

Functional response and predatory interactions in conspecific and heterospecific combinations of two congeneric species (Coleoptera: Coccinellidae)

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Abstract. In the present study, the predatory interactions between two locally abundant large lady beetles, *Coccinella septempunctata* L. (C7) and *Coccinella transversalis* F. (Ct) provided with either an extremely scarce, scarce, sub-optimal, optimal or abundant supply of the pea aphid, *Acyrtosiphon pisum* (Harris) were investigated. For this, three 2-predator combinations (conspecific C7+C7 and Ct+Ct, and heterospecific C7+Ct) of 10-day-old unmated adult females were used. The relationships between the proportion of prey consumed by the predators in the conspecific and heterospecific combinations when provided with five different abundances of prey were similar in consisting of a decelerating (type II) functional response when provided with an extremely scarce to optimal supply of prey, followed by an accelerating (type III) functional response when provided with an optimal to abundant supply of prey. It is likely that the modified type II functional response recorded in the present study was a consequence of using a small experimental arena (Petri dish). The multiplicative risk model analysis revealed that the predators interacted antagonistically except in the C7+C7 combination provided with an extremely scarce supply of prey where the predators had an additive effect. The predators in the heterospecific C7+Ct combination consumed fewer aphids when provided with an extremely scarce, scarce, sub-optimal or optimal supply of prey than the predators in conspecific C7+C7 combination but a similarly high number when provided with an abundant supply of prey. In addition, the efficiency of converting prey biomass into their own biomass was higher in the heterospecific C7+Ct than in the conspecific C7+C7 or Ct+Ct combinations at all prey densities. Although the conversion efficiency of the predators in the conspecific and heterospecific combinations provided with an extremely scarce supply of prey was the highest recorded, their growth rate was the lowest. In contrast, both the conversion efficiency and growth rate of the predators in the three combinations were highest when provided with an abundant supply of prey. It may, therefore, be concluded that when the predators in the combinations occupy similar ecological niches, they will probably consume less prey than they require when prey is both scarce and abundant because the predators interact antagonistically.

INTRODUCTION

Coccinellids, commonly known as lady beetles, are an important group of predatory insects with considerable potential for use as biocontrol agents of aphids and other pest species (Hodek & Honek, 1996; Michaud, 2012). They feed on a wide range of prey, tend to be very voracious and exhibit rapid numerical responses (Hodek & Honek, 1996; Bayoumy, 2011a; Bayoumy & Michaud, 2012). However, they do not always control prey populations (Northfield et al., 2010; Michaud, 2012). Thus, it is necessary to evaluate particular aphidophagous species in specific agronomic situations in order to assess their biological control potential.

Since the abundance of aphids in agroecosystems fluctuates in space and time their availability severely affects the life attributes of lady beetles (Santos-Civdanes et al., 2011). As a result, the predatory responses of lady beetles differ depending on the biomass of aphid prey. Such variations in predation by lady beetles govern the complex interactions (e.g. con- and heterospecific competition) between them when feeding on the same prey source. Studies on the dependency of the intensity and nature of such interactions

between lady beetles on the abundance of prey, therefore, is important as it affects the structure of predatory guilds.

One of the most informative ways of studying predator-prey interactions is to measure the functional response of a predator to prey abundance, which can be used as a measure of searching efficiency and is often correlated with its biocontrol efficiency (Fathipour et al., 2006; Bayoumy, 2011a, b; Bayoumy & Michaud, 2012; Omkar & Kumar, 2013). There are five types of functional responses, viz. Type I, which is linear; Type II, which is curvilinear; Type III, which is sigmoidal (Holling, 1959); Type IV, which is dome shaped (Luck, 1985) and Type V, which is negatively exponential (Watt, 1959). Several studies on different species of lady beetles, however, reveal that in lady beetles the type II functional response predominates (e.g., Omkar & Pervez, 2011; Osman & Bayoumy, 2011; Gupta et al., 2012). It is characterized by a curvilinear increase in predation with increase in prey density, becoming asymptotic at high prey densities and thereafter remaining constant due to satiation (Mills, 1982; Jalali et al., 2010) and is not stage specific (Omkar & Srivastava, 2001; Omkar & Kumar, 2013). However, some studies also report type III

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responses in lady beetles, i.e. a sigmoidal functional response (e.g., Sarmiento et al., 2007; Abdollahi et al., 2010; Bayoumy, 2011a).

Several studies have evaluated the functional responses of particular species of lady beetles (Sarmiento et al., 2007; Abdollahi et al., 2010; Bayoumy, 2011a; Osman & Bayoumy, 2011; Gupta et al., 2012; Omkar & Kumar, 2013) and the nature of their predator-predator interactions (synergistic/additive/antagonistic) within aphidophagous guilds (Losey & Denno, 1998, 1999; Snyder & Ives, 2003; Costamagna et al., 2007; Snyder, 2009; Grez et al., 2007, 2011, 2012). However, studies of the functional response of species within predatory guilds (Omkar & Pervez, 2011; Jamour & Shishehbor, 2012; Vanacloacha et al., 2013) coupled with that of the interaction between predators are scanty (Snyder & Ives, 2003; Omkar & Pervez, 2011). The few studies that assess the twin effects of the functional responses and interactions between predators within guilds do not evaluate the conversion efficiencies or growth rates of the different predators (e.g. Omkar & Pervez, 2011). In addition, there is no study on the effect of niche partitioning by predators on the shape of the type II functional response.

