EFFECT OF AGE ON THE REPRODUCTIVE FITNESS OF *PLATYGASTER DEMADES* AN EGG PARASITOID OF APPLE LEAFCURLING MIDGE

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ABSTRACT

Platygaster demades is an egg parasitoid of the apple leafcurling midge (ALCM), *Dasineura mali*. Each *P. demades* female carries a mean pre-ovipositional load of 1380 ± 263 eggs. Females fed with buckwheat nectar lived a maximum of 10 weeks at 15°C. The age-dependent reproduction of female *P. demades* was determined by a laboratory experiment at room temperature. ALCM eggs on apple shoots were exposed to 1, 2, 4, 8 and 10-week-old individual *P. demades* females, and ALCM larvae that emerged from the parasitised eggs were reared on fresh apple shoots and allowed to pupate on a layer of moist soil. The numbers and sex of adult *P. demades* that emerged from the ALCM pupae were recorded. Older females (8 and 10 weeks old) were able to locate and parasitise host eggs and produced as many offspring as younger females. The implication of the results in terms of the reproductive fitness of *P. demades* is discussed.

Keywords: *Platygaster demades*, apple leafcurling midge, parasitism, age, reproductive fitness.

INTRODUCTION

Platygaster demades Walker (Hymenoptera: Platygasteridae) was introduced to New Zealand from Europe in 1925 to control pear leafcurling midge (*Dasineura pyri* (Bouché) (Diptera: Cecidomyiidae)). Apple leafcurling midge (ALCM) (*Dasineura mali* Kieffer) was first recorded in New Zealand in 1950 (Berry & Walker 1989) and *P. demades* was found to be established on ALCM during the earlier part of the 1954-55 season (Todd 1956).

Female ALCM lay eggs on the young shoots of apple trees. The larvae emerge from the eggs after 3-4 days and start feeding inside the curled leaves. Feeding prevents the growing leaf from unfolding, with the roll becoming tighter as the leaf growth continues. Mature ALCM larvae then drop from the leafroll to pupate in the soil. Female *P. demades* oviposit into ALCM eggs but the embryonic development of the parasitoid is delayed until the ALCM larva spins its pupal cocoon (Todd 1956).

Four to five generations of both ALCM and *P. demades* develop every year in New Zealand starting from early October. The emergence of *P. demades* adults is well synchronized with the first generation of ALCM. However, the size of the ALCM population substantially increases in the second generation as a result of asynchrony between the two species, leading to considerable damage to the apple trees (Shaw & Wallis 2004). Studies have been conducted to understand the reasons behind the asynchrony between ALCM and *P. demades* populations in the second generation. He & Wang (2007) reported that the high parasitism rate in first generation *P. demades* was caused by aestivation. They suggested that *P. demades* aestivated as eggs in a high proportion of the second and third generations and emerge with over-wintering generations. *Platygaster demades* took longer to develop than ALCM at spring soil temperatures (at 11-15°C) in many apple growing areas in New Zealand; and adult *P. demades* emerged over a longer period at those temperatures and survived for longer when fed with buckwheat nectar (Sandanyaka & Ramankutty 2007; Sandanyaka & Charles 2007).

The female *P. demades* is pro-ovigenic and carries a mean load of 1380 ± 263 eggs (Sandanayaka & Ramankutty 2007). It is hypothesised that female *P. demades* emerging late in the first generation (at low temperatures in spring) could live and oviposit for longer if there was a suitable food source in the field. These females could then potentially bridge the gap between first and the second generations by being able to lay their entire egg load over this extended period. However, the reproductive fitness of older female *P. demades* needs to be determined. This paper discusses the effect of age of female *P. demades* on their reproductive success.

MATERIALS AND METHODS

Plants

Buckwheat (*Fagopyrum esculentum* Moench, Polygonaceae) plants were grown in a glasshouse from seeds and flowering shoots provided nectar for parasitoids. Potted 'Royal Gala' apple plants with new shoots maintained in the glasshouse were provided to mated ALCM females for oviposition.

Insects

Adult *P. demades* and ALCM were reared in the laboratory from field-collected ALCM larvae. Apple leaves infested with mature ALCM larvae were placed on a layer of moist soil in plastic boxes (with ventilated lids) and held at $21 \pm 1^\circ\text{C}$ and a 16:8 h light:dark cycle. Adult ALCM and *P. demades* emerging from the boxes were collected in a fine net cage and used in the experiments.

