

# Effect of prey density on intraguild interactions among foliar- and ground-foraging predators of aphids associated with alfalfa crops in Chile: a laboratory assessment

Audrey A. Grez<sup>1\*</sup>, Tania Zaviezo<sup>2</sup> & Angélica Mancilla<sup>1</sup>

<sup>1</sup>Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile, and <sup>2</sup>Facultad de Agronomía e Ingeniería Forestal, P. Universidad Católica de Chile, Santiago, Chile

Accepted: 17 January 2011

**Key words:** *Acyrtosiphon pisum*, dropping behaviour, *Eriopis connexa*, *Hippodamia variegata*, *Trirammatus aerea*, predator–prey interaction, predator–predator interaction, Hemiptera, Aphididae, Coleoptera, Carabidae, Coccinellidae

## Abstract

Among the aphidophaga guild present in alfalfa [*Medicago sativa* L. (Fabaceae)], coccinellids forage on aphids most of the time on the foliage and carabids on the ground. The result of their combined effect can be synergistic, additive, or antagonistic, but this may depend on the prey density and interacting predatory species. In this study, we first determined, under laboratory conditions, the relative tendencies of *Therioaphis trifolii* (Monell), *Acyrtosiphon pisum* (Harris), and *Aphis craccivora* Koch (all Hemiptera: Aphididae) to drop in the presence and absence of two predators: *Eriopis connexa* (Germ) and *Hippodamia variegata* (Goeze) (both Coleoptera: Coccinellidae). These experiments showed that *T. trifolii* and *A. pisum* dropped more frequently than *A. craccivora*, and dropping was more frequent in the presence of *H. variegata*. We also determined the functional responses to aphid densities of five beetle species (Coleoptera: Carabidae) commonly found in alfalfa fields in Chile. All carabid species consumed aphids, with *Trirammatus aerea* (Dejean) being one of the most voracious. Then, we tested the hypothesis that the interactions between both coccinellid species and *T. aerea* would be antagonistic at low prey densities, because of competition or intraguild predation, and synergistic as prey density increases. For this, we recorded aphid consumption when predators were alone, or in combinations of a foliar and a ground predator, for five prey densities. For all predators and combinations, aphid consumption increased continuously with aphid density, and more prey were eaten when a foliar- and a ground-foraging predator were combined than when either predator was present. But, contrary to our expectations, we found that the interaction of these foliar- and ground-foraging predators was additive for all prey densities. Our results suggest that coexistence of these species would not interfere with aphid biological control in alfalfa.

## Introduction

The efficiency of natural enemies in controlling herbivorous insects may depend on intraguild interactions. If two species of natural enemies compete for the same prey, prey upon each other (i.e., intraguild predation), or interfere in their foraging behaviour, the interaction between natural

enemies will be antagonistic, and fewer prey than expected will be killed by their combined action (e.g., Rosenheim et al., 1993). If the foraging activity of one species of natural enemy alters the behaviour or feeding niche of the prey, making it more susceptible to attack by another species (i.e., predator facilitation; Soluk, 1993), the interaction between natural enemies will be synergistic and more prey will be killed when both species are present than the sum of their individual impacts (e.g., Losey & Denno, 1998a,b,c, 1999). If two species of natural enemies do not interact, then their combined effect on the prey population will be

\*Correspondence: A. A. Grez, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Casilla 2, Correo 15, La Granja, Santiago, Chile. E-mail: agrez@uchile.cl

additive and equal to the sum of their individual impacts (e.g., Chang, 1996).

Among aphidophagous predators, negative interactions occur, with fewer aphids consumed when more than one species of natural enemies co-occur (Lucas, 2005). Nevertheless, when aphids are in the presence of a combination of foliar- and ground-foraging predators, like coccinellids and carabids, synergism may occur. This is because aphids escape from coccinellids by dropping from their host plant to the ground, where they become more susceptible to attack by carabids, resulting in higher consumption rates than when no coccinellid predators are present (Losey & Denno, 1998a,b,c, 1999; Grez et al., 2007). Additionally, this predator avoidance behaviour has also other sublethal consequences like reduction in feeding time, and consequently lower reproduction and population growth (Nelson, 2007).