Studies of the functional responses and interactions between predators within guilds are essential as they are likely to provide a more realistic picture of the predator-prey or predator-predator relationships within natural agro-ecosystems in which the predators have overlapping niches. When prey is scarce, predators occupying similar niches are likely to compete for food and as a consequence of this interaction (antagonistic effect) they are likely to consume less prey. However, when prey is abundant there are likely to be fewer conspecific/heterospecific interactions (Dixon, 2000) and more prey are consumed (synergistic effect) (Losey & Denno, 1998). Therefore, it is important to determine whether: (i) variations in predatory interactions due to overlapping niches alter the form of the functional response curves recorded for the different predators in predatory guilds, (ii) the nature of the interactions between predators improve the performance of predatory guilds, in terms of guild conversion efficiency and growth rate, when prey biomass increases and (iii) consumption of prey by predators in heterospecific guilds with different feeding habits or conspecific guilds with similar feeding habits increases with increase in prey biomass?

In order to address the above we evaluated: (i) the functional response curves and (ii) the predatory interactions between 10-day-old virgin females of *Coccinella septempunctata* L. (C7) and *Coccinella transversalis* F. (Ct) in conspecific (C7+C7 and Ct+Ct) and heterospecific (C7+Ct) combinations when the biomass of prey in the form of the pea aphid, *Acyrtosiphon pisum* (Harris) was either extremely scarce, scarce, sub-optimal, optimal or abundant.

Coccinella septempunctata, is of Palearctic origin and is now ubiquitous owing to its euryphagous nature, large size, ability to temporarily cease reproducing, heterogeneous voltinism and tendency to enter diapause, absence

of reproductive diapause in males, pre-hibernation mating, storage of sperm by females during winter and ecological plasticity in terms of both genetic and phenotypic polymorphism (Hodek & Michaud, 2008). *Coccinella transversalis* is also a large aphidophagous lady beetle of the Oriental region, native to India and found mainly in South Asia (Omkar et al., 2005). Both these lady beetles co-exist as predators of the numerous species of aphids that infest agricultural crops grown around Lucknow, India. Because they are polyphagous in the field, both lady beetles are recognized as effective biocontrol agents and used in aphid management programs.

MATERIAL AND METHODS

Stock cultures

Adults of *C. septempunctata* (C7) and *C. transversalis* (Ct) (n = 40) were collected from fields around Lucknow, India (26°50'N, 80°54'E) and allowed to mate. Mating pairs were separated and kept as pairs in plastic Petri dishes (14.5 × 1.5 cm) under constant conditions (27 ± 2°C temperature; 14L : 10D photoperiod) in Biochemical Oxygen Demand (BOD) incubators (Yorco Super Deluxe, YSI-440, New Delhi, India). They were provided with an ad libitum supply of the aphid, *A. pisum* (Ap), reared on broad bean, *Vicia faba* L. (Fabaceae) in a green house (22 ± 1°C temperature; 65 ± 5% relative humidity and 14L : 10D photoperiod). The eggs they laid were collected every 24 h, kept until they hatched and then the neonates were reared individually in Petri dishes under the same conditions as above. The larvae were provided daily with an ad libitum supply of aphids until they reached stages required for use in the experiments.

As they are the most voracious stages, fourth instar larvae (12 h after moulting) and adult females were used in the experiments (Omkar & Srivastava, 2001). However, when the biomass of prey provided to the larvae of C7 and Ct was either extremely scarce or scarce high levels of cannibalism were recorded in the conspecific (C7+C7 and Ct+Ct) and intraguild predation in the heterospecific C7+Ct combinations, in which the C7 larvae consumed the Ct larvae. Therefore, only adult females were used in subsequent experiments.

An age of 10-days was selected because females (C7 and Ct) of this age are (i) still in their pre-oviposition periods when fed on *A. pisum* and kept at 27 ± 2°C and under a 14L : 10D photoperiod (Bista & Omkar, 2013), (ii) more voracious than middle aged (Mishra et al., 2012) and old females, and (iii) probably less susceptible to environmental stress than younger females. A preliminary trial was also conducted using 10-day old unmated females of C7 (n = 20) and Ct (n = 20) to ensure that they did not lay eggs. For this they were kept individually in Petri dishes for 24 h and provided with an abundant supply of prey and kept at 27 ± 2°C and under a 14L : 10D photoperiod in BOD incubators. Petri dishes were checked every hour for eggs.

The status of females was unmated because (i) unmated females do not lay eggs, which may introduce errors due to variations in the reproductive performance of these lady beetles (Omkar et al., 2014) and (ii) mated females might consume their own eggs or those of other females resulting in a reduced consumption of prey and an underestimate of potential biomass of prey that can be consumed. Moreover, unmated females are more voracious than mated females (Rhamhalingham, 1987).

