EFFECT OF HOST AGE ON SEARCHING AND OVIPOSITION BEHAVIOUR OF *DIAERETIELLA RAPAE* (M'INTOSH) (HYMENOPTERA: APHIDIIDAE)

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ABSTRACT

Diaeretiella rapae is an important biological control agent of cabbage aphid. Host searching, handling and oviposition behaviour were investigated in relation to host age. The parasitoid spent 61% of her foraging time searching for hosts. Host handling time of the parasitoid decreased with increased number of host encounters. The females were more successful in finding older hosts (7 days old) and spent more time (94.9 ± 20.5 s/encounter) and did more stings (9.9 ± 1.4/encounter) on them than the younger hosts. They preferred to sting the abdomen rather than the thorax, head or legs of the host. The average number of eggs laid per host was highest (1.4 ± 0.2 eggs) in 7-day-old hosts. When attacking 7-day-old hosts, they gained 42% success in ovipositing the host compared to 10, 18 and 30% success in 1-, 3- and 5-day-old hosts, respectively. Results suggest that *D. rapae* may have adaptive preference for larger hosts and mass production could be more efficient by using 7-day-old aphids.

Keywords: *Diaeretiella rapae*, *Brevicoryne brassicae*, host age, foraging, oviposition, searching, encounter.

INTRODUCTION

Cabbage aphid, *Brevicoryne brassicae* (L) (Homoptera: Aphididae), is a cosmopolitan pest of cruciferous crops (Hughes 1963). *Diaeretiella rapae* (M'Intosh) (Hymenoptera: Aphidiidae) is the most common natural enemy of the cabbage aphid (George 1957) and can also be used for controlling other aphids like *Myzus persicae* (Sulzer) and *Diurpahis noxia* (Mordvilko). It is solitary species, i.e. it lays one or more eggs in a host but only one develops into an adult (Godfray 1994), and females emerge with fully developed eggs (Flanders 1950). *Diaeretiella rapae* allows its hosts to feed and develop after becoming parasitised. Foraging behaviour, including searching, handling and oviposition, varies both among and within species (Lewis et al. 1990). Parasitoids may use chemical cues (semiochemicals) and/or physical features like host size to select hosts for oviposition (van Driesche & Bellows 1996).

Once a parasitoid encounters a host, the amount of time it spends on the host depends on host quality (Godfray 1994). Host age or body size may reflect the quality of the hosts for parasitoid development (Charnov et al. 1981; He & Wang 2006). Although in some parasitoids, host age or body size has no effect on oviposition decisions (Mackauer 1973; Phillips 2002), many parasitoids prefer a particular host stage for oviposition (Vinson & Iwantsch 1980). Larger hosts contain more resources and may give rise to large offspring (Bennett & Hoffmann 1998) and are often preferred by parasitoids for oviposition (Charnov 1982; Harvey et al. 2004). Host size can also affect a parasitoid's attack rate (Charnov 1982; Islam & Copland 1997) and its host handling time (Drost et al. 2000).

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Time is considered as a limited resource for parasitoids so host handling time influences the efficiency of parasitoids (Hudak et al. 2003). Understanding the foraging behaviour of parasitoids is important for the success of biological control programmes (Lewis et al. 1990), especially for optimising methods for mass production of biological control agents. Some studies on foraging behaviour of *D. rapae* have been done in relation to host plants (Gentry & Barbosa 2006) and pesticides (Umoru et al. 1996), but foraging behaviour of *D. rapae* in relation to host age or size has not been studied in detail. To help develop efficient biological control programmes for cabbage aphid and other aphid species, host searching and oviposition behaviour of *D. rapae* was studied in relation to host age.

MATERIALS AND METHODS

Insect colonies

Colonies of *B. brassicae* and its parasitoid *D. rapae* were established from parasitised and unparasitised adults of *B. brassicae* collected 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). Two holes, each 13 cm in diameter, were made on opposite sides of the cage and covered with metal mesh (aperture size of the mesh <0.5 mm) for ventilation. One of the two remaining sides of the cage was fitted with small circular opening (16 cm in diameter) for handling plants and insects in the cage. The colonies were maintained at 22 ± 2°C, 60-70% RH and 16:8 h light:dark and reared for 20 generations before use in the experiments. The sizes of the aphids were measured under a stereomicroscope (Leica MZ12, Germany) using a micrometer with a readability of 0.01 mm. Body length of 15 aphids of each age class, i.e. 1, 3, 5 and 7 days old, was measured, with means (± SE) of 0.60 ± 0.01, 1.13 ± 0.02, 1.50 ± 0.02 and 1.92 ± 0.04 mm, respectively (ANOVA: $P<0.001$). The host sizes were positively correlated with their age (analysis of regression: $R^2=0.9$, $F_{1,59}=1708.82$, $P<0.0001$).

