Colony optimisation of *Mastrus ridens* (Hymenoptera: Ichneumonidae), a potential biological control agent of codling moth in New Zealand

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Abstract *Mastrus ridens* (Horstmann) (Hymenoptera: Ichneumonidae) was imported from Argentina into New Zealand as part of a classical biological control programme against codling moth (CM), *Cydia pomonella* (L.) (Lepidoptera: Tortricidae). A laboratory colony was successfully established and maintained in quarantine, and experiments were conducted to understand the biology and behaviour of the parasitoid to maximise its efficiency prior to release. Naïve females parasitised a maximum of four CM larvae/day, laying a total of 12.9 ± 1.5 eggs. However, a single CM larva could support the development of a maximum of 10 parasitoids. The body sizes of emerging *M. ridens* females in the colony decreased with an increase in the number of pupae developing on a single CM larva, because of competition. Female *M. ridens* preferred larger CM larvae to smaller larvae in cocoons for oviposition. Superparasitism, host feeding and the number of CM larvae hosting damaged parasitoid eggs increased with decreasing host/parasitoid ratios.

Keywords: *Mastrus ridens*, biological control, codling moth, laboratory rearing, oviposition behaviour.

INTRODUCTION

Codling moth, (CM) *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), is a significant pest of apples in all apple growing areas of the world except Japan and eastern China (Kuhlmann & Mills 1999). *Mastrus ridens* (Horstmann) (Hymenoptera: Ichneumonidae) is a synovigenic (female continues to produce and mature eggs throughout adult life), gregarious, ectoparasitoid of prepupal CM larvae and is also an idiobiont parasitoid (prevents further development of the host after parasitism) (Mills 2005; Charles & Dugdale 2011). CM larvae complete their larval development inside fruit and pupate within cocoons under the bark of the host trees (apple,

pears and walnuts). Pupation sites are usually aggregated on the lower trunk of fruit trees. Volatiles released from CM cocoons are used by female *M. ridens* as attractants and/or arrestants in their search for hosts (Jumeau et al. 2005).

Mastrus ridens, originally identified as *M. ridibundus*, was collected from Kazakhstan in the 1990s (Horstmann 2009). *Mastrus ridens* was released in California, Argentina and Chile to control codling moth (Hougardy & Mills 2007; Devotto et al. 2010). Following approval from ERMA, New Zealand's regulatory agency for biocontrol agents, *M. ridens* was brought into New Zealand quarantine in 2009 from Argentina

(descendants of the insects established in California) for tests to determine their behaviour, biology and ecological impacts.

The average development time of *M. ridens* was 24 ± 2 days and adults lived for 20 days at 25°C (Devotto et al. 2010). Female *M. ridens* emerge with ca five mature eggs and achieve a maximum egg load of about 17 mature eggs after 3-4 days of host deprivation (Hougardy & Mills 2007). Females produce approximately 60 eggs during their life time (Hougardy et al. 2005) and parasitise a maximum of four hosts/day in the laboratory (Bezemer et al. 2005) by laying clutches of 3-4 eggs on each host. The body size of females has no influence on the lifetime number of hosts attacked (Bezemer & Mills 2003; Bezemer et al. 2005; Hougardy et al. 2005). Hougardy & Mills (2007) found that the relative activity of *M. ridens* increased with temperature up to an optimum of $25\text{--}27^\circ\text{C}$ and declined at higher temperatures.

This paper describes a series of experiments on the oviposition biology of *M. ridens* conducted in quarantine in New Zealand, to determine the conditions that maximise fitness of the colony and the parasitoids prior to release.

MATERIALS AND METHODS

Colony maintenance

A colony of *M. ridens* was initiated from 111 females and 101 males that emerged from pupae shipped from Argentina. Emerging adults were examined by pathologists to confirm freedom from disease, and a colony was established in the quarantine facility at Plant & Food Research, Mount Albert Research Centre, at $22 \pm 1^\circ\text{C}$ and a 16 h photoperiod (provided by fluorescent lighting). The parasitoids were reared on diapausing CM larvae from a laboratory culture maintained at Plant & Food Research, Mount Albert Research Centre. This culture originated from field collected CM eggs and had been maintained for 60 generations on artificial diet (Singh 1983) at 25°C and a 12 h photoperiod. Final instar, diapausing larvae were provided with rolls (about 8 cm diameter) of strips of corrugated cardboard (2.5 cm wide) in which to

spin cocoons; these were then stored at 4°C until needed ('CM rolls'). Each CM roll nominally held 50 larvae.