Experimental design

Prior to the start of an experiment, 10-day-old unmated adult females (C7 = 21.0 ± 0.76^a mg; Ct = 19.4 ± 0.67^a mg; F_{one way ANOVA}

= 2.49; $P = 0.132$; $df = 1, 19$; superscript “a” means the difference in weight is statistically insignificant) were starved for 12 h and weighed (Sartorius CP225-D; 0.01 mg precision). They (C7 or Ct) were kept singly in Petri dishes (size mentioned above) and provided with either an (i) extremely scarce supply of prey (12.5 mg ~25 third instars of *A. pisum*), (ii) scarce supply (25 mg ~50 third instars of *A. pisum*), (iii) sub-optimal supply (37.5 mg ~75 third instars of *A. pisum*), (iv) optimal supply (50 mg ~100 third instars of *A. pisum*) or (v) abundant supply (75 mg ~150 third instars of *A. pisum*) for the next 24 h and kept in the above mentioned conditions. The females and aphids remains were separated and weighed after 24 h. There were 10 replicates per prey biomass per lady beetle species, giving a total of 100 adult females.

These prey biomasses were used to standardize the female predators (C7 or Ct) prior to their use in experiments. When fed an extremely scarce or scarce supply of prey they consumed all the aphids in 24 h. When fed a sub-optimal supply of prey either no prey or few prey (<0.005 mg) were left after 24 h. When fed an optimal supply of prey females left a few aphids (3.00 ± 0.05 mg) and from an abundant supply of prey they left more than twice the biomass of aphids (8.00 ± 2.00 mg) after 24 h.

To evaluate the combined predatory potential of females of the two species of lady beetle, two conspecific predators (C7+C7 and Ct+Ct; both individuals of the same predatory species) and heterospecific pairs of predators (C7+Ct; one individual of each predatory species) were provided with either an extremely scarce supply of aphids (25 mg), scarce supply (50 mg), sub-optimal supply (75 mg), optimal supply (100 mg ~200 third instars) or abundant supply (150 mg ~300 third instars) of *A. pisum* prey and kept in the above mentioned conditions for 24 h. The conspecific individuals were marked using a marker pen so that they could be individually recognized before releasing them into the experimental arena (Petri dish; 14.5×1.5 cm). Females of almost equal biomass (conspecific or heterospecific) were used in each of the experiments in order to nullify the effect of differences in body size. The females were weighed individually prior to and being provided with prey. After 24 h, any aphids remaining were separated and weighed. Each treatment was replicated ten times per prey biomass per species of predators; giving a total of 150 two-predator female interactions.

To determine the natural reduction in aphid biomass, if any, in the absence of predators, the following prey biomasses: 12.5, 25, 37.5, 50 and 75 mg of aphids for individual predators and 25, 50, 75, 100 and 150 mg for pairs of predators, were placed in Petri dishes and kept under similar conditions for 24 h, reweighed and used as controls. The average loss of biomass, if any, based on 5 replicates per standard aphid biomass, was used to normalize the data on consumption prior to calculating the various parameters.

STATISTICAL ANALYSIS

The data were analyzed to evaluate: (1) the functional responses of beetles in conspecific and heterospecific combinations, (2) predatory interactions between the different species and (2) the conversion efficiencies and growth rates of the two species of ladybirds fed the different biomasses of prey. The distributions of the results in all the data sets obtained in this study were checked for normality using the Kolmogorov-Smirnoff test. Means were separated using Tukey's test when data were normally distributed and variances were homogeneous (Bartlett's test for equal variances).

Functional responses of the predators in conspecific and heterospecific combinations

Analyses of functional responses recorded involved determining the type of functional response and estimating the param-

eters of functional response curves (Juliano, 2001). Initially, to discriminate between Type II and Type III responses, the shape of the functional response curve was analyzed using a logistic regression (Juliano, 2001) of the proportion of prey eaten (N_e) as a function of initial prey biomass (N_0). The data were fitted to a polynomial regression function using statistical software SAS (Version 9.0). The equation used was:

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

where, P_0 (intercept), P_1 (linear), P_2 (quadratic) and P_3 (cubic) are the parameters to be estimated. Positive linear ($P_1 > 0$) and negative quadratic ($P_2 < 0$) parameters indicate a Type III functional response. If $P_1 < 0$, the proportion of prey consumed declines monotonically with the initial number of prey offered, thus describing a Type II functional response (Juliano, 2001). The Proc CATMOD procedure was used to estimate these parameters. The second analysis used a nonlinear least squares regression, Proc NLIN (SAS Version 9.0), to estimate the parameter values (T_h and either a for Type II, or b, c and d for Type III). Because the experiment was carried out without replacing the prey that were eaten, the appropriate model for a Type II functional response is the “random-predator” equation (Rogers, 1972):

$$N_e = N_0 \{1 - \exp[-a(T_h N_e - T)]\} \quad \text{Type II}$$

$$N_e = N_0 \{1 - \exp[-(d + bN_0)(T_h N_e - T)/(1 + cN_0)]\} \quad \text{Type III}$$

where, N_e is the number of prey consumed, N_0 is initial prey biomass, a is the attack rate, T_h is the handling time per prey, T is the total time of exposure, and b , c and d are constants.