Laboratory trials carried out at a constant aphid density demonstrated that a higher proportion of *Aphis craccivora* Koch (Hemiptera: Aphididae) dropped from alfalfa plants [*Medicago sativa* L. (Fabaceae)] in the presence than in the absence of coccinellids, and that the interaction among *Eriopis connexa* (Germ), *Hippodamia variegata* (Goeze), and *Adalia bipunctata* (L.) (all Coleoptera: Coccinellidae) with *Notiobia cupripennis* (Germar) and *Metius flavipes* Dejean (both Coleoptera: Carabidae) were additive or synergistic, but never antagonistic (Grez et al., 2007).

Nevertheless, the outcome of predator–predator interactions may depend on prey availability. Antagonistic interactions may appear at low prey densities (Lucas et al., 1998; Jakobsen et al., 2004), whereas synergism frequently occurs at higher prey densities (Losey & Denno, 1998a; Chang & Eigenbrode, 2004). For example, Losey & Denno (1998a) found that at initial densities lower than 40 aphids per cage, the observed consumption when both predators were present was slightly higher than the expected consumption, but at higher aphid densities, the observed consumption was much higher than expected.

In this laboratory study, we first determined the tendencies of *Therioaphis trifolii* (Monell), *Acyrtosiphon pisum* (Harris) (both Hemiptera: Aphididae), and *A. craccivora* to drop in the presence and absence of coccinellids *E. connexa* and *H. variegata*. We also determined the functional responses to aphid densities of five carabid species commonly found in alfalfa fields in Chile. These experiments allowed us to choose an aphid species that presented a frequent dropping behaviour and a carabid species with high aphid consumption for the study of predator interactions under different prey densities. Thus, we tested the effect of density of *A. pisum* on the interactions among *Trirammatus aerea* (Dejean) (Coleoptera: Carabidae), *E. connexa*

and *H. variegata*. We expected that the interactions between these predator species would be antagonistic at low aphid densities, but synergistic at high densities.

## Materials and methods

### Comparative dropping behaviour of three aphid species exposed to two coccinellid species

For evaluating the dropping behaviour of *A. pisum*, *A. craccivora*, and *T. trifolii*, in the presence and absence of coccinellids, we used insects collected 1–4 days in advance from alfalfa fields near Santiago (Chile; 33°40'S, 70°35'W) that were maintained at 20 °C and L16:D8 in growth chambers (Bioref P+L, Santiago, Chile). Using insects collected from the field may add unknown sources of variability but also reflects the scenarios under which these interactions might occur.

Following Losey & Denno (1998b), we placed a small transparent plastic vial filled with moist sand in the middle of a plastic bowl (23 cm in diameter and 3 cm deep), in the centre of which we placed two alfalfa stems (15 cm high) upright. The vial and the interior ring of the plastic bowl were ringed with fluon (BioQuip Products; Rancho Dominguez, CA, USA) to prevent fallen aphids from climbing back onto the plant. We added 30 conspecific aphids (fourth and fifth instars) to the stems and left them undisturbed for 30 min. Then, we added (1) a single adult of *E. connexa*, (2) a single adult of *H. variegata*, or (3) no predator (control). Coccinellids were held without food for 24 h before the experiments. All experimental arenas were individually covered with a screened cage (22 × 22 × 27 cm) and left near a window shelf. After 1 h, we removed the cages and counted the aphids on the stems and in the bowls to determine the proportion of the aphids that dropped to the ground [no. aphids in bowl/(no. aphids in bowl + no. aphids on stems)]. Also, with a chronometer, we estimated the time each coccinellid spent moving around the stems during the hour that the experiment lasted. Trials including each species of aphid, coccinellids, and controls were replicated 10 times, between 9 May and 3 June 2008, using a complete randomized factorial design.

The effect of aphid species, predator treatment, and their interaction on the proportion of dropped aphids (arcsine  $\sqrt{x}$  transformed) was analysed using analysis of variance (ANOVA). Treatment means were compared using Tukey's honestly significant difference (HSD) test ( $\alpha = 0.05$ ). The average movement times of the coccinellid species were compared with a t-test. Finally, the relationship between coccinellid movement time and the proportion of dropped aphids was analysed with non-parametric Spearman correlation in STATISTICA 7.0 (StatSoft, 2001).

