Interspecific competition between *Mastrus ridens* and *Liotryphon caudatus*, ectoparasitoids of codling moth *Cydia pomonella*

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Abstract *Mastrus ridens* is a gregarious ectoparasitoid and *Liotryphon caudatus* is a solitary ectoparasitoid of cocooned codling moth larvae. These two parasitoid species could potentially compete for resources because they oviposit and develop on the same life-stage of their host. Competition experiments in the laboratory showed that females of both species oviposited into host cocoons regardless of previous parasitism, but adults of only one species ever emerged. *Liotryphon caudatus* larvae were highly aggressive. They preyed upon eggs and larvae of *M. ridens*, as well as conspecifics, such that only a single adult *L. caudatus* emerged. *Mastrus ridens* larvae survived competition with *L. caudatus* if they had a sufficient time to develop to a size that could attack *L. caudatus* eggs. However, *L. caudatus* was often still able to outcompete *M. ridens* even when *M. ridens* eggs were laid first. *Mastrus ridens* hyper-parasitised *L. caudatus* larvae that had themselves cocooned within the host cocoon, but no parasitoids emerged. *Liotryphon caudatus* out-competed *M. ridens* in an enclosed space, but that ability in a natural ecosystem remains to be investigated.

Key words *Cydia pomonella, Liotryphon caudatus, Mastrus ridens*, interspecific competition, biological control.

INTRODUCTION

Codling moth (*Cydia pomonella* L.: Lepidoptera: Tortricidae) has been a serious pest of apples in New Zealand since the 1860s. It has been subjected to classical biological control programmes since the 1890s. The parasitoid *Liotryphon caudatus* (Hymenoptera: Ichneumonidae) was released in New Zealand (from California in 1906-07 and from Canada in 1942-44), but establishment appeared to be patchy. By 1989, it was known only from a few records from Auckland, Waikato and Hawke's Bay regions (Wearing & Charles 1989). In 2011, parasitism by *L. caudatus* was recorded from abandoned apple orchards in Hawke's Bay (Cole & Walker 2011). *Liotryphon caudatus* is a solitary ectoparasitoid of cocooned codling moth larvae. It may complete a generation in 3-4 weeks depending on temperature (Mills et al. 1996), although individual adult females can survive for nearly 2 months (Clausen 1978). Females lay a life-time average of 150 eggs (Davis et al. 2016).

To improve codling moth control in New Zealand, a second classical biocontrol programme using another parasitoid of mature larvae, *Mastrus ridens* (Hymenoptera: Ichneumonidae), was initiated in 2009 (Charles & Dugdale 2011). *Mastrus ridens* was quite recently discovered, and is a gregarious ectoparasitoid of cocooned codling moth larvae (Mills 2005; Devotto et al. 2010). *Mastrus ridens* was originally collected from Kazakhstan and brought to New Zealand from Argentina in 2009. Up to ten *M. ridens* larvae can successfully complete development on a single codling moth larvae (Sandanayaka et al. 2011). Favourable rearing temperatures for *M. ridens* lie between 15°C and 25°C (Devotto et al. 2010) and, at 22±0.5°C with adequate food and water, females can live for a mean of 27.2±1.2 days and are able to produce a mean of 58.5±4.5 eggs during an oviposition period of 14.3±0.6 days (Sandanayaka et al. 2015).

Mastrus ridens and *L. caudatus* attack and develop on the same life-stage of codling moth i.e. cocooned larvae, and so there is potential for interspecific competition between them both by females searching for a host on which to oviposit, and by parasitoid larvae consuming the host. The existence of competition between females for cocooned codling moth larvae centres on whether they can detect previous parasitism and if that deters them from ovipositing on that host larva. If a female cannot determine if a larva has already been parasitized (or even if it can, but is not deterred from oviposition), then there is no behavioural competition *per se* for oviposition sites, and the consequent 'winner' of interspecific competition is determined by larval competition. Because both species are known to readily superparasitise larvae conspecifically (Cole & Walker 2011; Sandanayaka et al. 2011), we tested the null hypothesis that females do not compete for oviposition sites, and will oviposit on codling moth larvae regardless of whether they have already been used for oviposition by another parasitoid of either species. We also investigated larval competition between *M. ridens* and *L. caudatus* when developing on the same host larva. The goal of this study was to provide insights into how the interactions between the two species might influence their relative abundance in the field and effectiveness as biocontrol agents, assuming that they will increasingly come into contact with each other as *M. ridens* becomes more widely distributed throughout New Zealand.