Data on prey consumption and proportion of prey consumed by the different predators when supplied with different biomasses of prey were subjected to two way ANOVA with prey biomass, predator combinations (C7+C7, Ct+Ct and C7+Ct) and their interaction as independent factors and prey consumption and proportion of prey consumed as dependent factors, followed by Tukey's post hoc comparison of means. Statistical analyses were performed using SAS (Version 9.0) and MINITAB 16.

Multiplicative risk model and predatory interactions

The predicted/observed prey consumption recorded over a period of 24 h for each of the predators was assessed using a “multiplicative risk model” [using equations (1), (2), (3) and (4)], which correct for prey depletion within an additive experimental design (Sih et al., 1998; Omkar et al., 2014).

(1) Proportion of prey consumed by either predator a or b (P_a or P_b) equals to:

$$\frac{\text{Prey consumption (mg) by predator a or b alone}}{\text{Prey (mg) provided to predator a or b alone}}$$

The proportion of prey consumed when different biomasses of prey were provided:

(i) Extremely scarce supply: P_a (C7) = 0.92 ± 0.03 ; P_b (Ct) = 0.89 ± 0.02

(ii) Scarce supply: P_a (C7) = 0.98 ± 0.00 ; P_b (Ct) = 0.66 ± 0.04

(iii) Sub-optimal supply: P_a (C7) = 0.88 ± 0.02 ; P_b (Ct) = 0.83 ± 0.02

(iv) Optimal supply: P_a (C7) = 0.89 ± 0.04 ; P_b (Ct) = 0.70 ± 0.04

(v) Abundant supply: P_a (C7) = 0.75 ± 0.03 ; P_b (Ct) = 0.59 ± 0.01

(2) Predicted proportion of prey consumed equals to:

$$1 - [(1 - P_a)(1 - P_b)]$$

where P_a and P_b are the proportions of prey consumed by predators “a” and “b”, respectively.

(3) Observed proportion of prey consumed by the two predators equals to:

TABLE 1. Maximum likelihood estimates derived from the logistic regression of proportion of prey eaten as a function of the initial prey biomass by predators in the three combinations.

Predator combinations	Parameters	Estimates	SE	χ^2 -value	P-value
C7+C7	Intercept (P_0)	12.4465	1.4613	72.55	<0.0001
	Linear (P_1)*	-0.4169	0.0532	61.50	<0.0001
	Quadratic (P_2)	0.00488	0.000599	66.36	<0.0001
	Cubic (P_3)	-0.00002	2.068E-6	70.33	<0.0001
Ct+Ct	Intercept (P_0)	3.2874	0.4929	44.48	<0.0001
	Linear (P_1)*	-0.1117	0.0205	29.60	<0.0001
	Quadratic (P_2)	0.00138	0.000253	29.58	<0.0001
	Cubic (P_3)	-4.97E-6	9.256E-7	28.84	<0.0001
C7+Ct	Intercept (P_0)	4.4926	0.5268	72.73	<0.0001
	Linear (P_1)*	-0.1600	0.0216	54.98	<0.0001
	Quadratic (P_2)	0.00191	0.000264	52.63	<0.0001
	Cubic (P_3)	-6.59E-6	9.624E-7	46.88	<0.0001

C7 and Ct represent *Coccinella septempunctata* and *C. transversalis*, respectively. *A significant negative estimate for parameter P_1 indicates that the slope of the functional response curve is declining, which indicates a type II functional response.

$$\frac{\text{Combined prey consumption by both predators (mg)}}{\text{Total biomass of prey provided (mg)}}$$

(4) Predicted/Observed proportion of prey consumed (%) equals to:

Predicted/Observed proportion of prey consumed*100

Variation in the predicted and observed proportion of prey consumed by the predators in each of the combinations was analyzed using one way ANOVA, followed by Tukey's post hoc comparison of means. All data were subjected to arcsine square root transformation prior to ANOVA. The statistical software MINITAB 16 (Minitab Inc., State College, Pennsylvania, USA) was used for the analyses.

Conversion efficiencies and growth rates of the predators

The conversion efficiencies and growth rates of the predators were calculated using the following formulae:

(1) Conversion efficiency (modified after Dixon, 2000) equals to:

$$\frac{\text{Change in combined biomass (mg) of both predators within guilds}}{\text{Biomass (mg) of prey consumed by both predators within guilds}}$$

(2) Growth rate (day^{-1}) (modified after Waldbauer, 1968) equals to:

$$\frac{\text{Change in combined biomass (mg) of both predators within guilds}}{\text{Feeding period (1 d)} \times \text{Mean biomass of both predators within guilds (mg)}}$$

Variations in conversion efficiency and growth rate were analyzed using two way ANOVA with prey biomass, predator combinations (C7+C7, Ct+Ct and C7+Ct) and their interaction as independent factors and predator conversion efficiencies and growth rates as dependent factors, followed by a Tukey's post hoc comparison of means. Statistical analyses were performed using MINITAB 16.