### Comparative aphid consumption by five carabid species

To select the carabid species for the following experiments, we compared aphid consumption of the five more abundant species of carabids found in alfalfa fields in Chile [mean body length in mm ( $\pm$  SEM) is given within parentheses]: *N. cupripennis* ( $11.55 \pm 0.24$ ), *T. aerea* ( $13.21 \pm 0.39$ ), *Trirammatus striatula* (Fabricius) ( $13.44 \pm 0.32$ ), *Tetragonoderus viridis* Dejean ( $6.49 \pm 0.20$ ), and *Calosoma vagans* (Dejean) ( $21.23 \pm 0.38$ ). All insects were collected 1–4 days in advance from alfalfa fields near Santiago, and maintained in a growth chamber, food-deprived. In glass Petri dishes (10 mm diameter) with moist filter paper, we added one adult carabid with 5, 10, 20, or 40 individuals (a mixture of fourth and fifth instars) of the aphid *A. pisum*. This aphid was selected because of its frequent dropping as demonstrated in the previous experiment (see Results), and also in other studies (Losey & Denno, 1998a,b; Nelson & Rosenheim, 2006; Nelson, 2007; Francke et al., 2008). Each aphid density was replicated 10 times in a complete randomized design. After 24 h in a growth chamber (at 20 °C and L16:D8), we removed the predator and counted the remaining aphids in each Petri dish to estimate the number of aphids that were eaten by each carabid. Data were arcsine  $\sqrt{x}$  transformed and analysed using a two-factor ANOVA with carabid species and aphid density as factors. Treatment means were compared with Tukey's HSD test ( $\alpha = 0.05$ ).

### Effect of prey density on intraguild interactions among foliar- and ground-foraging aphidophagous predators

To evaluate the effect of aphid prey density on the interaction among foliar- and ground-foraging predators through their aphid consumption, we used experimental arenas similar to those used to evaluate aphid dropping, but in this case, the bowls were filled with sand to simulate a natural substrate for carabids. Based on our previous results reported here, the species used for this experiment were the aphid *A. pisum*, the coccinellids *E. connexa* and *H. variegata*, and the carabid *T. aerea*. In each arena, we carefully added aphids to the alfalfa stems to have five prey density treatments (5, 10, 20, 40, or 80 late-instar aphids) and kept them inside a clip cage for 12 h, to allow their settlement on the plant, before adding the predators. Thereafter, the clip cages were removed and 24-h-starved adult predators were added, generating three predator treatments: (1) predation from the foliar forager (one *E. connexa* or one *H. variegata*), (2) predation from the ground forager (one *T. aerea*), and (3) predation from one foliar + the ground forager. Experiments were carried out independently for the combinations including *E. connexa* and *H. variegata*. To accommodate our experiments to the time when each coccinellid species is abundant in the field,

experiments were run between 17 November 2008 and 15 January 2009 (spring-early summer) for the predator combinations including *E. connexa*, and between 16 March 2009 and 14 April 2009 (fall) for those including *H. variegata*. Thus, each experiment followed a  $5 \times 3$  randomized factorial design, with five aphid densities and three predator combinations, and was replicated 10 times.

The experimental arenas were left in a growth chamber at 20 °C and L16:D8, and after 24 h, we removed the predators and counted the remaining aphids on the stems and in the bowls. Predator consumption was estimated by subtracting the final from the initial numbers of aphids in each experimental arena (discounting the newly born aphids).

The effect of aphid density, predator treatment, and their interaction on the number of aphids eaten [ $\log(x + 1)$  transformed] was analysed using a factorial ANOVA. The effect of aphid density on dropping, in the no-predator treatment, was compared with ANOVA. Treatment means for the experiments were compared using Tukey's HSD test ( $\alpha = 0.05$ ).

To determine whether the interaction between the predator species studied was antagonistic, additive, or synergistic, the observed predation when both predators were present was compared with the predation expected following Soluk (1993):

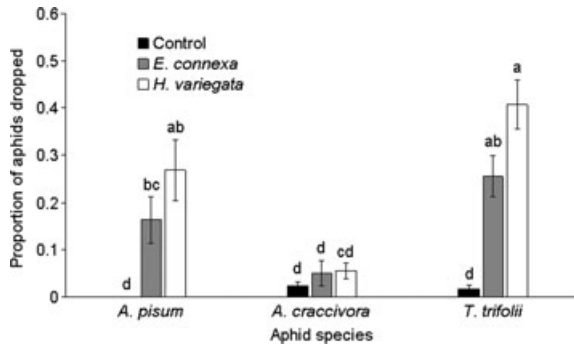
$$C_{fs} = N_{\text{aph}} (P_{\text{coc}} + P_{\text{car}} - P_{\text{coc}} \times P_{\text{car}}), \quad (1)$$