MATERIALS AND METHODS

Insects

Laboratory cultures of both *M. ridens* and *L. caudatus* were established on codling moth larvae from a laboratory culture reared on artificial diet (Singh 1983) held at the Mt Albert Research Centre, Auckland. About 50 final instar, diapausing codling moth larvae were provided with a roll of corrugated cardboard (about 8 cm diameter and 2.5 cm wide) in which to spin their cocoons, after which they were stored at 4°C until required for experiments. *Mastrus ridens* and *L. caudatus* were reared at 22±1°C and 16 h photoperiod (provided by fluorescent lighting) in separate buildings, using the same methodology: the corrugated cardboard rolls containing cocooned codling moth larvae were placed into nylon mesh covered cages (40x40x40 cm) containing adult parasitoids and honey-agar diet and water. Cardboard rolls with parasitised codling moth larvae were removed and held in emergence containers (see Sandanayaka et al. 2011 for full details of parasitoid rearing). Emerging adults were fed with honey-agar diet and water before being deployed in experiments. All the experiments were conducted at 23±1°C and 16 h photoperiod. Adults of both species were provided with cocoons of diapausing codling moth larvae retained in corrugated cardboard.

Experiments with *Mastrus ridens* and *Liotryphon caudatus*

Experiments were conducted to establish the outcomes of exposing: (1) unparasitised codling moth larvae to competing female parasitoids of the two species in the same test arena; (2) parasitised codling moth larvae, with <3-h-old or <24-h-old eggs of one species, to experienced parasitoids of the other species; (3) codling moth larvae parasitized with *L. caudatus* larvae exposed to

M. ridens females; and (4) codling moth larvae parasitized with *L. caudatus* pre-pupae exposed to *M. ridens* females. Outcomes were measured either by counting the numbers of eggs laid by females of each species, or by counting the numbers of adult parasitoids that subsequently emerged from each codling moth larva. Females of both parasitoid species that were ≥5-day-old were used in the experiments because pilot experiments showed that younger females did not lay as many eggs.

1. Competition for oviposition on unparasitised codling moth larvae

Although *M. ridens* females are capable of oviposition within 48 h of emergence, *L. caudatus* females do not start oviposition until they are more than a week old (Davis et al. 2016). Five cocooned codling moth larvae (ccml) in a vented transparent plastic container (16x16x18 cm) with honey-agar and water were exposed simultaneously to a mated, 10- to 12-day-old and experienced (previously exposed to ccml for 48 h) *M. ridens* and a similar *L. caudatus* female for 28 h. The ccml were then removed to individually labelled containers until adult parasitoids (or moths) emerged. This was repeated ten times and the numbers and species of each emerging parasitoid were recorded.

2. Competition for oviposition on ccml with <3-h-old or <24-h-old eggs of the other parasitoid species, and competition between parasitoid larvae

A single ccml was exposed in a vented plastic vial (11 x 4 cm), containing honey agar and water, to either a 7- to 9-day-old *M. ridens* or *L. caudatus* female with previous oviposition experience (exposed to ccml for 48 h prior to the experiment). After 3 h, the ccml was transferred to a similar vial with a single female of the other species for a further 3 h period. The codling moth cocoon was then carefully opened using forceps and the number of parasitoid eggs of each species recorded. The eggs of the two parasitoids were readily distinguishable under the binocular microscope. *Liotryphon caudatus* eggs were longer (1.7±0.03 mm) than *M. ridens* eggs (1.0±0.01 mm) and were distinctly sculptured. The experiment was repeated 22 times for each parasitoid species attacking first. The ccml with eggs of both species were held individually until adult parasitoids emerged to determine the outcome of larval competition. Using the same procedure as above, but with a 24 h exposure period to each parasitoid, the responses were measured of *M. ridens* or *L. caudatus* females to ccml containing parasitoid eggs less than 24 h old. This experiment was repeated 16 times for each parasitoid species exposed first. The ccml with eggs of both species were held individually until adult parasitoids emerged.

An analysis of the mean numbers of eggs laid by each parasitoid species (using ccml with eggs from both species) during sequential oviposition periods of 3 h and 24 h, was carried out by analysis of variance followed by Fisher's protected least significant difference test (5% level), using GenStat statistical progamme. Fisher's exact test was used to test the null hypothesis of no difference in the number of ccml resulting in the emergence of either *M. ridens* or *L. caudatus* after 3-h and 24-h sequential oviposition periods.

