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Intra-guild interactions between the parasitoid *Aphytis lingnanensis* and the predator

***Chilocorus circumdatus*: Implications for the biological control of armoured scales**

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Abstract

The parasitoid *Aphytis lingnanensis* and the predator *Chilocorus circumdatus* are used for releases in different crops to control armoured scales. Both natural enemies compete, to some extent, for the same resource and therefore they can incur intraguild predation interactions (IGP). In the present work, the consequences of these interactions on the parasitism and predatory efficiency of these natural enemies were assessed under laboratory conditions by studying potential changes in their functional responses.

A type II functional response to host/prey density was observed in *A. lingnanensis* and *C. circumdatus* when acting alone. The predatory efficiency of *C. circumdatus* was not affected by the presence of *A. lingnanensis* in the same arena. Conversely, the parasitism efficiency of *A. lingnanensis* was affected by the presence of the predator. Due to IGP at low host densities there was a shift from functional response type II to type III. No changes in the handling time when the predator was present suggested that parasitism behaviour was not influenced by the presence of the predator. *Chilocorus circumdatus* did not discriminate between parasitised and unparasitised scales. A recommended strategy in biological control programs could be the use of *A. lingnanensis* at low infestation levels and to reinforce these releases with *C. circumdatus* when high pest densities are given.

1 INTRODUCTION

Armoured scales such as the California red scale, *Aonidiella aurantii* (Maskell), citrus snow scale, *Unaspis citri* (Comstock) and oleander scale, *Aspidiotus nerii* Bouché, (Hemiptera: Diaspididae) are among the principal armoured scale pests which have been recorded from hundreds of host species in more than 100 plant families (Beardsley and Gonzalez, 1975; Rosenheim and Rosen, 1991; Smith and Peña, 2002). These phytophages are common pests in citrus groves (Rosenheim and Rosen, 1991) where they can induce important economic losses (*A. nerii* mainly in lemons) (Knapp et al., 1996; Jacas et al., 2010; Lucas et al., 2009). Natural enemies can cause high mortality to armoured scales and in many cases they are able to regulate their populations. Parasitoid species of the genus *Aphytis* (Hymenoptera: Aphelinidae) are considered the most efficient natural enemies of *A. aurantii* and *A. nerii* in different climatic and agronomic conditions (Moreno and Luck, 1992; Reeve and Murdoch, 1986; Smith et al., 1997; University of California (UC), 1991). In addition, predators may also help to reduce armoured scales being able to cause a rapid decline of their populations at high prey density levels (Samways, 1984). The most important predators on armoured scales are the ladybirds beetles (Coleoptera: Coccinellidae), such as *Rhyzobius lophanthae* (Blaisd.) and *Chilocorus* spp. (Debach and Rosen, 1976; Drea and Gordon, 1990; Sorribas and Garcia-Mari, 2010; Vanaclocha et al., 2009).

Augmentative releases of *Aphytis* ssp. are considered a key component of IPM programs in many citrus regions around the world. *Aphytis melinus* DeBach is released in Mediterranean climate citrus areas such as California (Rosen and Debach, 1979), Southern Australia (Smith et al., 1997), South Africa (Bedford, 1998), Spain (Vacas et al. 2012) and Sicily (Siscaro et al., 1999) whereas *Aphytis lingnanensis* Compere is mainly released in sub-tropical climatic zones such as Queensland (Australia) (Smith et al., 1997). Both species are mass-reared in either public or private insectaries.

Predators can also play an important role on armoured scales biological control by complementing the actions of *Aphytis* species (Rosen and Debach, 1978; Omkar and Pervez, 2003). On occasion, *Aphytis* ssp. releases could be reinforced with augmentative or inoculative releases of Coccinellidae predators, especially *Chilocorus* species (Bedford and Cilliers, 1994; Hattingh and Samways, 1991). In Australia, *Chilocorus circumdatus* Gyllenhal (Coleoptera: Coccinellidae) releases are occasionally used against *U. citri* (Smith et al., 1995).

