noldus *et al.*, 1988), and understandably so because the minute size of this insect makes it difficult to observe, even within a small enclosed space in the laboratory. In the field, *T. minutum* Riley is considered an arboreal species (Thorpe, 1985) and flight as part of its biology, has been inferred (Hendricks, 1967; Smith, 1988; Yu *et al.*, 1984).

Our interest in flight of *T. minutum* is based on reports that *Trichogramma* species have displayed more dispersal power in field experiments than can be accounted for by walking alone (Stern *et al.*, 1965). Because of the large areas of forest being attacked by our targets for inundative release in Canada (Smith *et al.*, 1990), those factors influencing the dispersal and flight of *T. minutum* are of particular interest. An understanding of these factors may enable us to better target released insects, and reduce release rates.

This paper describes emergence, preening, and flight initiation in laboratory reared *T. minutum* and quantifies the influence of selected environ-

mental conditions on the timing and propensity for flight.

Material and methods

Parasitoid colony

T. minutum used in this study were reared from a laboratory culture of the Angoumois grain moth, *Sitotroga cerealella* (Olivier). The parasitoids were collected in July 1988 near Quetico Park, Ontario, Canada (48.7° N, 91.1° W) from five spruce budworm egg masses on balsam fir. They had been reared in 25 × 95 mm glass vials at 16L:8D and 20 °C for about 45 generations, after which they had been reared at 25 °C for more than 30 generations.

Experimental design

When conducting the experiments, the parasitoid colony was sampled as follows; fresh host eggs of *S. cerealella* were glued to a card and exposed for three hours to newly emerged *T. minutum*. Adult parasitoids were then removed from the egg card and it was transferred to an empty vial. These freshly parasitized eggs were incubated in 25 °C at 16L:8D for 8 days and then transferred to 15 °C and 0L:24D for about 36 h. The cold and dark treatment was done to synchronize emergence in order to get a higher rate of emergence on the testing day.

Emergence and preening behaviour

Preening behaviour and the timing of emergence were examined using parasitoids held individually in gelatin capsules. At two days prior to emergence, parasitized host eggs were gently brushed off an egg card and put individually into gelatin capsules which were then placed at 15 °C and 24 h dark to synchronise emergence. On the day of emergence, the gelatin capsules were transferred to 23 °C and parasitoids were observed from the time the lights came on until they had fully developed their wings. Our previous observations of preening behaviour indicated that a shift in behaviour to continuous walking marked the end of the preening period. Our data collection consisted of the time of emergence and the time when continuous walking started for each parasitoid. When more than one parasitoid emerged from the same egg, that capsule was discarded.

Flight test

The flight chamber consisted of a clear polyacetate cylinder (30 cm high) with a diameter of 15 cm. Both the bottom and top of the cylinder were sealed with a tightly fitting clear plastic petri dish. Light was prevented from reaching the inside of the cylinder by surrounding the sides and the top with an outer shell of black paper and aluminium foil. A 25 × 95 mm glass vial was fit tightly into a hole in the centre of the cylinder top. An open-ended cardboard cylinder (toilet paper roll), placed over the vial, screened the incoming light so that the only direct light going into the cylinder came from the top of the vial. A 1–2 mm wide barrier of Stickem Special® was placed around the inside of the cylinder at mid height to prevent parasitoids from walking to the top of the cylinder. The standard temperature for the flight tests was 25 °C (*ca.* 50% r.h.).

On the day of each test, the egg cards were cut into pieces which were introduced singly into an individual flight chamber. Experiments were always initiated at lights on (*ca.* 07:00 EST). The vial on the top of the flight cylinder was changed each hour during the flight test; parasitoids that had flown into the vial were counted and sexed. At the end of the test period (16 hours), the vial was replaced with a cork and the flight cylinder transferred to a freezer for later assessment of parasitoid emergence. Experiments were run in incubators under constant temperature and with cold white fluorescent lights.

Flight behaviour: effect of mates

The influence of mating status on flight initiation was assessed by either isolating individual para-

sitoids before emergence to prevent mating or allowing parasitoids to emerge normally and mate. Unmated parasitoids were obtained by placing individual parasitized eggs in gelatin capsules before their transfer to 15 °C for synchronization of emergence. On the morning of the test, emerging parasitoids were sexed and transferred to separate 25 × 95 mm vials (50 unmated males or 50 unmated females per vial). Mated females were obtained from an eggcard in a vial where both sexes had emerged together; between 50 and 100 females were placed in new vials. Mating status of the female parasitoids was confirmed by placing 20 individuals in separate vials with host eggs. For the flight test, vials containing either mated or unmated individuals were fixed in an upright position on the bottom of the flight chamber. The flight test was initiated within two hours from lights on; based on previous observations no flights occur during this period. There were 3 flight cylinders for each treatment (mated females, unmated females, and unmated males).