3. Oviposition of M. ridens on codling moth larvae with developing L. caudatus larvae

Two ccml, each parasitised by *L. caudatus* five days previously, were exposed to a single 5- to 7-day-old, experienced female *M. ridens* within a glass arena (10.5x7.5 cm). Each ccml held either 1 or 2 *L. caudatus* larvae. Visual observations were used to record the moment in time when the female *M. ridens* laid eggs, and the cocoon was then opened and the numbers of *M. ridens* eggs recorded before any predation by *L. caudatus* larvae could occur. The timing and the sequence of the main oviposition behaviours (examination, acceptance and oviposition) were recorded, as described by Charles et al. (2013). The experiment was repeated ten times (i.e. using 20 ccml), and the ccml tested were held in the laboratory to identify the parasitoids that emerged.

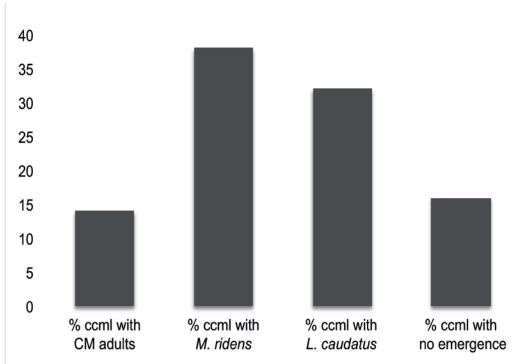

Figure 1 Parasitism of cocooned codling moth larvae (ccml) exposed simultaneously to *Mastrus ridens* and *Liotryphon caudatus*. CM = codling moth.

4. Oviposition of M. ridens on codling moth larvae with L. caudatus pre-pupae

A ccml, with a *L. caudatus* pre-pupa within its own cocoon, was exposed to a single, 5- to 7-day-old, experienced female *M. ridens* for 16 h in a vented plastic vial (11x4 cm) containing honey-agar diet and water in a cotton wick. The host cocoon was then cut open and the numbers of *M. ridens* eggs and the morbidity of the *L. caudatus* pre-pupa was recorded. The ccml was then held in the laboratory and emerging parasitoids identified. The experiment was repeated fifteen times.

RESULTS

1. Competition for oviposition on unparasitised cocooned codling moth larvae

Adult parasitoids emerged from 35 out of 50 ccml (70%): *M. ridens* emerged from 19 ccml (38%) and *L. caudatus* from 16 ccml (32%). Adult codling moth emerged from 7 ccml (14%). No emergence was recorded from the 16% of ccml that had been host-fed i.e. the parasitoid fed on the host larva rather than ovipositing (Figure 1).

2. Competition for oviposition on ccml with <3-h-old or <24-h-old eggs of the other parasitoid species, and competition between parasitoid larvae

Eggs of both species were found on most of the ccml regardless of the sequence or timing of oviposition, and the numbers laid per ccml were, in general, little changed for either a 3-h or a 24-h oviposition period (Table 1). The numbers of eggs of each species found on ccml that had been parasitized by both species were not significantly different (P>0.05) in all treatments (Table 1). The numbers of *L. caudatus* eggs per host larva indicated multiple parasitism over both 3-h and 24-h oviposition periods, and the numbers of *M. ridens* eggs, were somewhat lower than expected for this gregarious parasitoid (Table 1).

Only one parasitoid species emerged from any of the ccml which were parasitized by both species, and only a single *L. caudatus* emerged from an individual ccml. The mortality of both parasitoid species was high on all occasions, with no survival of *M. ridens* from ccml exposed first to *L. caudatus* and then *M. ridens* for 24 h (Table 1). The numbers of ccml from which either *M. ridens* or *L. caudatus* adults emerged were almost identical when ccml were initially exposed to *M. ridens* in 3-h and 24-h sequential oviposition periods. More ccml gave emergence to *L. caudatus* than *M. ridens* from eggs laid during the first oviposition phase of either 3-h (P = 0.0154) or 24-h (P = 0.003) sequence, indicating that *L. caudatus* is a superior competitor (Table 1). Host-feeding of ccml by *L. caudatus* females was observed. No parasitoids emerged from 23 – 56% of the ccml containing eggs of both parasitoid species, and some of those larvae showed obvious signs of host feeding (Table 1).