The *M. ridens* culture was maintained as follows. A known number of female and male parasitoids were released into a sleeved, nylon mesh-covered oviposition cage ($40 \times 40 \times 40$ cm) containing CM rolls suspended across the cage, honey-agar diet (Sandanayaka & Ramankutty 2007) and water from a moist cotton wick dipped in a water reservoir. Mating and oviposition were uncontrolled within the cage for 14 days before the CM rolls were removed and the surviving adult *M. ridens* frozen (at -20°) within the mesh cover of the cage for at least 24 h. The dead parasitoids were then disposed of and the mesh cover washed for re-use. Parasitised CM rolls were kept in a sealed container for another 4-5 days before pupae were removed from the cardboard rolls. Approximately 50 parasitoid pupae were transferred into emergence cages (Sandanayaka et al. 2011) containing honey agar droplets to provide food for emerging adults. The sex ratio of the adults emerging from each cage was approximately 1:1 (Sandanayaka et al. 2011). Twenty-four generations have been reared, producing sufficient numbers of adults from two cages/week both to maintain the colony and provide a supply of parasitoids for experiments.

Oviposition biology of *M. ridens*

The number of *M. ridens* pupae developing on a single codling moth (CM) larva was monitored from a random sample in each generation. A total of 1545 parasitised CM larvae were checked in 23 generations. The body length (from the head to the end of the abdomen) of 30 *M. ridens* females that emerged from the selected groupings of pupae (a single pupa/CM larva, and 2, 4, 7-8 and 10 pupae/CM larva) was also measured.

To allow measurement of reproductive parameters of *M. ridens* females, CM larva cocoons within two layers of white tissue paper ('CM in tissue paper') were prepared by providing layers of tissues to 50 final instar, diapausing codling moth larvae in a plastic box ($220 \times 135 \times 8$ mm). When the codling moth had cocooned, they were

stored at 4°C until needed in the experiments outlined below.

Oviposition behaviour

Direct visual observations of the behavioural responses of individual naïve mated *M. ridens* females (n=16) to a single CM in tissue paper in a Petri dish (85 mm diameter × 25 mm high) were carried out until the process of oviposition was complete. Oviposition was confirmed by *M. ridens* eggs inside the cocoon.

The impact of host body size (large – body length 16.1±0.52 mm and small – body length 8.45±0.25 mm) on *M. ridens* oviposition was tested in choice and no-choice experiments. A naïve mated female was given a choice of two large and two small CM cocoons in tissue paper for oviposition over a 72-h period in a Petri dish (85 mm diameter × 25 mm high) containing a droplet of honey-agar. Simultaneously, in the no-choice test, a single naïve mated female was exposed to either four large CM or four small CM in tissue paper in a similar Petri dish for 72 h. The choice test was repeated 20 times and each no-choice test was repeated 15 times. After 72 h, the fate of each CM larva was recorded and the total numbers of *M. ridens* larvae and eggs on each parasitised CM larva were counted. The numbers of eggs laid (from the total counts of eggs and larvae) on large CM larvae were compared with those on small CM larvae in both choice and no-choice tests using a two-sample *t*-test.

Superparasitism

The occurrence of superparasitism in restricted arenas (85 mm diameter × 25 mm high Petri dishes with honey-agar and water) under laboratory conditions was examined in two different tests. For the conspecific superparasitism test, direct visual observations were carried out to investigate the behaviour of a mated, naïve *M. ridens* female when exposed to a choice of either an unparasitised CM in tissue paper or a CM in tissue paper already parasitised by a different female within the previous 2 h. Observations were continued until an oviposition event was completed. The choice of the CM larva

for oviposition and the number of *M. ridens* eggs found on that larva were recorded. This test was repeated with 15 females. For the self superparasitism test, a mated, naïve female was exposed to three individual CM in tissue paper for 96 h. This was repeated with 10 different females. The numbers of *M. ridens* eggs or larvae on each CM larva was recorded, including three development stages of *M. ridens* larvae (eggs, white small larvae that were estimated as being <48 h old and larger yellowish larvae >48 h old).

