

## Functional response of the lady beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) on the aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae)

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### Abstract

The functional responses of each instar larva and adult of the coccinellid *Harmonia axyridis* to adults of the green peach aphid *Myzus persicae* were estimated under laboratory conditions. Linear parameter estimates from a logistic model of the proportion of *M. persicae* consumed by *H. axyridis* were negative at all development stages. Although a realistic estimate for the handling time of first instar larvae could not be produced, functional response curves of fitting the data with a random predator equation exhibited a Type II curve in most development stages. Fourth instar larva had the highest attack rate and shortest prey handling time. The implications of these results are discussed with more effective stages of *H. axyridis* in the context of biological control.

**Key words:** Functional response; biological control; *Harmonia axyridis*; *Myzus persicae*

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### INTRODUCTION

The coccinellid *Harmonia axyridis* Pallas has been used extensively as an excellent prospective biological control agent of aphids in Asia, North and South America and Europe (Koch, 2003). There are three types of release methods for *H. axyridis*, egg masses, larvae and adults (Teddars and Schaefer, 1994; Trouve et al., 1997; Ferran et al., 1998; Kitagami and Ohkubo, 1998; Kuroda and Miura, 2003). The release stage of predators is an important factor that influences its effectiveness and potential economic benefits because the search rates and handling times of predators should vary with their development (Varley et al., 1973; Hassell, 1978; Dixon, 2000).

Functional responses at each development stage on major pests provide insights into determining the stages for the release of predators. Those of *H. axyridis* have been reported on *Aphis gossypii* Glover (Lee and Kang, 2004), and on *Aphis craccivora* Koch (Mogi, 1969); however, most other studies have only researched its functional response at the fourth instar or adult stages. In *H. axyridis*, functional responses of each instar larva and adult should be considered for each prey

species, because *H. axyridis* exhibit different responses to various prey species (Pervez and Omkar, 2005), i.e. Type I (Luo, 1987), Type II (e.g. Mogi, 1969; Lee and Kang, 2004), and Type III (Hu et al., 1989). The green peach aphid *Myzus persicae* Sulzer is a major insect pest due to the transmission of plant viruses (Harris and Maramorosch, 1977; Matthews, 1991), and resistance to insecticides (Devonshire, 1989; Quacchia et al., 1993); however, no detailed study has been conducted on its functional response to *M. persicae*, although *H. axyridis* is a well-known predator of *M. persicae* (e.g. Hokusima and Kamei, 1970; LaRock et al., 2003).

In this study, in order to determine the effectiveness of the various stages released, the functional responses of each instar larva and adult of *H. axyridis* on *M. persicae* were estimated under laboratory conditions.

### MATERIALS AND METHODS

**Insect culture.** Fifty adults of *H. axyridis* were collected in Fukuyama (western Japan, Lat. 34°28'N, Long. 133°23'E) during late April 2003. A stock culture was established in the laboratory

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and about 100 individuals were maintained per generation. Approximately 40 larvae or adults were reared per plastic container (16×22×8 cm high) at LD 16:8, 25°C and 40–60% RH. Stock at the fifth generation from the field was used in this study. The stock was reared on the pea aphid *Acyrtosiphon pisum* Harris from a field population. *A. pisum* was reproduced on broad bean seedlings in plastic containers in the laboratory. *M. persicae* was from the stock culture at the National Institute of Vegetable and Tea Science. *M. persicae* was reared on leaves of the Japanese radish at 22–28°C and 30–60% RH in the greenhouse.

**Functional response.** The experiment was performed to calculate the functional responses of each instar larva and adult of *H. axyridis* on *M. persicae* as follows. Twenty egg clusters of *H. axyridis* were collected from the stock culture. First instar larvae were removed one by one soon after hatching in order to exclude the effect of sibling cannibalism. A less than 12-h-old first instar larva was placed on wet paper in a Petri dish (9 cm diameter) after allocating adults of *M. persicae* at each prey density. Prey densities examined were 1, 2, 4, 8, 16, 32, 64, and 128 aphids. The number of predation events was calculated by counting the number of aphids surviving after 24 h. Hatchlings from other egg clusters were individually reared on wet paper in a Petri dish with about 100 *A. pisum* provided daily as food until individuals achieved the second instar. Each larva was starved for 18–24 h and placed on wet paper in a Petri dish after allocating adults of *M. persicae* at each prey density. The same experiments were performed at the third and fourth instar larval, and adult stages. Six-day-old adults of *H. axyridis* were starved for 18–24 h.

These experiments were conducted under LD 16:8 at 25°C and 40–60% RH. Ten replicates of each prey density were performed for all instar larval, and adult stages of *H. axyridis*. Control treatments with no predator were also replicated 10 times for each prey density to consider the natural mortality of the prey and the number of first instar nymphs produced by adults of *M. persicae*. They were assessed with a binocular microscope after 24 h.