Flight behaviour: effect of temperature, host eggs, and food

The effect of temperature on the flight behaviour of *T. minutum* was tested on two separate occasions; the first experiment was run at 15, 20 and 25 °C, and a second experiment run at 25 and 30 °C. Emergence at the different temperatures was monitored using parasitized eggs from the same cards that were used in that flight test.

The effect of host availability on flight initiation was tested by comparing the flight response of emerging parasitoids, in the presence or absence of fresh host eggs. The host egg treatment consisted of placing a standard rearing card (*ca.* 4000 eggs fixed with white glue on white cardboard *ca.* 20 × 80 nm) at the bottom of the flight cylinder about 5 cm from the card where parasitoids were emerging. Control cylinders had cards with glue but no eggs.

To examine the effect of food on flight initiation, the flight response of emerging parasitoids were compared in the presence or absence of honey. Pieces of filter paper soaked in a 50% honey/water solution were placed in a circle around the emerging parasitoids at the bottom of the treatment cylinder. Control cylinders received filter paper soaked in water.

Statistical analysis

There were 6 flight cylinders for the treatments and 6 for the controls in the temperature, host egg, and food experiments. We pooled the data obtained from the control cylinders associated with the above experiments to compare the timing of male and female flight and to examine the effect of the number of individuals of one sex, on timing and flight propensity of the other sex.

The timing of flight activity was calculated using weighted means computed over the 16 h period of the flight experiment. Weighted means were compared using a 2-factor-analysis of variance with treatment and sex as factors.

Flight propensity was measured by the number of individuals flying to the top of the cylinder divided by the total number of parasitoids that emerged during the 16 h experiment. Data on the percentage parasitoids flying were arcsin transformed to achieve homogeneity of the variance, and means were compared using a t-test. Unless otherwise indicated, all values in the text and tables are means ± se.

Results

Emergence and preening behaviour

Emergence started about half an hour after the lights came on (7am) and continued throughout the day. Most individuals emerged during the first two hours of light (Fig. 1A). Although emergence of male and female parasitoids overlapped, males emerged earlier proportionally than females. After emergence, both male and female parasitoids preened for approximately one hour until the wings were fully developed (mean = 62 ± 2 min, n = 43). During this period, male parasitoids made

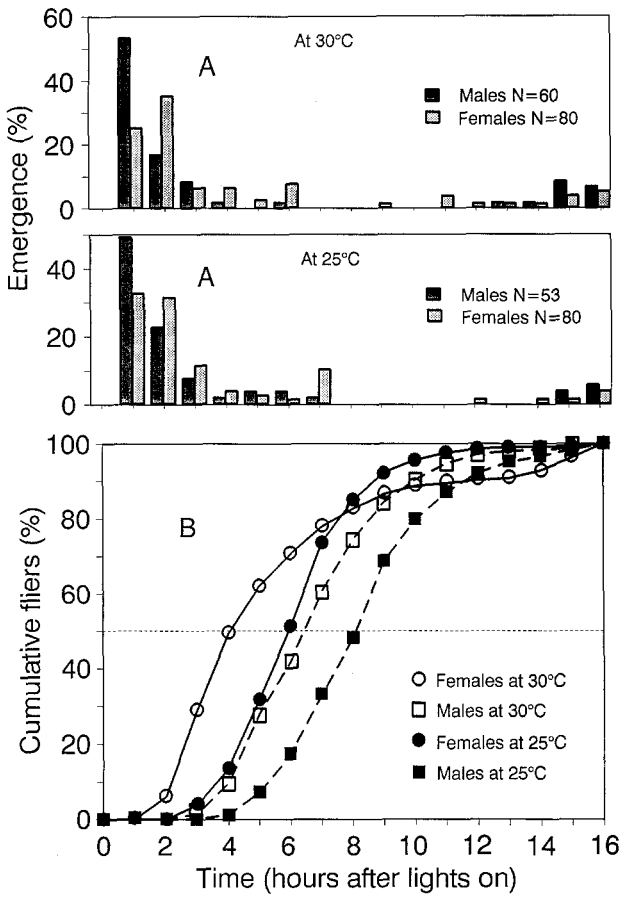

Fig. 1. The effect of temperature of the emergence (A) and flight activity (B) of male and female *Trichogramma minutum* reared on Angoumois grain moth eggs at 25 °C. Each curve in (B) represents a mean of six cylinders over a 16 hour light photoperiod.