ALCM adults that emerged from the laboratory colony were allowed to mate in the fine net cage until midday. Ten mated females (non-calling females) were released onto an apple plant with new shoots in a mesh cage at $21 \pm 1^\circ\text{C}$ and 16:8 h light:dark. The shoots of the apple plants were checked after 24 h for ALCM eggs, using a hand lens. Only apple plants with ca 100 ALCM eggs were used for the experiment, and the number of eggs per plant was restricted to avoid excessive ALCM larval competition for resources.

Five each of newly emerged female and male *P. demades* were held in a plastic Petri dish (15 x 85 mm diameter) with a flowering shoot of buckwheat. The shoot with flowers and buds was placed into the vertically held Petri dish as described in Sandanayaka & Charles (2007). Petri dishes with parasitoids and flowers were held at 15°C and 14:10 h light:dark (spring conditions). Fresh flowers were provided as needed until the female parasitoids could be used in the experiment or died. Ten female parasitoids of each age group (1, 2, 4, 8 and 10 weeks old) were individually tested for their reproductive fitness and progeny sex ratio.

Two female *P. demades* of a known age (for each age group: 1, 2, 4, 8 and 10 weeks old) were released into a cage containing one of the apple plants previously infested with ALCM eggs. A flowering shoot of buckwheat with its stem in a glass vial filled with water was placed inside the cage to provide nectar for the parasitoids. After 4–5 days, when the young apple leaves had started to roll up indicating that the emerging larvae had begun to feed, the apple plants were transferred to a glasshouse ($27 \pm 2^\circ\text{C}$). This environment provided optimal growing conditions for the plants, which were also protected from other pests and diseases, and were watered onto soil rather than from overhead. The leaves were removed after 12–15 days, when the leaf rolls became brittle and had started to turn brown, and ALCM larvae had matured. The leaf rolls were opened and then laid on a layer of moist soil in small ClickClack® boxes (110 x 75 x 50 mm) with ventilated lids and maintained in the laboratory at $21 \pm 1^\circ\text{C}$ and 16:8 h light:dark until the adults emerged. Ten replicates were completed; each replicate took 57–191 days to complete.

The numbers of ALCM and *P. demades* that emerged from each box were recorded as well as the date of emergence and the sex of emerged adult *P. demades*. Two female parasitoids of the same age were released to each cage, to minimise the possibility of ALCM eggs not being parasitised. It was assumed that both parasitoids performed equally. Parasitism by *P. demades* was calculated as a percentage of total ALCM and *P. demades*

emerged, and the mortality that occurred during rearing was ignored. Data collected were analysed using analysis of variance (ANOVA), followed by means separation with Tukey's LSD ($P<0.05$) (Origin 7.5).

RESULTS

Adult *P. demades* reared on buckwheat in the Petri dishes began to die within 3 weeks and only a few healthy females remained after 10 weeks. All the males had died by the end of the sixth week. Initially, around 100 ALCM eggs were exposed to *P. demades* females and it was expected that most would develop to either adult ALCM or *P. demades*. However, none of the egg batches had 100% emergence. The highest total emergence (both ALCM and *P. demades*) was recorded from the 2-week-old females, which was significantly different ($P<0.05$) from that of 1-week-old females, although it was not significantly different from those of 4, 8 and 10 week-old females (Table 1).

TABLE 1: Emergence (total and *Platyaster demades* emergence as % of total), offspring sex ratio and duration of development (days from egg to adult) (female:male) of five age groups of females tested against apple leafcurling midge (ALCM) (mean $\pm$ SEM)¹.

	Female <i>P. demades</i> age when first exposed to ALCM eggs				
	1 week	2 weeks	4 weeks	8 weeks	10 weeks
% emergence (total ALCM and <i>P. demades</i>)	33.4 $\pm$ 2.4 a	53 $\pm$ 6.9 b	39.8 $\pm$ 4.2 ab	39.9 $\pm$ 3.8 ab	37.5 $\pm$ 3.5 ab
<i>P. demades</i> emergence (% of total ALCM and <i>P. demades</i> emergence)	42.25 $\pm$ 2.44	40.52 $\pm$ 2.30	40.12 $\pm$ 2.67	40.02 $\pm$ 2.8	37.06 $\pm$ 1.84
Sex ratio	7.85 $\pm$ 1.1 a	6.32 $\pm$ 0.94 a	5.49 $\pm$ 0.76 a	0.17 $\pm$ 0.04 b	0.21 $\pm$ 0.04 b
Duration of development	59.70 $\pm$ 1.09 a	63.05 $\pm$ 0.98 ab	64.01 $\pm$ 1.15 b	54.64 $\pm$ 0.92 c	52.67 $\pm$ 0.77 c

¹Values in the same row with the same letters indicate that the means are not statistically different at $P<0.05$ (Tukey's LSD).