Despite the demonstrated effectiveness of these natural enemies, the simultaneous use of these two kind of biological control agents could result in reduction of their efficiency, as occur in other parasitoid-predator systems (Martinou et al., 2010; Meyhöfer and Klug, 2002; Snyder and Ives, 2003). When two species fed upon a common resource they may incur what is defined as intra-guild predation (IGP) (Borer et al., 2003; Kindlmann and Houdková, 2006; Lucas et al., 1998; Polis and Holt, 1992; Rosenheim et al., 1995). This kind of trophic interaction is considered one of the most important mortality factors among coexistent natural enemies. Between parasitoids and predators the most frequent form of IGP is asymmetric; that means one of the two competitors is always inferior to the other. Predators are able to kill parasitoids but not vice versa (Meyhöfer and Klug, 2002; Müller and Brodeur, 2002; Taylor et al., 1998). Several studies reported the interactions between parasitoids and predators when they shared a prey. Densities of parasitoids declined in the presence of predators (Snyder and Ives, 2003). In addition, other studies have demonstrated how the presence of predators can negatively influence the behavior of parasitoids by increasing handling times and therefore probably reducing searching times (Martinou et al., 2010). As most biological control agents that share a given host or prey, *A. lingnanensis* and *C. circumdatus* may compete for it, engaging in some sort of trophic interaction (Müller and Brodeur, 2002; Rosenheim et al., 1995). As a consequence, the efficiency of biological control programs that combine the use of both natural enemies could be compromised under certain conditions. Additionally, indigenous predators of armoured scales have demonstrated to be a key component on their natural

mortality in the field (Samways 1985; Smith et al. 1995). IGP between indigenous predators and released *Aphytis* ssp. could therefore also impact on the efficacy of the armoured scales biological control programs. To our knowledge, no studies have evaluated how predators and parasitoids of these citrus pests interact when competing for the same resource and how this could affect to their performance. In the present work, the consequences of these interactions on *A. lingnanensis* parasitism and *C. circumdatus* predatory efficiency were assessed under laboratory conditions by studying potential changes in their functional responses. A better understanding of this kind of interactions will help for decision-making when these two biological control agents are to be used.

2 MATERIALS AND METHODS

2.1 Plant and insects

Aphytis lingnanensis and *C. circumdatus* reared on *A. nerii* on butternut squash (*Cucurbita moschata* Duchesne) (Cucurbitales: Cucurbitaceae) were obtained from the commercial mass-rearing facilities of Bugs for Bugs (Mundubbera, QLD, Australia). Squash infested with *A. nerii* obtained from the same commercial facilities were used for the experiments. The environmental conditions in all the experiments were 25 ± 1ºC, 65 ± 5% RH and a photoperiod of 16:8 h (L:D).

2.2 Functional response of *C. circumdatus*

Chilocorus circumdatus adults were deprived of food for 48 h in individual plastic cages before commencing the experiment to standardise their hunger. During this period and in the course of the experiments, a water-soaked piece of cotton wool was supplied as a water source. Different densities of third-instar nymphs of *A. nerii* (2, 6, 10, 20, 30, 60, 120 and 220) were exposed to starved *C. circumdatus* in twenty replicates per density, in each experimental unit. One *C. circumdatus* adult was used per replicate. The experimental unit consisted of a transparent plastic jar of 7 cm in diameter closed with a tight-fitting lid that had a 2.5 cm diameter gauze-covered hole for ventilation. Inside the plastic jar a piece of squash (3.5 × 3.5

cm) was placed. Each piece had fixed on its peel the assigned density of *A. nerii* third-instar nymphs. These densities were selected from a denser population and the rest of scales were removed. The pieces of squash, except the peel containing the scales, were covered with absorbent paper to prevent predators from feeding on the vegetable. After 24 h, predators and the slice of scale infested squash were removed from the experimental unit and the number of scales preyed upon was recorded. Prey insects were not replaced during the experiment. A control treatment with ten replicates and a density of 10 scales per piece of squash was used to assess natural mortality rates in the prey.

2.3 Functional response of *A. lingnanensis*

Aphytis lingnanensis pupae were isolated from beneath *A. nerii* scale covers and placed individually in plastic tubes of 2 ml in volume. A small drop of honey was provided as a food source onto the sides of the tube. The tubes were sealed with a piece of cotton. The pupae were checked daily for emergence of adult parasitoids and then they were sexed and paired off. After that, the new pairs were left undisturbed for 48 hours to ensure female mating. During this time the parasitoids were fed with honey.