RESULTS

Functional responses of predators in conspecific and heterospecific combinations

Prey consumption by the predators increased from 24.42 ± 0.12 to 112.60 ± 4.13 mg, 19.20 ± 0.25 to 101.00 ± 3.27 mg and 21.69 ± 0.85 to 119.00 ± 3.07 mg in the C7+C7, Ct+Ct and C7+Ct combinations, respectively, when the biomass of prey provided was increased from 25 to 150 mg. In contrast, the proportion of the prey con-

sumed by the predators in these combinations decreased from 0.98 ± 0.00 to 0.75 ± 0.03 , 0.77 ± 0.01 to 0.67 ± 0.02 and 0.87 ± 0.03 to 0.79 ± 0.02 , respectively, when the biomass of prey provided was increased.

The decrease recorded in the proportion of the biomass of prey consumed when biomass of prey provided was increased indicates a type II functional response. Results of the maximum likelihood analyses of the results confirmed this for the C7+C7, Ct+Ct and C7+Ct combinations (Table 1). By plotting a type II functional response and fitting polynomial logistic regression models to the biomass and proportions of prey consumed, respectively, the resulting regression lines fitted the data well (Fig. 1). However, the curves of the proportion of prey consumed were similar for the predators in all three combinations, initially decreasing (as in type II) as the biomass of aphids provided was increased from extremely scarce to optimal, followed by an increase (as in type III) when the food supply was increased from optimal to abundant (Fig. 2). The significantly negative P_1 parameters recorded when different biomasses of prey were provided, suggest that in this case

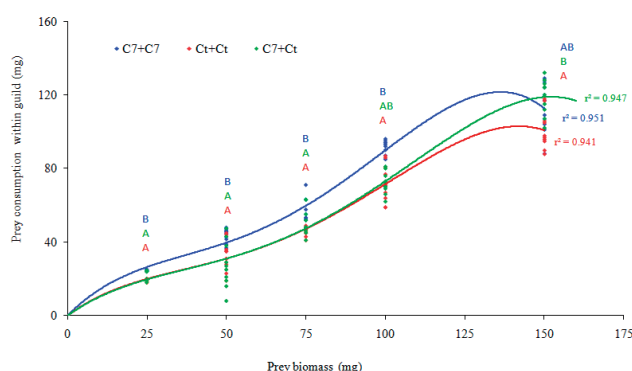


Fig. 1. Fitted relationships for prey consumption by two predators in conspecific and heterospecific combinations of two predators for data recorded when provided with five different abundances of prey. C7 and Ct represent *C. septempunctata* and *C. transversalis*, respectively. Large letters represent comparison of means between predatory guilds.

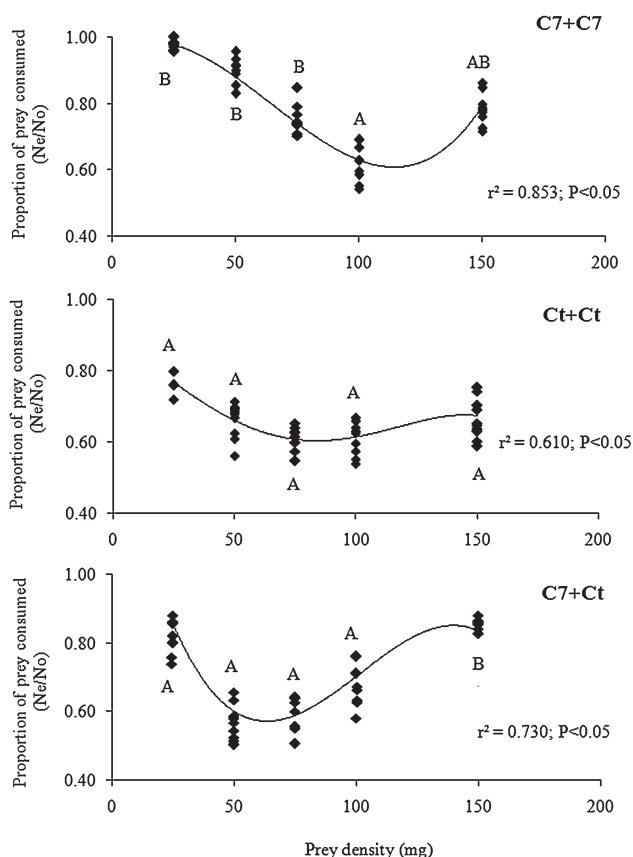


Fig. 2. Fitted relationships for percentage of prey consumed by conspecific and heterospecific combinations of two predators for data recorded when provided with five different abundances of prey. C7 and Ct represent *C. septempunctata* and *C. transversalis*, respectively. Large letters represent comparison of means between predatory guilds.

the slopes of the functional response curves decline, which is characteristic of a type II functional response (Table 1).