occasional short walks, while females tended to remain in one place. Once preening was completed, both sexes assumed nearly continuous walking with occasional jumps being observed. In other experiments, when both sexes were present in emergence vials, we observed males, with their wings still only partially developed, searching for females and mating.

Flight behaviour: effect of mates

At 25 °C, flight activity started two to three hours after lights on and continued throughout the day (Fig. 1B). When male and female parasitoids were allowed to emerge, mate, and then fly within a flight cylinder, female parasitoids flew before males (Fig. 1B; mean time to flight for females = 6.13 ± 0.27 h; males = 8.75 ± 0.21 h; $t = 7.7$, $P < 0.001$, $n = 18$ cylinders). Generally, flight propensity was higher for females than for males (females = 84%; males = 79%; $t = 2.69$, $P = 0.011$, $n = 18$ cylinders). There was no significant difference in the flight times of mated female, unmated male or unmated female parasitoids ($F = 3.6$, $P = 0.095$, $n = 3$). There was a tendency, however, for unmated females to fly later than the other two groups (Fig. 2) and this was supported by preliminary experiments which showed the same consistent trend. A significantly higher proportion of mated than unmated females flew (flight propensity of mated females = 96%, unmated females = 74%, $t = 16.8$, $P < 0.001$).

There was a weak but statistically significant, negative relationship between the flight propensity of males and the total number of females in a cylinder ($r^2 = 0.322$, $P = 0.017$, $n = 17$ cylinders). Newly emerged males were observed sitting on the egg masses waiting to mate with emerging females. The association between male flight propensity and the number of females caused us to restrict further analysis of flight propensity in the temperature, host egg, and food experiments to the females only. Any effects of the treatments on male flight propensity were confounded by the number of females present in the cylinders. There was no reciprocal relationship between the flight propensity of females and the total number of males in the cylinder. There was also no relationship between the number of individuals of either sex in a cylinder and the timing of flight by either sex.

Flight behaviour: effect of temperature, host eggs and food

Both *T. minutum* males and females had a flight threshold between 20 and 25 °C. At 25 °C, 81.7% of the parasitoids flew, whereas at 20 °C only 3.4% flew (mean number of parasitoids per cylinder = 323 ± 63, n = 4, 96 ± 21, n = 6 at 25 and

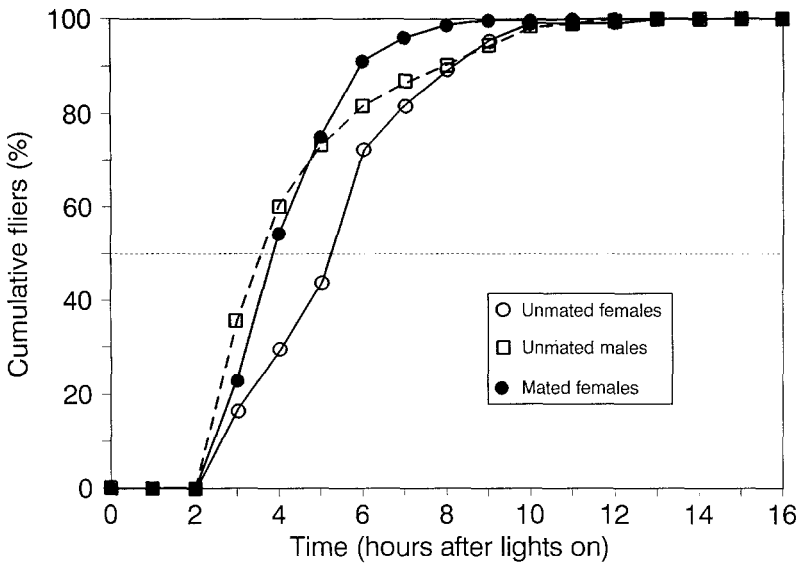

Fig. 2. The effect of mates at the time of parasitoid emergence on flight activity in *Trichogramma minutum* reared on Angoumois grain moth eggs at 25 °C. Each curve represents a mean of three cylinders.