In addition, different host/parasitoid ratios were tested to investigate the effect of competition on superparasitism and host feeding. Ten CM in tissue paper were placed in a box (160 × 110 × 70 mm in size, containing honey-agar and water) and exposed to 1, 5, 10 or 20 mated female *M. ridens* for 24 h. CM larvae were then checked for parasitism, superparasitism, host feeding and damaged eggs. This experiment was repeated 10 times. The total number of *M. ridens* eggs found on each CM larva was recorded and the percentage of damaged eggs calculated. The percentages of superparasitised and host-fed CM larvae were also recorded for each host/parasitoid ratio. Data were analysed first using the Kruskal-Wallis test (non-parametric ANOVA test). If the *P*-value was significant at the 5% level, the Wilcoxon rank sum test (pair-wise non-parametric two sample test), was applied to test the differences between ratios. The statistical package R 2.12.0 was used in the computation.

RESULTS

Number of *M. ridens* per codling moth larva

In measurements made on the whole colony, the number of *M. ridens* pupae recorded from a single CM larva ranged from 1–10, but most CM larvae held 2–5 *M. ridens* (Figure 1). The body length of the emerging female parasitoids decreased with an increasing number of pupae developing on a single CM larva. The parasitoids that emerged from single *M. ridens* pupa/CM larva were the largest in body size (6.3±0.06 mm). The body length of the females emerged from 2 pupae/CM larva (6.0±0.05 mm) and 4 pupae/CM larva (6.0±0.08 mm) were not significantly

different from each other ($P>0.05$). Females that emerged from 7–10 pupae/CM larva were smaller than the rest of the females (Figure 2).

Visual observations of oviposition behaviour

When a *M. ridens* female reached the CM larva, it first examined the cocoon with its antennae and then touched the surface of the cocoon with the tip of the ovipositor. This behaviour (1.5–21 min) was followed by a quick penetration of the ovipositor into the cocoon when it ‘stung’ the larva with venom. Soon after, the female became motionless for 23–78 min except for occasional checks with her antennae and ovipositor. Occasionally this behaviour (the stinging action followed by remaining motionless for some time) was repeated for a few times before oviposition occurred. To commence egg laying, the female

raised her abdomen in an arc to enable full insertion of the ovipositor into the cocoon and stayed still with an inserted ovipositor for 2–8 min. After oviposition, the female lingered near the parasitised larva for 1–2 min before leaving in search of food or another host. After a single oviposition event, *M. ridens* eggs were found as a mass on the CM larva, either on the head or somewhere on the abdomen. The CM larva was observed to be paralysed from the venom injected by the female. Host feeding (adult females feeding on the contents of the larval body through a puncture made by the ovipositor) was occasionally observed and it could reliably be identified by a prominent scar left on the body of the CM larva and a stain of larval body fluid on the white tissue paper.

Figure 1 Frequency diagram of *Mastrus ridens* pupae that had developed on a single codling moth larva. Data were collected from parasitised codling moth larvae in one cage per generation for 23 generations.

Figure 2 Mean body length ($\pm$ SE) of adult female *Mastrus ridens* emerged from single and multiple pupae developed on a codling moth larva. Different letters indicate values that are significantly different ($P<0.05$).

Host body size

Significantly more eggs were laid on a larger CM larva than on a smaller CM larva in both choice and no-choice tests ($P<0.05$) (Table 1). Host feeding was more common with smaller CM larvae. From the 33.3% of host-fed smaller larvae, 16.7% were paralysed and 6.6% were found dead.