Searching and oviposition behaviour

The behaviour of *D. rapae* was observed during the light period under a light intensity of 430 Lux supplied by broad-spectrum, high-frequency tube-lights (Osram L36W/72-965). Four aphids, each of a known age (1-, 3-, 5- and 7 days old), were released to a Petri dish (5.5 cm in diameter and 1.3 cm in height). To allow parasitoids to mate, newly emerged male and female parasitoids were put together for 24 h in a glass vial (2.5 cm in diameter and 7.5 cm in height) containing a cotton bud soaked in honey solution. The mated and honey-fed female parasitoid was released in the aphid arena, through a small hole (1 cm in diameter) in the lid of the Petri dish. Before observation, the lid was replaced with an intact lid.

Host searching and oviposition behaviour was recorded for 20 min using a video camera (JVC-TK-C1380E, Digital ½ inch CCD) connected to VCR and TV monitor. The behaviour and length of time for host searching and handling were documented manually via TV monitor and stop watch. The time budget of the parasitoid was divided into searching and host handling periods. The documented searching time of the parasitoid included walking, standing and preening between two host encounters. The host handling time refers to the time period between the first and last stings (inserting ovipositor) on the same aphid. The host handling behaviour of the parasitoid was quantified based on the amount of time the parasitoid spent per host and number of stings done per host. The number of stings on head, thorax, abdomen and legs was also recorded to determine the body part preference by the parasitoids for attack.

After these observations the aphids were transferred to cabbage leaves for feeding and development, and were dissected 4 days after parasitism to find the number of larvae in the hosts, which was assumed to be equivalent to the number of eggs laid (Bueno et al. 1993). Twenty four parasitoids were tested in this experiment.

Statistical analyses

A goodness-of-fit test was used to test the distribution of the data. The percentage data were arcsin transformed prior to analysis of variance (ANOVA). Analysis of regression

(AOR) was used to determine the relationship between the number of hosts a parasitoid encountered in 20 min and time spent per encounter. Data on handling time per encounter, stings per encounter, time taken per sting, body part preference for stings, eggs laid per host and number of stings required per egg laid were not normally distributed and were analysed by non-parametric Kruskal-Wallis test (KWT) followed by multiple comparisons using Bonferroni (Dunn) t test. Searching and oviposition success of parasitoids on hosts of different sizes was estimated by Mann-Whitney U Test (MWT).

RESULTS

Host searching and handling behaviour

The foraging time of *D. rapae* was divided into searching and host handling time. Parasitoids spent 61 ± 3.1% (mean ± SE) of their total foraging time searching for hosts, significantly more than that on handling hosts (P<0.001). Host handling times decreased as host encounter rates increased (P<0.001) (Fig. 1). During host searching, parasitoids were significantly more likely to encounter older than younger hosts (MWT: U_0=36.76> $\chi^2_{3,0.05}$ =7.82, P<0.0001) (Fig. 2).

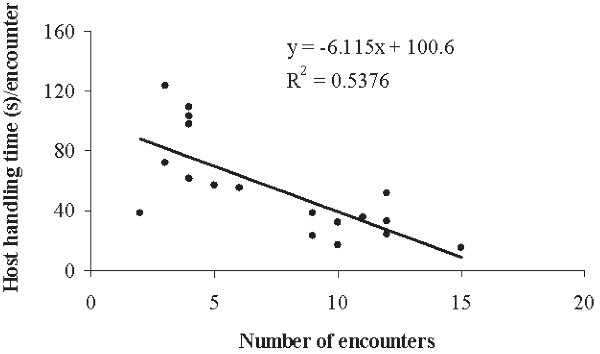

FIGURE 1: Relationship between the time (seconds) spent on an encounter by *Diaeretiella rapae* and number of encounters in 20 min.

