DEVELOPMENT AND REPRODUCTION OF *DIADEGMA SEMICLAUSUM* (HYMENOPTERA: ICHNEUMONIDAE) ON DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (LEPIDOTERA: PLUTELLIDAE)

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ABSTRACT

This study investigated the development, emergence and mating of *Diadegma semiclausum* Hellen, an important parasitoid of diamondback moth in the laboratory at $21 \pm 1^\circ\text{C}$ and 50-60% RH, with a photoperiod of 12:12 h (light:dark). The parasitoids' developmental period was significantly shorter and pupal weight significantly higher if their mothers parasitised the fourth instar larvae of DBM ($P < 0.01$). The offspring sex ratio was strongly male-biased (<20%) but no significant difference was detected between host instars. Most adults emerged during the first few hours of the photophase and none emerged during the scotophase. Mating had no effect on the longevity of both sexes, but small females lived significantly longer than the larger ones ($P < 0.05$). Male age and size had no effect on mating success, whereas small females achieved higher mating success than large ones ($P < 0.05$). The implication of this study in mass-rearing in the laboratory is discussed.

Keywords: *Diadegma semiclausum*, *Plutella xylostella*, emergence, body size, mating.

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (L.), is the most destructive pest of cruciferous crops worldwide including New Zealand (Talekar & Shelton 1993; Cameron et al. 1997). *Diadegma semiclausum* Hellen is one of the most important, solitary larval endoparasitoids of DBM (Talekar & Shelton 1993), and was introduced into New Zealand from England in 1936 to manage the DBM problems (Beck & Cameron 1992; Talekar & Shelton 1993). *Diadegma semiclausum* has great prospects for the biological control of DBM because of its high specificity and searching ability (Shi et al. 2004). The parasitism rate of DBM by *D. semiclausum* can be as high as 70% (Iga 1997; Walker et al. 2002; Chen et al. 2003; Kwon et al. 2003), but the rate is only 12-27% in commercial crops in spring (Cameron et al. 1998). The high resistance of DBM to the major classes of insecticides (Cameron et al. 1997) and biopesticides (Shelton et al. 1993) has led to increasing interest in the role of parasitoids in the biologically based integrated management of DBM aiming to reduce reliance on insecticides (Talekar & Shelton 1993; Liu & Sun 1998).

The biology and ecology of *D. semiclausum* have been studied to some extent (Abbas 1988; Yang et al. 1993; Kwon et al. 2003; Winkler et al. 2006). The male-biased sex ratio for this species (Yang et al. 1993; Kwon et al. 2003) may contribute to the failure in suppressing the spring population and restrict the quality of mass-rearing in the laboratory. However, the reasons for the male-biased sex ratio are poorly understood. The aim of this research was to investigate the development, emergence and mating behaviour of *D. semiclausum*, information of which is vital to the understanding of its basic biology and improvement of mass-rearing programmes.

MATERIALS AND METHODS

Breeding colony

The breeding colonies of DBM and *D. semiclausum* started from DBM larvae collected from a commercial farm in Palmerston North in January 2008 and reared at the Entomology and IPM Laboratory, Massey University, at $21 \pm 1^\circ\text{C}$ and 50–60% RH, with a photoperiod of 12:12 h (light:dark). Ten DBM larvae were transferred to each potted cabbage seedling (4–5 leaves), and the plants were individually maintained in transparent plastic jars (25 cm in height $\times$ 17 cm in diameter) with two holes (5 cm in diameter) covered with metal mesh (aperture size of the mesh <0.5 mm) on opposite walls for ventilation. The parasitoid colony was maintained in a similar way where a mated parasitoid female was released into a jar with DBM larvae each day. DBM and parasitoid adults were provided with 10% honey solution soaked in cotton balls (0.5 cm in diameter) as food.

Development and emergence

To determine whether hosts of different instar stages affected the development and body weight of *D. semiclausum*, a mated parasitoid female was released into the jar where 10 second, third or fourth instar larvae of DBM were feeding on a cabbage seedling. The parasitoid was allowed to stay for 24 h for oviposition and then removed. There were 6, 6 and 10 mated female parasitoids used to parasitise the second, third and fourth instar larvae of DBM, respectively. Parasitised DBM larvae were allowed to feed on cabbage seedlings until pupation. Once the parasitoids pupated, they were removed from the silken web and weighed using an electric balance (Mettler Toledo, AG135, Switzerland) with a readability of 0.01 mg. Parasitoid pupae were individually maintained in glass vials (4 cm in height $\times$ 2 cm in diameter) for emergence. Emergence was recorded hourly during the photophase, and the sexes of adults and the developmental period of parasitoids from eggs to emergence were also calculated. Emergence observation was also performed immediately after light-on to determine whether parasitoids emerged during the scotophase.