The instantaneous rate of attack rate (a) and handling time (T_h) were highest in the two conspecific, C7+C7 and Ct+Ct, combinations, respectively; and lowest in the heterospecific C7+Ct combination. Predicted consumption of prey over a period of 24 h (T/T_h) was greatest for the predators in the C7+Ct combination, followed by the C7+C7 and Ct+Ct combinations (Table 2).

Two way ANOVA further revealed a significant effect of both the predators and the biomass of prey provided on the amount of prey consumed ($F_{\text{interaction}} = 5.86$; $P < 0.0001$; $df = 8, 149$) and the proportion of prey consumed ($F_{\text{interaction}} = 6.33$; $P < 0.0001$; $df = 8, 149$). The predators in the C7+C7 combination provided with either an extremely

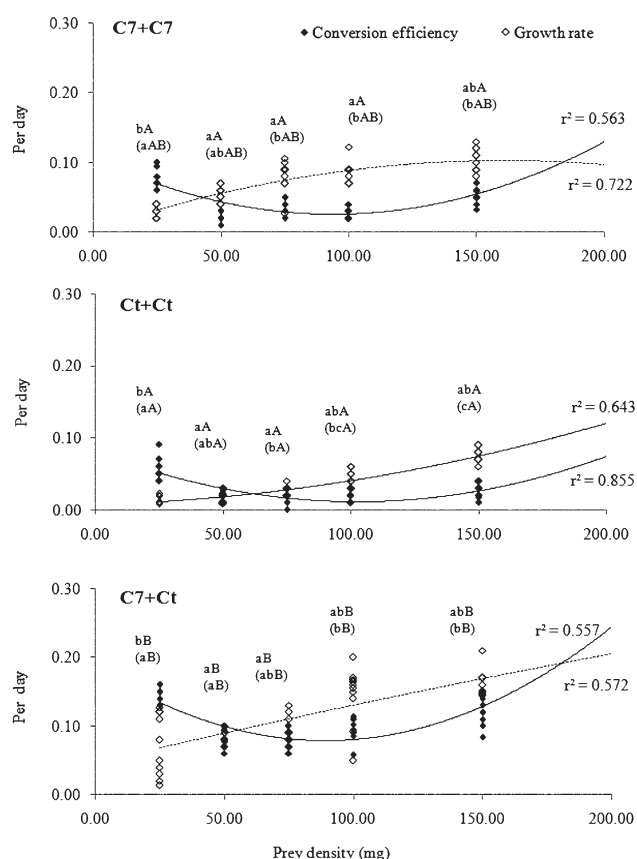


Fig. 3. Fitted relationships for conversion efficiency and growth rate of conspecific and heterospecific combinations of two predators for data recorded when provided with five different abundances of prey. C7 and Ct represent *C. septempunctata* and *C. transversalis*, respectively. Small and large letters without parentheses and within parentheses are for conversion efficiency and growth rate, respectively. Small and large letters represent comparison of means between prey density and between predatory guilds, respectively.

scarce, scarce, sub-optimal or optimal supply of prey had the highest prey consumptions and proportions of prey consumed. However, when provided with an abundance of prey, predators in the C7+Ct combination consumed more prey and a greater proportion of prey than the predators in the C7+C7 and Ct+Ct combinations (Fig. 2).

Multiplicative risk model and predatory interactions

The multiplicative risk model followed by a one way ANOVA revealed that the observed consumption of prey by the predators (except C7+C7 provided with an extremely scarce supply of prey) was significantly lower than

TABLE 2. Estimates (\pm SE) of the attack rate (a) and handling time (T_h) plus the 95% confidence limits (CL) for the different predator combinations derived from their functional responses.

Predator combinations	a	95% CL		T_h (hours)	95% CL		T/T_h
		Lower	Upper		Lower	Upper	
C7+C7	0.002340 ± 0.000332	0.00167	0.00301	0.1683 ± 0.01310	0.1419	0.1947	142.60
Ct+Ct	0.001040 ± 0.000177	0.000801	0.00127	0.1777 ± 0.00915	0.1593	0.1961	135.06
C7+Ct	0.000826 ± 0.000099	0.000626	0.00103	0.0985 ± 0.01790	0.0626	0.1345	243.65

C7 and Ct represent *Coccinella septempunctata* and *C. transversalis*, respectively. Here, $T = 24$ h.

TABLE 3. Observed and predicted prey consumptions (%) and predatory interactions between the females in the different combinations predicted by the Multiplicative Risk model.