Different densities of third-instar nymph of *A. nerii* (2, 4, 6, 8, 10, 20, 30, 60 and 120) were exposed to *A. lingnanensis* mated females in twenty replicates per density. One *A. lingnanensis* mated female was used per replicate. The experimental unit was similar to the one described for the *C. circumdatus* functional response experiment. In each replicate a light streak of honey was provided onto the side of the plastic jar as a source of food for the parasitoids. After 24 h the parasitoids were removed from the experimental units and the number of *A. nerii* scales parasitised (scales containing parasitoid eggs beneath the scale cover) was evaluated under binocular stereoscope. Host scale insects were not replaced during the experiment. A control treatment with ten replicates and with a density of 10 scales was used to assess natural mortality rates in the host.

2.4 Functional response of *C. circumdatus* and of *A. lingnanensis* when sharing the same prey/host

Starved *C. circumdatus* adults and *A. lingnanensis* mated females were obtained following the same procedure explained above in the relevant sections, and the experimental units used were the same as explained above. Different densities of third-instar nymphs of *A. nerii* (2, 4, 6, 8, 10, 20, 30, 60, 120 and 220) were exposed to *C. circumdatus* adults and to *A. lingnanensis* mated females in ten replicates per density. One *C. circumdatus* adult and one *A. lingnanensis* mated female, both sharing the same experimental unit, were exposed to the prey/host in each replicate. After 24h the predators and the parasitoids were removed from the experimental units and the number of scales parasitised or preyed upon was counted in each replicate. Hosts/prey insects were not replaced during the experiment. In each experimental unit a water-soaked piece of cotton wool was supplied as water source for predators, and a light streak of honey was provided as a source food for parasitoids. A control treatment with ten replicates and with a density of 10 scales was used to assess natural mortality rates in the prey/host.

2.6 Prey preference of *C. circumdatus*

Prey preference of *C. circumdatus* between parasitised and unparasitised scales was also evaluated at the densities of 60 and 120 scales when both the parasitoid and predator were sharing the experimental unit. The number of preyed scales parasitised and unparasitised was evaluated for each density by counting the number of non-preyed scales that were parasitised and unparasitised. The initial number of prey parasitised for each density was considered as the number of parasitised scales obtained when the parasitoid was acting alone for the corresponding densities.

2.5 Data analysis

In order to evaluate the type of functional response that best fitted the data in the different experiments a logistic regression of the relative proportion of scales parasitised or predated was

performed (Juliano, 2001; Trexler et al., 1998). Data were fitted to a polynomial function with intercept, linear and quadratic coefficients using the maximum likelihood method. A positive linear coefficient and a negative quadratic coefficient imply that data fit a type III functional response whereas a negative linear coefficient means a better adjustment to type II functional response. Once this preliminary analysis was done, each set of data was fitted to its corresponding functional response equation. Because the experiments were conducted without prey/host replacement the 'random-predator equation' for a type II functional response (Rogers, 1972; Royama, 1971) and the 'Hassell equation' for a type III functional response (Hassell, 1978) were used. The data were fitted through a non-linear least-squares regression by means of the Levenberg–Marquardt iterative estimation procedure. The functional response parameters, attack rate (a') and handling time (T_h), were extracted from this regression. In the sigmoid functional response (type III), the attack rate varies with prey/host density ($a' = b \times x / (1+c \times x)$) where '$x$' is the host density and 'b' and 'c' are constants of itself (Hassell, 1978).

To evaluate interactive effects on predatory and parasitism behaviours, when both natural enemies are sharing the same experimental unit and host/prey, the attack rates were compared. Additionally, the number of preys/hosts preyed or parasitised was also compared for the highest densities tested between the experiments where parasitoids and predators were acting alone and the experiment where they were sharing the experimental unit. Significance was assessed using Student's t-test ($P < 0.05$).

The preference for parasitised *A. nerii* was determined by calculating the Manly's preference index (α_p) (Chesson, 1983; Manly et al., 1972) for each replicate of the two scale densities tested. The equation for the preference index was:

$$\alpha_p = \frac{\ln\frac{n_p - r_p}{n_p}}{\ln\frac{n_p - r_p}{n_p} + \ln\frac{n_u - r_u}{n_u}}$$

The parameters 'n_p' and 'n_u' were the initial numbers of parasitised and unparasitised scales, respectively and 'r_p' and 'r_u' were numbers of parasitised and unparasitised scales consumed over a 24-h period, respectively. The average preference index is predicted to be 0.5 if no preference is exhibited (Krebs, 1999). Significance of no preference for parasitised and unparasitised scales was assessed using Student's *t*-test ($P < 0.05$).