Conspecific superparasitism

Six of the 15 females chose previously parasitised CM larvae over unparasitised larvae, showing that conspecific superparasitism may occur even in the presence of unparasitised CM larvae. Two clutches of *M. ridens* eggs were found on different parts of the bodies of those larvae. The remaining nine females oviposited on previously unparasitised larvae, and only single clutches of eggs were found. Host feeding was observed in two larvae that were already parasitised. Ovicide (the destruction of the eggs of the first clutch) was encountered on one occasion. A distinctive drilling-type action with the ovipositor was observed with one female and damaged eggs were subsequently found in one clutch, presumably laid by the first parasitoid.

Self superparasitism

Self superparasitism was confirmed by the presence of three different developmental stages of *M. ridens* (eggs (2.5 ± 0.3 /CM larva), white small larvae (2.3 ± 0.3 /CM larva) and larger

yellowish larvae (3.3 ± 0.3 /CM larva)) found on the codling moth larvae exposed to a single female for 96 h. Twenty-eight of the 30 larvae were parasitised and 1-7 *M. ridens* eggs or larvae were found on each parasitised CM larva. In total, 9-15 eggs were laid by each female over the 96-h period. A few damaged eggs (mean of 2.3 ± 0.3 /CM larva) were also found, indicating some ovicide.

Effect of competition on superparasitism and host feeding

Super-parasitism, host feeding and the number of CM larvae with destroyed eggs all increased with decreasing host/parasitoid ratios. Naïve females parasitised a maximum of four CM larvae/day, laying a total of 12.9 ± 1.5 eggs. No destroyed eggs were recorded from the ratio of 1 *M. ridens*:10 CM and only 1% of CM larvae were either superparasitised or fed upon at this ratio (Table 2).

DISCUSSION

Parasitoids distribute their eggs over resources that occur in discrete units of limited size, and so confine their offspring to a fixed amount of food (Zaviezo & Mills 2000; Silva-Torres et al. 2009). The body sizes of emerging *M. ridens* females in the colony decreased with an increase in the number of pupae developing on a single CM larva because of competition among individual parasitoid larvae. Bezemer et al. (2005) reported

Table 1 The percentage of codling moth larvae that were not parasitised or paralysed with or without egg laying by *Mastrus ridens* in choice or no-choice tests. Also shown are the percentage of codling moth larvae with host feeding scars and the mean number of eggs per larva ($\pm$ SE).

Body size of CM larvae	Not parasitised	Paralysed		Host feeding scar	No. eggs per larva
		Eggs present	No eggs present		
Choice test					
large	0.0	100.0	0.0	27.5	5.8±0.3
small	47.5	30.0	22.5	17.5	0.8±0.2
No-choice test					
large	15.0	80.0	5.0	20.0	4.4±0.4
small	25.0	51.7	16.7	33.3	1.7±0.3

Table 2 Effect of competition on *Mastrus ridens* parasitism, superparasitism, host feeding and destruction of eggs.

	Number of female <i>M. ridens</i> /10 codling moth larvae			
	1	5	10	20
Mean no. of <i>M. ridens</i> eggs/CM	4.4 ± 0.3 a ¹	6.5 ± 0.4 ab	9.2 ± 0.6 b	15.3 ± 0.8 c
% parasitised	29.0 a	72.0 b	91.0 bc	96.0 c
% super-parasitised	1.0 a	46.0 b	76.0 bc	94.0 c
% host fed	1.0 a	14.0 ab	34.0 bc	45.0 c
% CM with damaged eggs	0.0	3.0 a	18.0 ab	38.0 b
% eggs damaged	0.0	2.0 a	6.0 a	10.0 a

¹Means followed by the same letters in each row are not significantly different (Wilcoxon Rank Sum test, $P < 0.05$).

that larger *M. ridens* females were able to store more metabolic resources than smaller females. They also found that the body size of a female had a positive relationship with longevity, number of mature eggs and initial egg load. Part of the maintenance protocol for the parasitoid colony was to select only larger females for release into rearing cages, and to ensure that there were sufficient numbers of CM larvae in each cage to support adequate numbers of large adult females and hence maintain the fitness of the colony.

According to the *M. ridens* rearing protocol, females were removed from emergence cages twice a week, so that the females released into rearing cages were less than 4 days old. Older females could suffer from host deprivation, which may have an effect on oviposition (Hougardy & Mills 2007). *Mastrus ridens* females deprived of hosts had a greater egg load than females provided with excess hosts (Hougardy et al. 2005). However, increasing the duration of host deprivation did not influence the host-finding ability of *M. ridens* (Hougardy & Mills 2007).