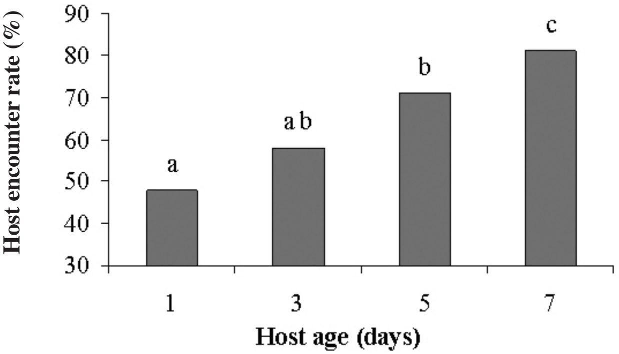

FIGURE 2: Host encounter rate by *Diaeretiella rapae* in relation to host age. Columns with the same letters are not significantly different (P>0.05).

Parasitoids spent significantly more time handling older hosts ($P<0.0001$), and stung them significantly more often than younger ones ($P<0.0001$) (Table 1). They took a significantly longer time to sting 7-day-old hosts than younger ones ($P<0.0001$) (Table 1). Older hosts appeared to attempt to defend themselves from parasitism by shaking, swinging their antennae and kicking, and these behaviours appeared to disturb parasitoids. With younger hosts, however, these behaviours were not as effective and did not disturb the parasitoid. Parasitoids also appeared to use more power to penetrate older hosts with their ovipositors.

TABLE 1: Time spent and number of stings by *Diaeretiella rapae* per host and time taken per sting in hosts of different ages.

Host age (days)	Mean time (s) per encounter	Mean number of stings per encounter	Time (s) per sting
1	15.1 ± 3.0 a	2.4 ± 0.3 a	5.5 ± 0.6 a
3	21.4 ± 4.6 ab	4.4 ± 0.9 ab	6.0 ± 0.9 ab
5	42.3 ± 7.2 b	6.5 ± 1.0 bc	8.6 ± 0.8 b
7	94.9 ± 20.6 c	9.9 ± 1.4 c	11.5 ± 2.2 c
H (df = 3)	33.96	26.75	28.43

Means (±SE) with same letters in columns are not significantly different ($P>0.05$).

Preference for stinging different body positions in hosts

Parasitoids significantly preferred the abdomen over other body parts of the host for stinging (KWT: $H=28.66>\chi^2_{3,0.05}$, $P<0.0001$) (Fig. 3). Legs were the body position least preferred by the parasitoids.

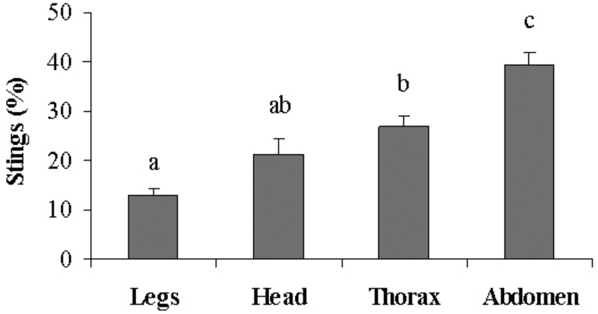

FIGURE 3: Proportion of stings performed by *Diaeretiella rapae* on different body parts of hosts. Columns with the same letters are not significantly different (P>0.05).

Oviposition success in hosts of different ages

Females laid significantly more eggs in older hosts than in younger ones ($P<0.0001$). However, the number of stings required by the parasitoid for ovipositing an egg in younger hosts was significantly less when compared to older ones ($P<0.001$) (Table 2). Females were significantly more likely to lay eggs upon encountering older than younger hosts (MWT: $U_0=10.38 > \chi^2_{3,0.05}=7.82$, $P<0.001$) (Fig. 4).

TABLE 2: Mean number of eggs laid by *Diaeretiella rapae* per encounter and number of stings required to lay an egg in hosts of different ages.

Host age (days)	Parasitoid larvae found/host	Stings required to lay an egg
1	0.3 ± 0.1 a	3.0 ± 0.9 a
3	0.5 ± 0.1 ab	6.5 ± 1.1 ab
5	0.9 ± 0.1 bc	9.8 ± 2.0 b
7	1.4 ± 0.2 c	21.8 ± 5.2 c
H (df=3)	19.57	15.56

Means (±SE) with same letters in columns are not significantly different (P>0.05).