3 RESULTS

3.1 Functional response on *A. lingnanensis*

A type II functional response was obtained from the logistic regression because estimation of the linear coefficient was negative and the quadratic coefficient was positive (Table 1). The estimated attack-rate coefficient was 1.150 ± 0.267 days $^{-1}$ and the estimated handling time was 0.090 ± 0.006 days (Table 2). The estimated maximum number of scales parasitised in 24h was of 10 scales (Figure 1).

3.2 Functional response on *C. circumdatus*

A type II functional response was obtained from the logistic regression because estimation of the linear coefficient was negative and the quadratic coefficient was positive (Table 1). The estimated attack-rate coefficient was 2.083 ± 0.679 days $^{-1}$ and the estimated handling time was 0.017 ± 0.001 days (Table 2). The estimated maximum number of scales preyed on in a 24h period was 53 (Figure 2).

3.3 Functional response on *A. lingnanensis* in the presence of *C. circumdatus*

A type III functional response was obtained from the logistic regression because estimation of the linear coefficient was positive and the quadratic coefficient was negative (Table 1). The estimate 'b' and 'c' parameters were 0.002 ± 0.001 and 0.004 ± 0.009 respectively (Table 2). The estimated handling time was 0.075 ± 0.017 days (Table 2). The estimated maximum number of scales parasitised in 24 h was of 12 scales (Figure 1). No significant differences were found in the number of scales parasitised for the highest density tested (120 scales) between

the treatment where *A. lingnanensis* was alone and the treatment where the parasitoid was sharing the experimental unit with the predator (t_{28} = 1.535; *P* = 0.1359) (Figure 1).

3.4 Functional response on *C. circumdatus* in the presence of *A. lingnanensis*

A type II functional response was obtained from the logistic regression because estimation of the linear coefficient was negative and the quadratic coefficient was positive (Table 1). The estimated attack-rate was 1.520 ± 0.309 days $^{-1}$ and the estimated handling time was 0.018 ± 0.001 days (Table 2). The estimated maximum number of scales preyed on in 24 h was of 52 scales (Figure 2). No significant differences were found in the number of scales preyed on for the highest density tested (220 scales) between the treatment where *C. circumdatus* was alone and the treatment where the predator was sharing the experimental unit with the parasitoid (t_{28} = 0.2478; *P* = 0.861).

3.5 Prey preference of *C. circumdatus*

Chilocorus circumdatus did not discriminate between parasitised and unparasitised scales at 60 and 120 scales densities since the Manly's average preference index (α_p) was 0.4512 ± 0.06831 (n = 7) (t_{12} = 1.011; *P* = 0.3320) and 0.5950 ± 0.1794 (n = 10) (t_{18} = 0.7487; *P* = 0.4637).

4 DISCUSSION

In this study a type II functional response to host/prey density has been observed in *A. lingnanensis* and *C. circumdatus* when they acted alone, showing an asymptotic relationship with host/prey densities. In both cases, efficiencies declined with increasing *A. nerii* densities. The type II functional response is the most common of invertebrate parasitoids and predators (Fernández-arhex and Corley, 2003; Matadha et al., 2005; Monzó et al., 2009). Several studies reported a type II functional response by *Aphytis* species such as *Aphytis holoxanthus* DeBach or *Aphytis proclia* (Walker) (Matadha et al., 2005; Podoler et al., 1978). In the case of *A. melinus* and *A. lingnanensis*, Podoler (1981) found an increase in the levels of parasitism with increasing *A. nerii* densities. Despite this, some works reported later pointed out that type III functional response could also be characteristic of invertebrate natural enemies, especially

under field conditions where numerous factors are able to affect their behaviour (Hassell et al., 1977; Montoya et al., 2000). The predatory coccinellid, *C. circumdatus*¸ showed a higher attack rate and a lower handling time than the parasitoid, resulting in a more efficient functional response. However, other factors such as the numerical response, habitat complexity or environmental preferences have to be taken into account before making decisions about which natural enemy is most appropriate for a release program.

The predatory efficiency of *C. circumdatus* was not affected by the presence of *A. lingnanensis* at any density tested. In fact, the estimated handling times and attack rates were similar independent of the presence of the parasitoid. Because coccinellids are superior competitors than parasitoids when sharing the same patch and resource (Meyhöfer and Klug, 2002; Müller and Brodeur, 2002; Taylor et al., 1998) no negative IGP effects on the predator were expected. On the other hand, *C. circumdatus* did not seem to have any preference between parasitised and unparasitised scales.