Host body size had an impact on *M. ridens* oviposition. Results of the present study demonstrated that female *M. ridens* selectively lay more eggs on larger hosts and fewer eggs on smaller hosts, presumably to allocate equal resources for offspring. Taking this into account, an integral part of maintaining the health of the parasitoid colony is to ensure that the diapausing CM hosts were not only healthy but also not subjected to competition during their

development (as this could result in smaller-sized CM).

Most parasitoids are able to recognise hosts previously parasitised by conspecifics or by themselves and either reject those hosts (discrimination) or lay a second clutch of eggs in or on them (superparasitism) (van Alphen & Visser 1990). Superparasitism is an important factor governing stability of host-parasitoid interactions especially in gregarious parasitoids, where the number of progeny per host may vary because of this superparasitism (Taylor 1988). Results of the host/parasitoid ratio test demonstrated that superparasitism was common amongst *M. ridens* females because of competition in a restricted arena (Table 2). An increased number of parasitoid progeny, from either large egg-clutch size or super parasitism, often results in smaller adults because they compete for a finite food source. Larvae of innately gregarious species tend to respond to competition for food by developing into smaller adults (Silva-Torres et al. 2009), compared with larvae of solitary parasitoids, which may kill smaller and weaker competitors. More than six *M. ridens* pupae could develop from a single CM larva, either from a larger clutch of eggs laid by a female or as a result of superparasitism. Females that emerged from the groups of 7–10 pupae/CM larva were smaller than those from CM larvae supporting fewer parasitoids and were not used for colony maintenance.

Although a maximum number of 38 *M. ridens* eggs/CM larva was recorded as a result

of superparasitism, only a maximum of 10 *M. ridens* pupae were observed to develop fully on a single CM larva. A single female might parasitise a maximum of four CM larvae by laying a total of 6–20 eggs within 24 h at the beginning of the reproductive period. This agrees with Bezemer et al. (2005), who also found that female *M. ridens* could parasitise a maximum of 4 hosts per day. Hence attempts were taken to avoid superparasitism in the *M. ridens* colony by providing sufficient numbers of CM larvae in rearing cages.

In some parasitoids, superparasitism is preceded by ovicide. Frequency of ovicide will increase with increasing proportion of hosts already parasitised (van Alphen & Visser 1990). Destroyed *M. ridens* eggs on CM larvae were occasionally found in this study, but only on superparasitised larvae. Results of the host/parasitoid ratio test indicate that superparasitism, host feeding and ovicide increasingly occurred with competition. At the same time the results indicate that ovicide is not a generalised trait to avoid competition.

Host feeding is confined to hymenopteran female parasitoids. The quantity of material removed from an individual host varies both between and within parasitoid species. Females may simply imbibe the small amounts of fluid that exude from punctures, or they may consume the entire contents of a host (Jervis & Kidd 1986). Although *M. ridens* has previously been recorded as a non-host feeding species (Hougardy & Mills 2007), host feeding by females was observed in the laboratory under competitive conditions in restricted arenas (Table 2). Since test females were provided with sufficient food, host feeding seem to be a reaction to increasing numbers of competing female parasitoids. The ichneumonid parasitoid *Pimpla turionellae* mainly used hosts for oviposition when it had a high rate of host contact while those deprived of hosts showed a much greater tendency to host feed once they were exposed to hosts (Sandlan 1979). Jervis & Kidd (1986) suggested that host feeding might help female parasitoids to maximise egg production,

but this may not be the case with host feeding of *M. ridens*, as it was not commonly observed as a part of oviposition activity. Further investigation is required to explain factors involved with host feeding of *M. ridens*.

The present study demonstrates that the conditions in which *M. ridens* are reared play a decisive role in their reproductive behaviour. Information gathered from this study was used to improve the health and value of the laboratory culture of *M. ridens*, and studies are in progress to determine the optimum host/parasitoid ratio required in rearing cages for 14 day periods.

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