Longevity

To investigate whether mating and body size had any effect on the longevity of both sexes, mated and virgin adults were individually maintained in transparent plastic containers (10 cm in height $\times$ 7.5 cm in diameter) with two 3 cm mesh-covered ventilation holes. The parasitoids were fed with honey solution as above. Longevity of adults was recorded. The parasitoids used were those that emerged from DBM pupae parasitised at fourth instar larvae.

Mating behaviour

To investigate the effect of age and body size on mating success of both sexes, one male and one female *D. semiclausum* were paired in eight treatment combinations (n = number of replicates):

- (1) 12-h-old small female + 3-day-old small male, $n = 8$;
- (2) 12-h-old large female + 3-day-old large male, $n = 19$;
- (3) 24-h-old small female + 3-day-old small male, $n = 16$;
- (4) 24-h-old large female + 3-day-old large male, $n = 34$;
- (5) 12-h-old small male + 3-day-old small female, $n = 10$;
- (6) 12-h-old large male + 3-day-old large female, $n = 17$;
- (7) 24-h-old small male + 3-day-old small female, $n = 10$;
- (8) 24-h-old large male + 3-day-old large female, $n = 15$.

The body size of parasitoids was defined as small when pupal weight was less than 3.5 mg and large when pupal weight was greater than 3.5 mg. The mating behaviour of each pair was observed in a glass vial (7.5 cm in height $\times$ 2.5 cm in diameter) for 1 hour during the photophase. The number of mating successes, premating and mating periods were recorded.

Data analysis

A goodness-of-fit test was used to test the distribution of data before analysis. Developmental period data were not normally distributed even after transformation and

thus analysed using the nonparametric Kruskal-Wallis test (KWT) followed by Dunn's procedure for multiple comparisons. Data on pupal weight, adult longevity, and premating and mating periods were normally distributed and thus analysed using ANOVA followed by Tukey's studentized range test. Because mating had no effect on adult longevity, longevity of mated and virgin individuals was pooled for analysis of the effect of body size on longevity. Parasitoid emergence data from second, third and fourth instar larvae were pooled and the emergence pattern during the photophase was analysed using non-linear regression. Chi-square tests were applied to analyse the mating success.

RESULTS

Development and emergence

Parasitoids developed significantly faster ($P < 0.0001$) and resulting pupae were significantly heavier ($P = 0.006$) when developing on DBM fourth instar larvae (Table 1). No significant difference was found in developmental time and pupal weight between males and females ($P > 0.05$) (Table 1). The proportion of female progeny (13.9, 7.8 and 20.0% for second, third and fourth instar, respectively) did not vary significantly between the host larval stages at parasitisation ($P > 0.05$).

TABLE 1: Developmental period from eggs to emergence (days) and pupal weight (mg) of *D. semiclausum* that parasitised DBM larvae of different instars. Means ($\pm$ SE) followed by the same letters in columns are not significantly different ($P > 0.05$).

DBM instar	Developmental period			Pupal weight		
	Male	Female	Mean	Male	Female	Mean
2 nd	17.0 $\pm$ 0.1	17.4 $\pm$ 0.4	17.1 $\pm$ 0.1 b	3.4 $\pm$ 0.1	3.6 $\pm$ 0.2	3.5 $\pm$ 0.1 b
3 rd	16.8 $\pm$ 0.1	16.7 $\pm$ 0.3	16.7 $\pm$ 0.3 b	3.5 $\pm$ 0.1	3.5 $\pm$ 0.2	3.5 $\pm$ 0.2 b
4 th	16.2 $\pm$ 0.1	16.1 $\pm$ 0.1	16.2 $\pm$ 0.1 a	3.9 $\pm$ 0.1	3.9 $\pm$ 0.1	3.9 $\pm$ 0.1 a

No individuals were observed to emerge during the scotophase. The emergence of parasitoids was confined to the first 2 hours of photophase and then significantly decreased with time ($P = 0.03$; Fig. 1).

FIGURE 1: Emergence of *D. semiclausum* (number of adults/h) during the photophase.

Longevity

Mating did not significantly affect the longevity of either sex ($P=0.41$). However, the longevity of small females (34.6 ± 6.1 days) was significantly greater than that of large females (22.4 ± 3.5 days) and small males had greater longevity than large males (26.6 ± 2.78 and 24.6 ± 1.2 days) ($P=0.0129$).