The parasitism efficiency of *A. lingnanensis* was affected by the presence of the predator in the same patch only at low host densities. At these densities, the probability to encounter and feed upon parasitised *A. nerii* scales by *C. circumdatus* is high and as consequence parasitism efficiency drops off. Because immature parasitoids cannot escape or defend from the predator, they are extremely vulnerable to IGP (Lucas et al., 1998; Meyhöfer and Klug, 2002). When prey density increases, the encounter probability between the predator and parasitised scales starts to decrease and as a result, the IGP effect is mitigated to a large extent. IGP of *C. circumdatus* on *A. lingnanensis* was reflected in parasitism efficiency with a shift from functional response type II to functional response type III. A type III functional response requires an increase in the proportion of host/prey attacked over the lowest prey densities (Collins et al., 1981). At high densities, IGP on parasitoid immature stages is negligible and therefore has no effect on parasitism efficiency. The fact that the handling time as well as the maximum number of host parasitised at the highest density was not affected by the presence

of the predator suggests that parasitism behaviour is not influenced by the presence of the predator. These results are surprisingly different to the ones obtained in other studies evaluating interactions between predators and parasitoids. Martinou et al., (2010) found an increase in the handling time of the parasitoid *Aphidius colemani* Viereck (Hymenoptera: Aphidiinae) when the predator *Macrolophus caliginosus* Wagner (Hemiptera: Miridae) was present. The consequences for biological control of the fact that the parasitoid behaviour was not affected by the presence of the predator remain unclear. On the one hand, a reduction on handling time could lead to an increase of searching time. This would allow the parasitoid to find hosts safe from the presence of predators. On the other hand, a reduction of this parameter means a reduction on the number of eggs laid and as a consequence on the chances of obtaining a new progeny. *Aphytis lingnanensis* adults did not incur in IGP by *C. circumdatus*. The parasitoid may be faster than the predator and therefore able to develop escape strategies (Meyhöfer and Klug, 2002).

Interactive effects between *A. lingnanensis* and *C. circumdatus* seem to occur only when host/prey densities are low and IGP affects the parasitoid. Conversely, when host/prey densities are high and IGP does not occur, both natural enemies show a synergistic effect. Many species of parasitoids exhibit a pronounced type II functional response and they are candidates for synergistic effects with predators (Snyder and Ives, 2003).

According to the results obtained here, the use of both *A. lingnanensis* and *C. circumdatus* would be recommended when high scale infestations are occurring. The fact that *A. lingnanensis* did not modify its behaviour when *C. circumdatus* was present, and that this coccinellid seemed not to show any preference for parasitised scales make the use of both natural enemies compatible under these circumstances. On the contrary, at the early stages of pest infestation the synergistic benefits tend to disappear due to the combined effect of both natural enemies less than the sum of their separate effects. Experience with augmentative biological control has rarely produced evidence of pest outbreaks associated with releases,

indicating that IGP is rarely a cause of disruption (Rosenheim et al., 1995), the results here obtained suggest a loss of efficiency in additivity at low pest densities. A recommended strategy could be the use of *A. lingnanensis* at low infestation levels and to reinforce these releases with *C. circumdatus* when high pest densities are given. Further studies will be needed to investigate how *C. circumdatus* may affect the long-term population dynamics of *A. lingnanensis* by means of predator-parasitoid interactions over several parasitoid generations. Therefore it would be important to complement the information obtained in the functional response experiments with field trials to evaluate their additive performances.

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Table 1. Maximum likelihood estimate parameters from logistic regression of the proportion of host/prey eaten/parasitised as a function of initial host/prey densities by *A. lingnanensis* mated females, *C. circumdatus* adults, *A. lingnanensis* mated females sharing the experimental unit with *C. circumdatus* adults and *C. circumdatus* adults sharing the experimental unit with *A. lingnanensis* mated females.

	Parameter	Estimate	SE	χ^2	*df*	*P*
A. lingnanensis	Linear	-0.05090	0.00417	161.746	179	< 0.0001
	Quadratic	0.00023	0.00003	634.831		< 0.0001
C. circumdatus	Linear	-0.02579	0.00154	305.956	159	< 0.0001
	Quadratic	0.00005	0.000005	96.450		< 0.0001
A. lingnanensis/C. circumdatus	Linear	0.01011	0.00420	595.302	99	0.0147
	Quadratic	-0.00004	0.00002	63.408		0.0118
C. circumdatus/A. lingnanensis	Linear	-0.04731	0.00245	481.268	99	< 0.0001
	Quadratic	0.00013	0.00001	241.544		< 0.0001

Table 2. Functional response type (*FR*), attack rate (a') (days^{-1}) and handling time (T_h) (days) estimates obtained from non-linear regression of the number of host/prey by *A. lingnanensis* mated females, *C. circumdatus* adults, *A. lingnanensis* mated females sharing the experimental unit with *C. circumdatus* adults (*A. lingnanensis/C. circumdatus*), and *C. circumdatus* adults sharing the experimental unit with *A. lingnanensis* mated females (*C. circumdatus/A. lingnanensis*).