Statistical analyses were performed using the SAS statistical package (SAS Institute, 1989). In the first step, cubic logistic regression analysis (PROC CATMOD, SAS Institute, 1989) of the pro-

portion of prey consumed as a function of initial density was performed to distinguish the shape of the functional response curve (Type II or Type III) (Juliano, 2001) as follows:

$$N_e/N_0 = \frac{\exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)}{[1 + \exp(P_0 + P_1 N_0 + P_2 N_0^2 + P_3 N_0^3)]}, \quad (1)$$

where  $N_e$  is the number of prey consumed,  $N_0$  is the initial number of prey.  $P_0$ ,  $P_1$ ,  $P_2$  and  $P_3$  are parameters to be estimated. Significant negative or positive linear coefficients (i.e.,  $P_1$ ) from the regression indicate Type II or Type III curve, respectively (Juliano, 2001).

In the second step, nonlinear regression analysis (PROC NLIN, SAS Institute, 1989) was performed to estimate parameters of a Type II model by fitting the random predator equation (Royama, 1971; Rogers, 1972) as follows:

$$N_e = N_0 [1 - \exp(aT_h N_e - aT)], \quad (2)$$

where  $N_e$  and  $N_0$  are described in Eq. (1),  $T$  is the total time, which in this case was 1 day,  $a$  is the attack rate, and  $T_h$  is the handling time per prey.

Because the variance of data points about the curve was expected to be much lower at low prey densities than at high densities (see Fig. 1), weighted regression was performed using weighting factors  $w_j = 1/s_j$ , where  $s_j$  is the sampling variance in the number of prey eaten at density  $j$  (Houck and Strauss, 1985).

## RESULTS AND DISCUSSION

In control treatments with no predator, mortality rates of *M. persicae* (mean%±SE) were 0.0±0.0, 0.0±0.0, 2.5±2.5, 7.5±3.3, 3.8±1.7, 2.5±1.3, 3.8±0.8, and 3.8±0.7 at prey densities of 1, 2, 4, 8, 16, 32, 64, and 128, respectively. Few first instar nymphs were produced by adults of *M. persicae* at all prey densities. The number (mean±SE) was 0.0±0.0 at prey densities of 2, 4, 8, and 16, 0.1±0.3 at prey densities of 1, 32, and 64, and 0.4±0.7 at a prey density of 128; therefore, there was no need to correct obtained mortality data from functional response tests.

Parameter estimates from the logistic model of the proportion of *M. persicae* consumed by *H. axyridis* over a 24-h period versus prey density are shown in Table 1. Estimates of the linear parameter

Table 1. Logistic regression analyses of the proportion of *M. persicae* eaten by each development stage of *H. axyridis* against initial density

Stage of <i>H. axyridis</i>	Parameters	Estimates	SE	$\chi^2$	<i>p</i>
First instar	Constant	-1.3679000	0.3341000	16.76	<0.0001
	Linear	-0.0836000	0.0322000	6.72	0.0095
	Quadratic	0.0010900	0.0006710	2.63	0.1052
	Cubic	-0.0000040	0.0000035	1.35	0.2453
Second instar	Constant	1.4992000	0.2510000	35.66	<0.0001
	Linear	-0.0928000	0.0194000	22.96	<0.0001
	Quadratic	0.0011100	0.0003720	8.87	0.0029
	Cubic	-0.0000045	0.0000019	5.81	0.0159
Third instar	Constant	4.1809000	0.4950000	71.33	<0.0001
	Linear	-0.1793000	0.0318000	31.78	<0.0001
	Quadratic	0.0025400	0.0005540	21.01	<0.0001
	Cubic	-0.0000100	0.0000026	18.59	<0.0001
Fourth instar	Constant	28.4739000	0.0591000	232480.00	<0.0001
	Linear	-1.2572*	—	—	—
	Quadratic	0.0187*	—	—	—
	Cubic	-0.00008*	—	—	—
Adult (male)	Constant	6.5464000	0.6201000	111.46	<0.0001
	Linear	-0.1096000	0.0118000	85.93	<0.0001
	Quadratic	0.000423*	—	—	—
	Cubic	0.0000004	0.0000004	0.81	0.3691
Adult (female)	Constant	9.0523000	1.4854000	37.14	<0.0001
	Linear	-0.0617*	—	—	—
	Quadratic	-0.0015000	0.0006400	5.51	0.0189
	Cubic	0.0000110	0.0000043	7.09	0.0078

\* SE and  $\chi^2$  values of these estimates were not calculated because infinite parameters were estimated.

$P_1$  were negative for all stages, and were significant at the first, second, third instars, and in adult males (Table 1:  $p < 0.01$ ). The standard error and  $\chi^2$  values of each parameter were not calculated at the fourth instar and adult (female) stages, because infinite parameter estimates are shown (Table 1). Estimates of logistic regression coefficients can be infinite when zero cells or complete separation exist for the usual maximum likelihood estimation procedures, and infinite parameter estimates are desirable in logistic regression because it means that the prediction is perfect (David, 2002). Fourth instar larvae and female adults of *H. axyridis* fed on all aphids at low prey densities (1, 2, 4, 8, 16 aphids).