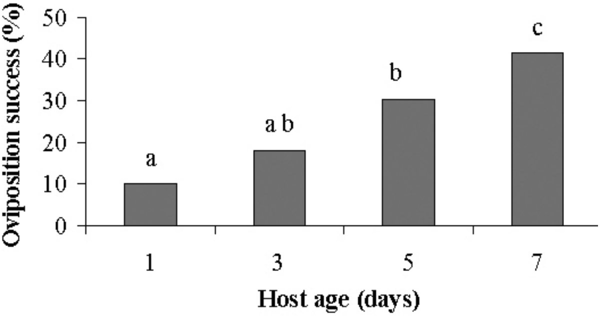

FIGURE 4: Proportion of *Diaeretiella rapae* laying eggs when encountering hosts of different ages. Columns with the same letters are not significantly different (P>0.05).

DISCUSSION

Most parasitoid species have limited time for foraging (Rosenheim 1999) and they show a great variability in searching and handling time (Vis et al. 2003). Female *D. rapae* spent twice as much time on searching for as on handling hosts. Similar behaviour was observed in *Encarsia formosa* (Hymenoptera: Aphelinidae) (Sutterlin & van Lenteren 1999) and *Eretmocerus eremicus* (Hymenoptera: Aphelinidae) (Hudak et al. 2003). Host handling time of *D. rapae* decreased even more when the female encountered a greater number of hosts. Hudak et al. (2003) also reported that the host handling time of whitefly parasitoids, *E. mundus* and *Amitus bennetti* (Hymenoptera: Platygasteridae), is affected by the number of hosts encountered.

The chance of finding a host during searching depends upon the size of host (van Roermund et al. 1994). In the present study, *B. brassicae* age was highly correlated with body length, and it was shown that *D. rapae* was more successful in encountering an older host than a younger one. This agrees with the findings of Drost et al. (2000) on parasitoids of *B. argentifolii*. It may be possible that parasitoids can detect larger hosts more easily than smaller ones. Another factor that increases the probability of encountering larger hosts is odours (semiochemicals) emanating from the host body (van Roermund et al. 1994). It is possible that larger hosts release odours in greater quantity.

Parasitoids prefer to oviposit in hosts of specific size to increase the oviposition success (Drost et al. 2000). In the present study, *D. rapae* spent more time and stung more while handling older hosts, which agrees with Godfray's (1994) suggestion that larger hosts have stronger defence capability. This may be due to frequent kicking and shaking by large hosts. The stronger cuticle of larger hosts probably makes it harder for the parasitoids to penetrate their ovipositors into hosts' bodies, which may result in the longer handling time. Ardeh et al. (2005) also observed the difficulty of *Eretmocerus sp*. in inserting its ovipositor in third instar compared to the younger instars of whitefly.

While attacking a host, *D. rapae* preferred to sting the abdomen of the host. A parasitoid of ants, *Elasmosoma sp*. (Hymenoptera: Braconidae), also preferred to sting and oviposit in the abdomen of its host (Poinar 2004). Phillips (2002) also observed preference of the parasitoid *Microctonus sp*. (Hymenoptera: Braconidae) for the host's abdomen compared to the head, thorax and legs. The preference for stinging the abdomen could be because it is a suitable site for oviposition. Stings on the thorax, head and legs could paralyse the hosts, which would reduce their defence capability and facilitate the oviposition process.

Although older hosts show stronger defence, *D. rapae* still prefers them for oviposition. This may be because these hosts are large and thus contain more resources for development (Charnov 1982) and produce large offspring which increases the fitness of the parasitoids (Bennett & Hoffmann 1998). *Diaeretiella rapae* deposited more than one egg in larger hosts to increase the chance of success in survival and emergence (Rosenheim & Hongkham 1996). Depositing more than one egg per host by *D. rapae* was previously observed by Lu et al. (1992). Therefore, when parasitoids encounter hosts of different quality, they adjust their foraging strategy and maximise their reproductive fitness per unit time by attacking larger hosts.

Results of this study indicate that the parasitoid *D. rapae* shows an adaptive preference towards older hosts for oviposition, which may result in larger offspring being produced. Older aphids may be provided as hosts for parasitoids to improve mass production in biological control programmes of cabbage aphids.

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