	FR	a'	SE	95% CI	T_h	SE	95% CI	R^2
A. lingnanensis	Type II	1.150	0.267	0.517-1.782	0.090	0.006	0.076-0.105	0,955
C. circumdatus	Type II	2.083	0.679	0.423-3.744	0.017	0.001	0.015-0.020	0,883
A. lingnanensis/ C. circumdatus	Type III	'b' = 0.002	0.001	6.737 E-5-0.003	0.075	0.017	0.034-0.116	0,992
		'c' =0.004	0.009	-0.017-0.026				
C. circumdatus/ A. lingnanensis	Type II	1.520	0.309	0.807-2.233	0.018	0.001	0.016-0.020	0,885

R^2 = are the coefficients of determination from R^2 each regression.

Figure 1.Functional response curves fit by non-linear least square regression of *A. lingnanensis* mated females acting alone (Type II), and *A. lingnanensis* mated females sharing the experimental unit with the predator *C. circumdatus* (Type III), exposed to different densities of *A. nerii* third-instar, during 24 h. Mean number (± SE) of *A. nerii* parasitised by *A. lingnanensis* is displayed for each host density and each treatment.
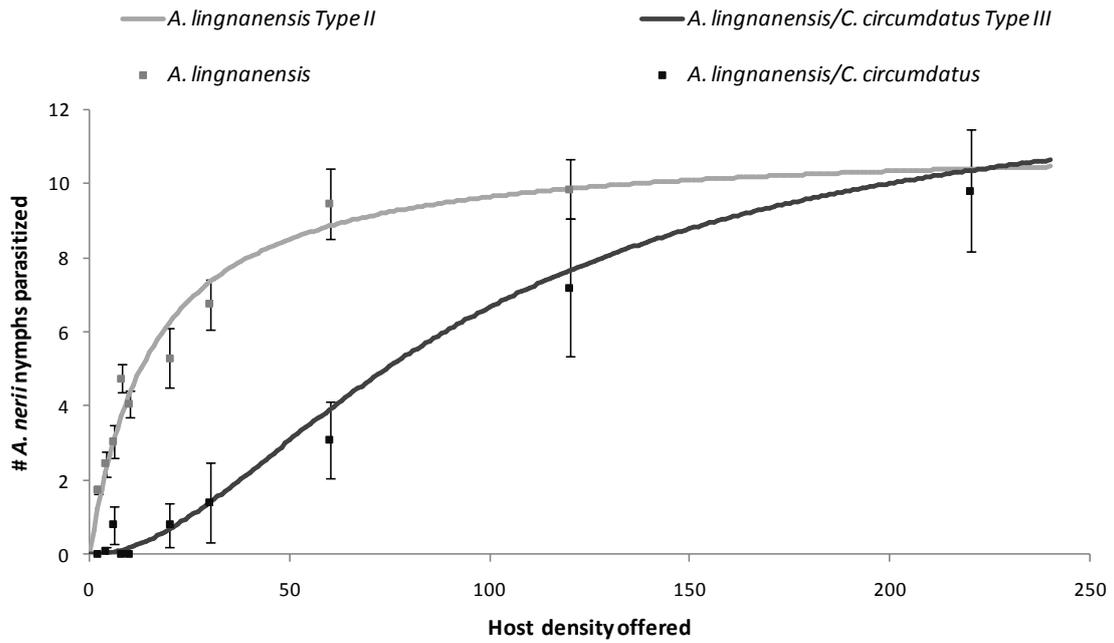
Figure 2. Functional response curves fit by non-linear least square regression of *C. circumdatus* adults acting alone (Type II), and *C. circumdatus* adults sharing the experimental unit with the parasitoids *A. lingnanensis* (Type II), exposed to different densities of *A .nerii* third-instar, during 24 h. Mean number (± SE) of *A. nerii* preyed upon by *C. circumdatus* is displayed for each host density and each treatment.