There was no significant difference ($P<0.05$) in parasitoid emergence between the five female age groups. However, significantly more female offspring emerged from the 1, 2 and 4-week-old females, while more male offspring emerged from 8 and 10-week-old females, as indicated by sex ratios (female to male) in Table 1. The sex ratio of the offspring from the 1, 2 and 4-week-old females was significantly higher ($P<0.05$) than that of the 8 and 10-week-old females. Although the highest mean ratio (7.85) was recorded from the 1-week-old females, it was not significantly different from that of the 2 or 3-week old females. The lowest ratio (0.17) was recorded from the offspring emerged from the 8-week-old females, which is not significantly different from that of the 10-week-old females.

The longest period taken for offspring to emerge (development period from egg to adult) was recorded from the 2 and 4-week-old females. The development time (egg to adult) for offspring to emerge from 1-week-old females was not significantly different from that of the 2-week-old females, but was significantly different ($P<0.05$) from that of 4-week-old females. The shortest duration taken for emergence was recorded from the offspring from 8 and 10-week-old females.

DISCUSSION

Platygaster demades has proved to be an effective parasitoid of pear leafcurling midge *D. pyri* (Dumbleton 1935). However, in apple orchards, the second generation asynchrony between the parasitoid and its host has reduced its ability to control ALCM populations. This study was aimed at determining whether it would be possible to reduce the effect of this asynchrony by utilising older *P. demades* females.

The parasitoids were reared at 15°C (average temperature in spring in many apple-growing areas in New Zealand) because adult *P. demades* survive better at lower temperatures (Sandanayaka & Ramankutty 2007). Buckwheat nectar has been shown to be a suitable source of food for *P. demades*, and females lived for 22.4 ± 0.7 days at 19°C (Sandanayaka & Charles 2007). In this study, ca 10% of female *P. demades* reared on buckwheat nectar survived for 10 weeks. The data of offspring emergence have demonstrated that the older females are as capable of locating and parasitising hosts as younger females.

The total percentage emergence of both ALCM and *P. demades* for all the age groups ranged from 33.4–53%. Although the remaining ALCM larvae/pupae were not checked, it was assumed they would have either aestivated (He & Wang 2007) or died during the process of rearing. He & Wang (2007) found 64% of live individuals of *P. demades* in the second generation aestivated and emerged with the over-wintering generation.

Multiple parasitism can occur in *P. demades* (Todd 1956), hence the limited number of ALCM eggs available in the cage may have led to competition between the two parasitoids released. In contrast, more adult ALCM emerged than *P. demades*. That could be explained by the limited time that *P. demades* females had to locate and oviposit in host eggs. The parasitoids were provided ca 100 ALCM eggs (24 h old) and expected to achieve the maximum level of parasitism in ca 48 h before ALCM larvae emerged. The percentage of unparasitised ALCM eggs (as recorded from emergence) was similar among all age groups, demonstrating the age of the female parasitoid had no effect on level of parasitism.

The results indicate that the age of the female *P. demades* does have an effect on the sex ratio of the progeny, as 8 and 10-week-old females produced more males than younger females. Hymenopteran parasitoids are usually arrhenotokous (when females arise from fertilised eggs and males from unfertilised eggs). It is hypothesised that sperm depletion resulted in older females laying more unfertilised eggs, with the consequent increased percentage of males. In this study, female *P. demades* were allowed to mate until males were dead. In most insect species, it is assumed that a single insemination can supply enough sperm to fertilise all eggs of a single female (Walker 1980; Jacob & Boivin 2005). Since very little is known about the mating behaviour of *P. demades*, it was assumed that the females successfully mated before males died (during the first 6 weeks). The reduced daughter production may be attributed to diminishing sperm viability and mobility in aging males and increased constraints in the fertilisation process in aging females (He & Wang 2008). The male-biased progeny in the second generation may provide females more opportunity to gain material to maximise their reproductive success through optimal sex allocation.

Results of this study demonstrate that there was no effect of female *P. demades* age on the percentage of offspring emerged, suggesting older females performed as well as younger females. Therefore, it is suggested that late emerging first generation *P. demades* females in the field with access to a suitable nectar source to increase their longevity could regulate the numbers of early emerging second generation ALCM, by bridging the first and second generations. This may improve parasitism rates limited because of asynchrony between the host and parasitoid in the second generation.

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