20 °C respectively). There was no flight by either sex at 15 °C.

The timing of flight by *T. minutum* in response to temperature, host eggs or food was dependent on the sex of the individuals; there was a significant interaction effect between sex and treatment in the 2-way ANOVAs for these experiments. Because of the significant interaction term, we examined the simple effects of the treatments within the factor sex using t-tests (Sokal & Rohlf, 1981).

Flight by both male and female parasitoids occurred faster at 30 °C than at 25° (Table 1, Fig. 1B). There was no significant difference in the flight propensity of female parasitoids at 25 and 30 °C. (Mean flight propensity of females 25 °C = 90%; at 30 °C = 85%; t = 1.95, P = 0.08. Mean number of female parasitoids per cylinder = 641 ± 64).

The presence of host eggs delayed the flight of both male and female parasitoids (Table 1). The flight propensity of female parasitoids was bimodal (Fig. 3) and almost halved in the presence of host eggs (mean flight propensity with host eggs = 47%; no host eggs = 86%; t = 19.0, P < 0.001. mean number of female parasitoids per cylinder = 462 ± 49).

Table 1. Effect of temperature, host eggs, and food[1] on the mean time of flight of male and female *Trichogramma minutum* in laboratory flight cylinders

Test parameter	Time of flight after lights on (h)	
	Males mean ± s.e.	Females mean ± s.e.
Temperature		
25 °C	9.75 ± 0.17	7.59 ± 0.11
30 °C	8.22 ± 0.22	6.89 ± 0.21
	P < 0.001[2]	P = 0.012
Fresh host eggs present	9.12 ± 0.19	8.80 ± 0.39
No fresh host eggs	8.36 ± 0.23	5.23 ± 0.21
	P = 0.028	P < 0.001
Food (honey) present	9.28 ± 0.32	5.71 ± 0.13
No food	8.13 ± 0.20	5.57 ± 0.13
	P = 0.02	P = 0.395

[1] Results are from 3 separate experiments on the effects of temperature, fresh host eggs, and food. Data from each experiment were analyzed using a 2-factor ANOVA, with sex and treatment as factors. There were significant interactions between sex and treatments in all experiments, thus treatment means were compared within the factor sex using t-tests (Sokal & Rohlf, 1981).

[2] Probability that 2 means immediately above the P-value are not significantly different based on t-test (Sokal & Rohlf, 1981).

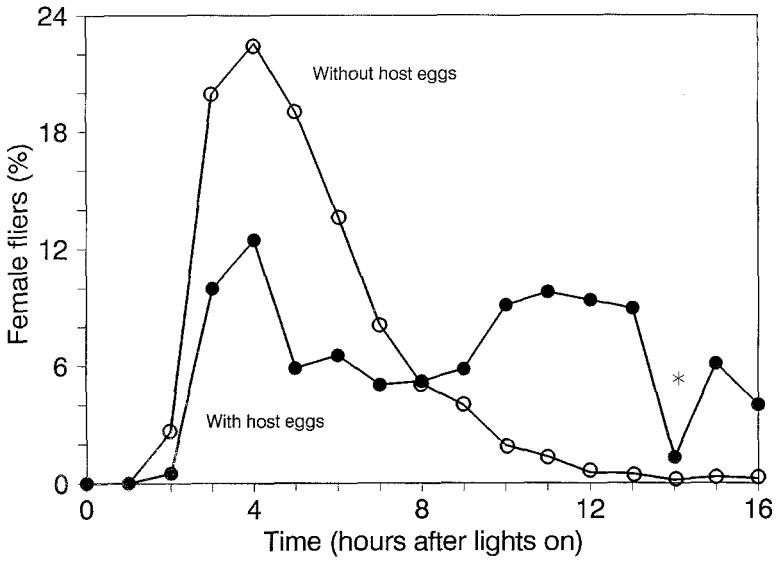

Fig. 3. The effect of fresh host eggs on flight activity of female *Trichogramma minutum* reared on Angoumois grain moth eggs at 25 °C. Each curve represents a mean of six cylinders with male parasitoids present in each cylinder throughout the experiment. The reduction in female fliers (*) at 14 h may be associated with a 30 min period of dark during which the lights were accidentally shut off.