Aphid prey supply	Predator combinations	Combined prey consumption (mg)	Sum of individual prey consumptions (mg)	Multiplicative Risk model			
				Observed prey consumption (%)	Predicted prey consumption (%)	F-value (P-value)	Predatory interaction
Extremely scarce	C7+C7	24.42±0.12	22.00±0.67	97.68±0.49	98.61±0.46	1.90 (0.185)	Additive
	Ct+Ct	19.20±0.25	21.40±0.52	76.80±1.00	98.40±0.40	403.41 (<0.0001)	Antagonism
	C7+Ct	21.69±0.85	21.70±0.40	86.76±3.40	99.17±0.37	13.13 (0.002)	Antagonism
Scarce	C7+C7	43.48±1.13	48.84±0.25	86.96±2.26	99.92±0.02	32.97 (<0.0001)	Antagonism
	Ct+Ct	32.70±2.60	33.10±1.98	65.22±5.17	87.16±2.28	15.11 (0.001)	Antagonism
	C7+Ct	27.34±3.92	27.32±3.87	54.64±7.74	99.20±0.22	33.16 (<0.0001)	Antagonism
Sub-optimal	C7+C7	56.35±2.33	64.80±1.53	75.13±3.10	98.07±0.59	52.77 (<0.0001)	Antagonism
	Ct+Ct	45.90±0.78	61.40±1.19	61.20±1.04	96.87±0.48	969.46 (<0.0001)	Antagonism
	C7+Ct	50.80±2.36	63.10±0.99	67.73±3.14	97.87±0.47	90.06 (<0.0001)	Antagonism
Optimal	C7+C7	91.10±1.30	89.00±4.00	62.53±1.72	97.44±2.07	168.14 (<0.0001)	Antagonism
	Ct+Ct	72.20±3.26	70.40±4.22	62.04±1.66	89.63±2.70	75.64 (<0.0001)	Antagonism
	C7+Ct	71.90±1.87	79.90±2.00	68.40±3.80	97.70±0.40	59.74 (<0.0001)	Antagonism
Abundant	C7+C7	112.60±4.13	112.70±4.65	79.08±3.82	92.95±1.37	11.69 (0.003)	Antagonism
	Ct+Ct	100.92±3.27	88.00±1.12	67.28±2.17	82.87±0.61	47.79 (<0.0001)	Antagonism
	C7+Ct	119.00±3.07	100.35±2.67	84.30±1.00	89.61±1.36	9.88 (0.006)	Antagonism

Values are means ±SE; F-values (of a one-way ANOVA between observed prey consumption and predicted prey consumption per predator combination) significant at $P < 0.05$; $df = 1, 19$. Additive effect was recorded when observed prey consumption (%) and predicted prey consumption (%) do not differ significantly. Antagonistic effect was recorded when observed prey consumption (%) was significantly lower than the predicted prey consumption (%) per predator combination. C7 and Ct represent *Coccinella septempunctata* and *Coccinella transversalis*, respectively.

their predicted prey consumption (sum of the consumption of prey by the females of the different species when kept separately), which indicates antagonistic effects when kept together in pairs and provided with one of the five different levels of prey abundance. The effect was additive when the predators in the C7+C7 combination were fed on extremely scarce supply of prey, i.e. the observed consumption of prey by the two predators was equal to the predicted consumption (Table 3).

Conversion efficiencies and growth rates

A two way ANOVA revealed that two independent factors, i.e. prey biomass and predator combination had significant effects on the conversion efficiency ($F_{\text{interaction}} = 2.83$; $P = 0.006$; $df = 8, 149$) and growth rate ($F_{\text{interaction}} = 4.49$; $P < 0.0001$; $df = 8, 149$).

A comparison of the means and regression lines further revealed that although the predators in the C7+C7, Ct+Ct and C7+Ct combinations had the highest conversion efficiencies when provided with an extremely scarce supply of prey, their growth rate was the lowest. However, when provided with an abundant supply of prey the predators in the three combinations had the highest conversion efficiencies and highest growth rates (Fig. 3). In addition, overall on the five biomasses of prey supplied as food, both the conversion efficiency and growth rate were highest for the C7+Ct combination followed by C7+C7 and lowest for the Ct+Ct combination.

DISCUSSION

In the present study, negative linear coefficients (P_1) for conspecific and heterospecific groups of two females reveal

that the rate of consumption of prey by these predators decreases with increase in abundance of prey, which indicate a Type II functional response (Bayoumy, 2011a, b; Osman & Bayoumy, 2011; Omkar & Kumar, 2013). The curves of the proportions of prey consumed by C7+C7, Ct+Ct and C7+Ct pairs of females are similar and appear to take the form of a decelerating type II functional response when provided with a low to optimal supply of prey, followed by an accelerating type III functional response when provided with an optimal to abundant supply of prey. This indicates that the 2-predator conspecific and heterospecific combinations exhibit modified type II functional responses. Similar results are reported for the parasitoid, *Aphytis diaspidis* Howard (Bayoumy, 2011b) and coccinellid predator, *Stethorus gilvifrons* Mulsant (Osman & Bayoumy, 2011).

Although (i) the attack rate of the C7+Ct combination of predators was the lowest recorded and (ii) they consumed a lower biomass of prey than the C7+C7 combination of predators when provided with either an extremely scarce, scarce, sub-optimal or optimal supply of prey, their predicted biomass of prey consumed in 24 h (T/T_h) was the greatest recorded in the present study. This may probably be due to their low prey handling time and high prey consumption when prey are abundant, as revealed by the present study. This further reveals that the heterospecific C7+Ct combination of predators is possibly more successful than the C7+C7 combination in consuming prey when prey is abundant but less so when prey is scarce. In addition, because of their higher attack rate and lower prey handling time, the C7+C7 combination of predators was predicted to consume a greater biomass of prey than the Ct+Ct combination of predators in 24 h (T/T_h).