Figure 1 shows the functional response curves of fitting the data at larval and adult stages with a random predator equation. The functional response curves fit Type II at all stages, except that of the first instar (Table 2). At these instar and adult stages, the coefficients of determination ( $r^2$ ) estimated by fitting random predator equations were more than 0.95 (Table 2). The attack rates ( $a$ ) of all

instars and adults had asymptotic 95% confidence intervals that did not include 0 (Table 2). The order of  $a$  was highest at the fourth instar stage, followed by adult female, adult male, and third, second, and first instar stages (Table 2). The order of  $T_h$  was shortest at the fourth instar stage, followed by adult male, adult female, and third, and second larval instar stages (Table 2). A realistic estimate for handling time ( $T_h$ ) could not be produced at the first instar stage (Table 2). This result may be due to the low predation rate of first instar larvae with their small body size and slow movement on *M. persicae* at all prey densities. First instar larvae of *H. axyridis* frequently failed to capture third instar nymphs of *M. persicae* (Kawai, 1978).

Our data provide insights to estimate more effective development stages for the release of *H. axyridis* against *M. persicae*. Firstly, we should be cautious about releasing first instar larvae (or egg masses) of *H. axyridis* because they tend not to succeed at predation, even in a small arena. Secondly, *H. axyridis* could be effective at suppressing

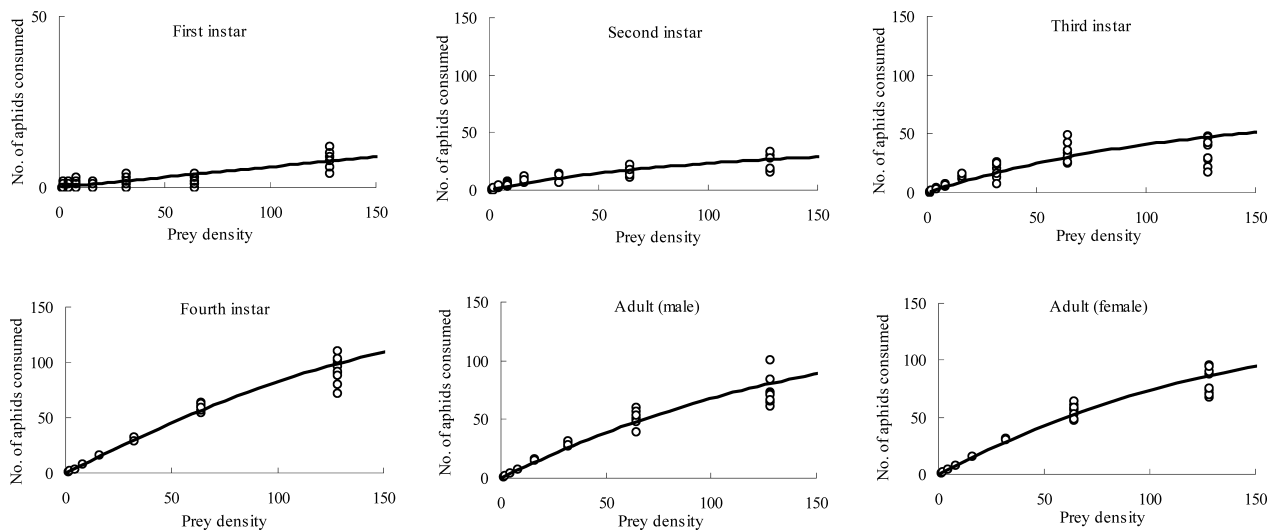


Fig. 1. Functional responses at each development stage of *H. axyridis*. Solid lines show the functional response curves of *H. axyridis* on *M. persicae* by fitting a random predator equation. Circles show the number of *M. persicae* consumed at each prey density.

Table 2. Type II functional response parameters of *H. axyridis* on *M. persicae*

Stage	Random predator equation		
	$a^a$	$T_h$ (day) <sup>b</sup>	$r^2$
First instar	0.0623*	$1.0 \times 10^{-8}$ ns	0.7470
Second instar	0.4849*	0.0194*	0.9500
Third instar	0.9486*	0.0108*	0.9824
Fourth instar	3.0894*	0.0053*	0.9995
Adult (male)	1.9485*	0.0061*	0.9982
Adult (female)	2.5498*	0.0065*	0.9990

\*Significant parameters have asymptotic 95% confidence intervals that do not include 0.

<sup>a</sup> Attack rate.

<sup>b</sup> Handling time per prey.

*M. persicae* density at the fourth instar and adult stages because of higher attack rates and shorter handling times. Use of third instar larvae may be cost-effective because mass rearing to the fourth instar and adult stages would be unnecessary; however, functional response experiments conducted under laboratory conditions in a small arena may have limited value for determining the characteristics of predation under field conditions (Gitonga et al., 2002; Lee and Kang, 2004). The functional response of ladybirds in patches containing low numbers of prey can appear irrelevant as they tend to avoid such patches and aggregate in those that con-

tain high numbers of prey in area-restricted search (Kawai, 1976; Nakamuta, 1985; Dixon, 2000). Further experiments incorporating a large spatial scale are needed to know the effective release stage of *H. axyridis* for *M. persicae*.

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