The presence of food (honey) had no effect on the timing of flight of female parasitoids but delayed the flight in males (Table 1). There was a significant, but small increase in the flight propensity of female parasitoids in the presence of food (mean flight propensity with honey = 88%; no honey = 82%; $t = 4.55$, $P < 0.001$; mean number of female parasitoids per cylinder = 405 ± 50).

Discussion

Emergence

Diel periodicities in insect activity are often generated by a combination of endogeneous and exogeneous rhythms (Saunders, 1982). In our study, emergence of *T. minutum* was synchronized with the onset of light and most individuals emerged at the beginning of the lightperiod. The relatively high and constant temperatures during the test may have caused the emergence of a small group of individuals towards the end of the lightperiod (Fig. 1). Alternatively, our attempt to synchronize emergence for the flight test might have offset the parasitoids' normal response and caused individuals that would have normally emerged the next day to emerge early. These late emerging females were able to reach flight maturity and initiate flight only at 30 °C (Fig. 1b; see dip in cumulative flight line for females 30 °C late in flight period).

Consequences of flight behaviour for mass rearing and release programmes

In many insects, flight can be delayed after emergence because flight muscles and enzyme systems are still immature and require some time for development (Johnson, 1976). Behaviourally, a delay in flight activity may be an adaptation for daytime (afternoon rather than early morning) flight when atmospheric conditions are favourable for dispersal (Johnson, 1969). Mean flight times of both male and female *T. minutum* (8.75 and 6.13 h after lights on respectively) were delayed indicating a possible adaptation by *T. minutum* for afternoon activity.

Male parasitoids fly less and later than females because they patrol the emergence site in order to

mate with emerging females. Pheromones probably play an important role in this interaction (Matthews, 1975) and might inhibit or delay flight initiation in male parasitoids in the test cylinder. The difference in flight propensity in mated and unmated female parasitoids suggests that the presence of males influences female flight behaviour; females may be less likely to disperse when unmated. This effect may not be important under natural conditions because females will usually emerge from an egg mass containing both sexes and will always be mated. It may have important implications, however, for mass-rearing programmes, where the goal is to produce a high proportion of females or pure female lines. When released in the field, female parasitoids from these lines might be less effective in dispersing or foraging because they are more likely to be unmated. Also, in a release programme where small host eggs are deposited individually in broadcast applications (Smith *et al.*, 1990), females are less likely to be mated than under natural conditions. Alternatively, unmated females may be more effective in localized pest outbreaks where releases can be targeted and dispersal is undesirable.

It is well known that kairomones emitted by the host, its eggs or scales inhibit or postpone flight in *Trichogramma* spp. and that this allows for local search and oviposition (cf Noldus *et al.*, 1988; Thomson & Stinner, 1990). Consequently, we were not surprised to find that the most significant delay in the timing of female flight and the largest difference in flight propensity of females occurred in the presence of fresh host eggs. The distinctive bimodal pattern in female flight activity (Fig. 3), however, was unexpected and indicated a degree of behavioural variability among females; a significant proportion of the population (the first peak) will ignore host cues and initiate flight despite the ready availability of fresh host eggs.

Temperature is the most important physical factor influencing flight in insects. Two thresholds are apparent; one below which insects do not initiate flight and a lower one below which they cannot maintain flight (reviewed in McManus, 1988). Our laboratory-reared (at 25 °C) *T. minutum* did not take off at temperatures below about 20 °C. Similar observations were made by Quednau (1957, Tab. 6 p 23) for *T. cacoeciae*. Although there are many examples of naturally high temperature thresholds for flight in insects, our result could be an artifact of long-term rearing at relatively high temperature. The influence of temperature on *Trichogramma* walking, fecundity and longevity has been widely studied (e.g. Boldt, 1974; Stinner *et al.*, 1974; Smith & Hubbes, 1986), however, the effect of temperature on flight has not been considered. If flight of inundatively released parasitoids is important for successful biological control, then the temperature threshold for flight will be an essential parameter to measure for quality control in mass rearing. When considering the use of *Trichogramma* in forestry, the dispersal process and thus flight is a critical aspect of the parasitoid's behavioural make-up because of the scale and heterogeneity of the forest.

Acknowledgements

The authors thank Drs P. Newton and D. Wallace for their comments on the manuscript and Ms Nalayini Maheswaran for technical assistance. This research was supported by a Natural Science and Engineering Research Council operating grant to SMS and contracts from COFRDA, Ciba-Geigy Canada, and the Premier's Council Technology Fund of Ontario.

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