Such functional response curves that are intermediate in form between a type II and III may be a consequence of using a small experimental arena (Petri dishes). In addition, variables like the attack rates and handling times of predators may be incorrectly estimated in Petri dish environments. It is highly likely that the functional responses of predators may be better estimated using more natural arenas like small plants. It is also likely that using a wider range and number of different prey abundances would greatly increase the accuracy with which functional responses can be estimated.

In the present study, the antagonistic effects recorded for the various combinations of predators (except C7+C7 provided with an extremely scarce supply of prey) when provided with different biomasses of prey is probably due to predator-predator interactions that result from either (i) exploitative competition in which a predator affects another predator by reducing the abundance of a shared prey, and/or (ii) interference competition in which the activity of one predator reduces the access of another predator to the shared prey (Mills, 2006). Studies have shown that in the presence of another predator(s) individual predators often consume less prey (Noia et al., 2008). The antagonistic effects recorded in the present study are in accordance with many earlier findings (Kaplan & Eubanks, 2002; Denno & Finke, 2006; Omkar et al., 2014). In contrast, the synergistic effects recorded when females of *C. transversalis* and *P. dissecta* share a common prey can be attributed to their occupying different niches (Omkar & Pervez, 2011).

In the present study, probably due to high voracity and energy requirements, both of the C7 predators (Mishra et al., 2012; Omkar et al., 2014) when provided with an extremely scarce supply of prey ate all the prey prior to interacting with one another. As a result, their recorded prey consumption was almost equal to their predicted prey consumption (prey consumption when kept individually), which resulted in the additive effect recorded for the C7+C7 combination. The higher prey consumption and proportion of prey consumed recorded for C7 in the C7+C7 combination when provided with an extremely scarce, scarce, sub-optimal or optimal supply of prey also reveals that C7 is more voracious than Ct.

Compared to the conspecific C7+C7 and Ct+Ct combinations the heterospecific C7+Ct combination consumed more prey when provided with an abundant supply of prey. This may be due to the lower interference recorded in predator-predator interactions when prey is abundant, which results in higher rates of prey consumption (Omkar & Pervez, 2011). Studies have shown that the predatory interactions between conspecific lady beetles are more frequent and intense, and result in the death and consumption of one of the antagonists than interactions between heterospecific ladybirds (Sloggett et al., 2009; Kajita et al., 2010). Thus, it is possible that when several conspecific predators are present in the same colony of aphids they spend more time defending themselves than consuming prey.

The higher proportion of prey consumed by the predators when provided with an abundant and a scarce supply

of prey recorded in the present study may be because they probably spent more time feeding on aphids than interacting with each other when prey was abundant and when scarce they quickly consumed all the aphids. The higher proportion of prey consumed recorded when prey is scarce is in accordance with results of a similar study on the larvae of *Adalia bipunctata* (Schuder et al., 2004).

In the present study, the low level of interaction between predators when provided with an abundant supply of prey probably enabled the predators to consume and utilize more prey, which resulted in their higher growth rate and conversion efficiency. Although, there are no studies on the conversion efficiency and growth rate of predators kept in conspecific or heterospecific combinations there are numerous studies on single species provided with abundant prey, conditions under which they perform best in terms of growth, development and reproduction (Schuder et al., 2004; Omkar et al., 2010). The highest conversion efficiency but lowest growth rates recorded when provided with an extremely scarce supply of prey support the findings of Schuder et al. (2004). It is likely that the higher conversion efficiency and growth rate of the C7+Ct combination recorded at all five different food supplies in the present study may be due to the weaker interactions between predators belonging to different species (Evans, 1991). Thus, the individual predators consumed more prey in the heterospecific than in the conspecific combinations.

The present study therefore indicates that: (i) the relationship between the proportion of prey consumed by C7 and Ct predators in conspecific and heterospecific combinations when provided with five different abundances of prey exhibited a wave form that appeared to be a composite of a decelerating (type II) functional response when provided with an extremely scarce to optimal supply of prey, followed by an accelerating (type III) functional response when provided with an optimal to abundant supply of prey. (ii) the C7+C7 combination provided with an extremely scarce supply of prey showed an additive effect, whereas the predators in all the other combinations showed antagonistic effects at all the prey abundances provided. (iii) predators in the C7+Ct combination consumed fewer aphids when provided with a low supply of prey than when supplied with an abundance of prey. In addition, their efficiency in converting prey biomass into their own biomass was highest in the C7+C7 and Ct+Ct combinations at all prey biomasses. (iv) In all the three combinations, predators had the highest conversion efficiencies but lowest growth rates when provided with an extremely scarce supply of prey. In contrast, conversion efficiencies were higher and growth rates were highest in the three combinations provided with an abundant supply of prey. It may, therefore, be concluded that when the combinations of predators include species that occupy similar niches, the predators will probably consume less prey than their nutritional requirements both at low and high prey biomasses as a result of interacting antagonistically.

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