Mating behaviour

When both sexes were released together, males always approached females with their wings vibrating. Upon female reception, they mated in tail to tail position. Mating success was significantly higher in small females than in larger ones when they paired with 3-day-old males ($P<0.05$) (Table 2). However, no significant differences were detected in mating success between ages or sizes of males when they were paired with 3-day-old females ($P>0.05$) and *vice versa* (Table 2). Parasitoid age and body size had no effect on the premating and mating periods (Table 2).

TABLE 2: Premating period (PMP, min), mating period (MP, min) and mating success (MS, %) of *D. semiclausum* at 12 or 24 h of age and with small or large body size.

12- or 24-h-old females mated with 3-day-old males						
Female age	Small female + small male			Large female + large male		
	PMP	MP	MS	PMP	MP	MS
12 h	21.0 ± 7.5	7.9 ± 0.6	75.0	13.3 ± 4.5	6.5 ± 0.2	36.8
24 h	12.0 ± 8.3	7.1 ± 0.5	75.0	16.5 ± 7.0	8.3 ± 0.3	38.2
12- or 24-h-old males mated with 3-day-old females						
Male age	Small female + small male			Large female + large male		
	PMP	MP	MS	PMP	MP	MS
12 h	16.4 ± 11.1	5.9 ± 1.5	50.0	20.3 ± 8.1	8.6 ± 1.2	41.2
24 h	13.3 ± 6.7	7.3 ± 0.6	42.9	14.2 ± 6.4	8.4 ± 0.8	47.1

DISCUSSION

Harvey & Strand (2002) suggested that parasitoids attacking foliar-feeding hosts may favour rapid development time over size while parasitoids that attack concealed hosts favour size over development time. In this study, when *D. semiclausum* attacked fourth instar larvae of DBM, it developed faster and produced larger progeny, indicating that *D. semiclausum* has the ability to adjust its development and growth rate according to host stage as reported in some other parasitoids, e.g. *Aphidius ervi* Haliday (Sequeira & Mackauer 1992). This may be because for the solitary parasitoids, large hosts contain more resources than small hosts (Charnov et al. 1981). The similar developmental period and body size for both male and female progeny from a given size of hosts suggests that sexes of *D. semiclausum* utilise similar amounts of host nutrients.

Diadegma semiclausum is a haplodiploid species where fertilised eggs develop to females and unfertilised eggs develop to males. Charnov et al. (1981) suggested that parasitoids can make efficient use of the size variation in the hosts by depositing fertilised eggs to large hosts to produce females and unfertilised eggs to small ones to produce males. However, in the present study, no significant difference in proportion of female progeny was found when *D. semiclausum* attacked hosts of different size, which does not support the parasitoid sex allocation strategy suggested by Charnov et al. (1981). The male-biased sex ratio of this species has been reported in both the laboratory (Yang et al. 1993) and field (Kwon et al. 2003). The fact that fertilised eggs of *D. semiclausum* may develop to either females or males (Noda 2000) may be attributed to the male-biased sex ratio in the laboratory and field populations.

The emergence pattern of parasitoids is usually linked to behavioural adaptations and reproduction strategy such as feeding, mating and oviposition (He et al. 2004). Results from this study and that of Yang et al. (1993) show that *D. semiclausum* mainly emerged in the first few hours in the photophase. Talekar & Yang (1991) reported that oviposition of *D. semiclausum* also occurs during the photophase. Furthermore, nectar is crucially important for *D. semiclausum* survival and reproduction in the field, and without nectar supply, females delay oviposition for 2-3 days after emergence in the laboratory (Winkler et al. 2006). Results from the present study show that *D. semiclausum* became sexually mature within 12 hours after emergence. Thus, morning emergence of *D. semiclausum* is in their favour in that it permits the search for food and mates.

In this study, smaller individuals, especially females, lived longer than the larger ones, which contrasts with the findings in other parasitoids where large individuals live longer than the smaller ones (Ueno 1998). The reason is unknown. Furthermore, small male and female parasitoids were more likely than large ones to achieve mating (Table 2). The lower mating success in larger males is also reported in *Hoplectis naranyae* Ashmead (Ueno 1998).

Due to the failure of *D. semiclausum* in suppressing the spring populations of DBM, augmentation of parasitoids in the spring must be done with caution. Results from this study imply that the production of small individuals of both sexes may improve the mating success. However, production of male-biased progeny is the major issue in laboratory mass-rearing and thus further investigation on how to produce female-biased progeny in mass-rearing programmes is warranted.

ACKNOWLEDGEMENTS

We thank Solar Produce Farm, Palmerston North, for allowing us to collect DBM and parasitoids from their vegetable farm, and Drs S. Zydenbos and S. Goldson for their constructive comments. This study was partly funded by New Zealand's International Aid and Development Agency.

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