3. Oviposition of *M. ridens* on codling moth larvae with developing *L. caudatus* larvae

Female *M. ridens* readily oviposited on ccml in which *L. caudatus* larvae were already developing. After a mean searching (pre-contact) period of 2.4 ± 0.9 min, all ten tested females completed the three main behavioural steps leading to oviposition: a host examination period of 1.2 ± 0.2 min; an acceptance period of 29.2 ± 1.7 min; and an oviposition period of 3.2 ± 0.2 min. In this experiment, only one of the paired ccml was ever attacked by a parasitoid, with 2-5 *M. ridens* eggs laid. Even though the codling moth

Table 1 Multi-parasitism on cocooned codling moth larvae (ccml) by *Mastrus ridens* and *Liotryphon caudatus.* Mean values (indicated *) were not significantly different from each other (P>0.05) (Fisher's protected least significant difference test). The number of ccml with different letters in the same column were significantly different (P<0.05) from each other (Fisher's exact test). (%) = as a percentage of ccml with eggs of both species.

First species > second species	*M. ridens > L. caudatus*		*L. caudatus > M. ridens*	
Sequential oviposition period for each species	*3 h*	*24 h*	*3 h*	*24 h*
Number of replicates	22	16	22	16
No of ccml with eggs of both species	16	13	13	10
*Mean no. *M. ridens* eggs/host larva	2.5 ± 0.32	3.23± 0.28	2.31 ± 0.35	3 ± 0.61
*Mean no. *L. caudatus* eggs/host larva	1.94 ± 0.25	3 ± 0.25	2 ± 0.36	2.5 ± 0.34
No (%) of ccml with no adult emergence	9 (56%)	5 (38%)	3 (23%)	3 (30%)
No (%) of host fed ccml with no adult emergence	4 (25%)	3 (23%)	2 (15%)	2 (20%)
No (%) of ccml from which *M. ridens* emerged	3 (19%) ***a***	3 (23%) ***a***	2 (15%) ***a***	0 ***a***
No (%) of ccml from which *L. caudatus* emerged	4 (25%) ***a***	5 (38%) ***a***	8 (62%) ***b***	7 (70%) ***b***
Mean number of *M. ridens* that emerged per host	0.31 ± 0.2	0.38 ± 0.21	0.15 ± 0.1	0
Mean number of *L. caudatus* that emerged per host	0.25 ± 0.11	0.38 ± 0.14	0.62 ± 0.14	1.7 ± 0.15

larvae were examined only a few minutes after oviposition, *L. caudatus* larvae were seen feeding on the *M. ridens* eggs on several occasions. Only *L. caudatus* adults emerged from the ccml tested.

4. Oviposition of *M. ridens* on codling moth larvae with *L. caudatus* pre-pupae

The codling moth larva was effectively an empty shrivelled sac in all 15 of the codling moth cocoons opened, while the codling moth cocoon was effectively filled by the *L. caudatus* cocoon. Within the 15 *L. caudatus* cocoons, six pre-pupae were paralysed and 1-4 *M. ridens* eggs were found on three of those six. Neither *L. caudatus* nor *M. ridens* adults emerged from any of the six paralysed *L. caudatus* pre-pupae. Adult *L. caudatus* emerged from the nine *L. caudatus* pre-pupae that were not attacked by *M. ridens* females.

DISCUSSION

Female parasitoids of both *M. ridens* and *L. caudatus* oviposited into ccml that contained eggs of the other species, regardless of the age of the eggs developing on the host, and no discrimination of oviposition sites was recorded. It appeared that the female parasitoids could not detect the presence of other parasitoid eggs, or if they could, this did not deter oviposition. Both species reproduced equally in the first experiment under the competitive environment (Figure 1), suggesting that the larger body size of female *L. caudatus* had no impact on oviposition success in presence of *M. ridens*. In the second experiment, regardless of the 3-h and 24-h sequential exposure periods, the mean numbers of eggs laid by both species on the same ccml were not significantly different from each other (Table 1). However, more *L. caudatus* adults emerged than *M. ridens* adults suggesting the *L. caudatus* larvae were more successful in competition with *M. ridens* larvae.

In this study, multiple eggs from both species (including more than a single clutch of *M. ridens* eggs) were often found on ccml, indicating that self-super parasitism (Sandanayaka et al. 2011) and conspecific super parasitism occurred. Multi-species parasitism is clearly a disadvantage for the species that does not have the same competitive abilities when they both develop on the same host. In the second experiment with 3-h and 24-h sequential exposure periods, adult *L. caudatus* emerged from the majority of the ccml from where interspecific and intraspecific competition occurred between larvae. Both *M. ridens* and *L. caudatus* larvae exhibited predation and cannibalism. In the same experiment, *L. caudatus* adults emerged from eggs laid during the second oviposition phase of the 24-h sequence, indicating an ability to outcompete the older *M. ridens* larvae. In contrast, no *M. ridens* adults emerged from eggs produced by *M. ridens* females during the second oviposition phase of 24 h, although they did emerge from some of the ccml exposed to *M. ridens* females for the second oviposition phase of 3 h. This suggests that *M. ridens* larvae would be able to survive competition with *L. caudatus* larvae for a restricted period when the age difference between them was less than 24h. Adult *M. ridens* emerged from ccml exposed to *M. ridens* females during the first oviposition phase of both 3 and 24 hours, indicating that older *M. ridens* larvae would be able to survive the competition. Significantly more *L. caudatus* adults emerged from ccml than *M. ridens* adults, suggesting the larger size of the *L. caudatus* larvae may also be a factor that helps them to outcompete *M. ridens* larvae. A single *L. caudatus* adult always emerged from a single ccml, regardless of the number of eggs originally laid, which was expected because the most successful *L. caudatus* larva is known to cannibalise conspecific eggs or larvae (Cole & Walker 2011).

Host feeding by *L. caudatus* females was commonly observed, although eggs from both species were found on ccml where host feeding had occurred. No offspring emerged from some of the ccml that had been parasitized by both species in the second experiment, and some of those ccml had been subject to host feeding. Host feeding may have had an impact on parasitoid larval development because no adults emerged from those ccml, possibly because the host died prematurely, thus depriving the parasitoid larvae of sufficient resources to complete development. Host feeding by both species (Sandanayaka et al. 2011) may have resulted from a number of conditions, including adult malnutrition (Liu et al. 2015). The failure of emergence from other ccml could not be explained.

In the third experiment, three main behavioural steps leading to oviposition were scored. Charles et al. (2013) described responses of *M. ridens* females to ccml in six behavioural steps (pre-contact, examination, acceptance (including stinging), guarding, oviposition and post-oviposition), with a mean guarding period of 78 ± 13.1 min. In the current study, the acceptance and stinging were followed by a much shorter guarding period (29.2 ± 1.7 min), presumably because the codling moth larva inside the cocoon was already paralysed by the venom injected by *L. caudatus* females.

The fate of *L. caudatus* eggs laid on ccml already parasitised by *M. ridens* larvae >24-h-old was not tested in this study. However, *M. ridens* larvae cannibalise eggs resulting from self- superparasitism (Sandanayaka unpublished data), hence we predict that older *M. ridens* larvae will prey on *L. caudatus* eggs. In addition, the oviposition behaviour of *L. caudatus,* particularly at the point of egg laying, was incompletely understood for us to be confident that the number of eggs laid could be determined prior to potential predation by *M. ridens* larvae. Further investigations are required to understand the differences in oviposition behaviour between *L. caudatus* and *M. ridens.*

In the fourth experiment, *M. ridens* females appeared unable to distinguish between a mature codling moth larva and a *L. caudatus* larva inside the codling moth cocoon, and attacked and oviposited on the latter. This may confirm that *M. ridens* females are attracted to the chemical cues on the codling moth silken cocoon (Jumean et al. 2009) but are unable to identify the type of larva on which they are ovipositing. However, the mature *L. caudatus* larva did not support development of *M. ridens* to maturity. This may be because the venom injected by the female *M. ridens* killed the *L. caudatus* larva, leading to the death of both parasitoids.

No inter-specific competition between *M. ridens* and *L. caudatus* for oviposition was recorded under laboratory conditions. However, *L. caudatus* females live longer (Clausen 1978), and are reproductively active for a longer period with a higher lifetime fecundity than *M. ridens* females (Davis et al. 2016). These laboratory studies indicate that *M. ridens* eggs, and at least young larvae, are vulnerable to competition from *L. caudatus* larvae. Nevertheless, both species co-exist in the wild in their native range (Kuhlmann & Mills 1999), suggesting that such inter-specific competition may be mitigated by some form of habitat niche-partitioning by females. Clearly, future ecological studies will be required to understand the development and dynamics of natural enemy guilds in New Zealand's regional pipfruit orchards.

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