where  $C_{fs}$  is the combined expected number of prey consumed,  $N_{\text{aph}}$  is the initial aphid number, and  $P_{\text{coc}}$  and  $P_{\text{car}}$  are the probabilities of an aphid being consumed by a coccinellid or a carabid, respectively, over the 24-h interaction period (number of aphids consumed/initial aphid number). This equation accounts for lack of independence in capture probabilities, because in finite populations, the capture of one prey by either predator lowers capture probability by the other (Soluk, 1993). Expected and observed values for each prey density were compared with a two-tailed paired t-test, because we did not have any previous information on how these species of coccinellids and carabids would interact (synergistically or antagonistically). All statistical analyses were carried out in STATISTICA 7.0 (StatSoft, 2001) and MINITAB (2000).

## Results

### Dropping behaviour of three aphid species exposed to two coccinellid species

In the presence of both coccinellid species, *T. trifolii* showed the highest frequency of dropping (mean  $\pm$  SEM =  $33.1 \pm 3.6\%$ ), followed by *A. pisum* ( $21.6 \pm 4.0\%$ ) and *A. craccivora* ( $5.3 \pm 1.5\%$ ; aphid species effect:  $F_{2,81} = 22.4$ ,  $P < 0.001$ ). Dropping was also affected by



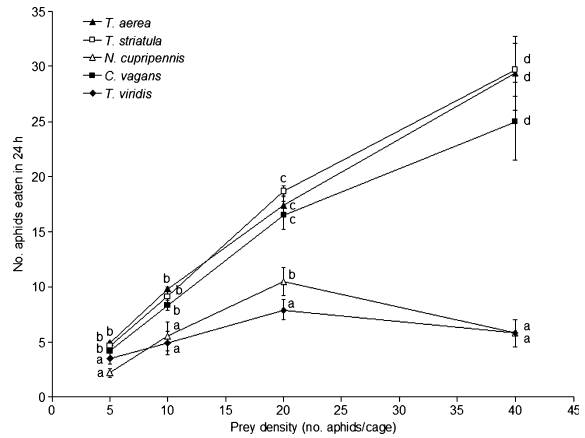
**Figure 1** Proportion of *Acyrtosiphon pisum*, *Aphis craccivora*, and *Therioaphis trifolii* dropping off alfalfa stems in the absence (Control) and presence of two foliar-foraging predators (*Eriopis connexa* and *Hippodamia variegata*). Different letters over bars denote significant differences (Tukey's HSD test:  $P < 0.05$ ).

predator treatment ( $F_{2,81} = 52.8$ ,  $P < 0.001$ ), with few aphids dropping when no predators were present ( $1 \pm 0.4\%$ ), and more aphids dropping in the presence of *H. variegata* ( $24 \pm 3.8\%$ ) than *E. connexa* ( $16 \pm 2.7\%$ ) (Tukey's HSD test:  $P < 0.05$ ). There was also a significant interaction between aphid species and predator treatment ( $F_{4,81} = 8.01$ ,  $P < 0.001$ ), where *A. pisum* and *T. trifolii* followed the general trend described, but *A. craccivora* showed low dropping frequency, even in the presence of the predators (Figure 1).

Predator movement on alfalfa stems during the experiment (1 h) differed marginally between coccinellid species, with *H. variegata* moving on average  $22.6 \pm 1.9$  min and *E. connexa*  $17.1 \pm 2.2$  min (t-test:  $t = -1.96$ , d.f. = 1,  $P = 0.054$ ). Also, there was a positive significant correlation between the proportion of aphids dropped (all three species combined) and coccinellid movement ( $R_s = 0.42$ ,  $P < 0.001$ ).

#### Aphid consumption by five carabid species

All carabid species consumed aphids, but the number eaten varied among predator species ( $F_{4,180} = 51.7$ ,



**Figure 2** Mean ( $\pm$  SEM) number of aphids eaten in 24 h at different prey densities (5, 10, 20, and 40 aphids per cage), by five common carabid species (*Notiobia cupripennis*, *Trirammatus aerea*, *Trirammatus striatula*, *Tretragonoderus viridis*, and *Calosoma vagans*) found in alfalfa fields in central Chile. Different letters denote significant differences between species within the same aphid density (Tukey's HSD test:  $P < 0.05$ ).

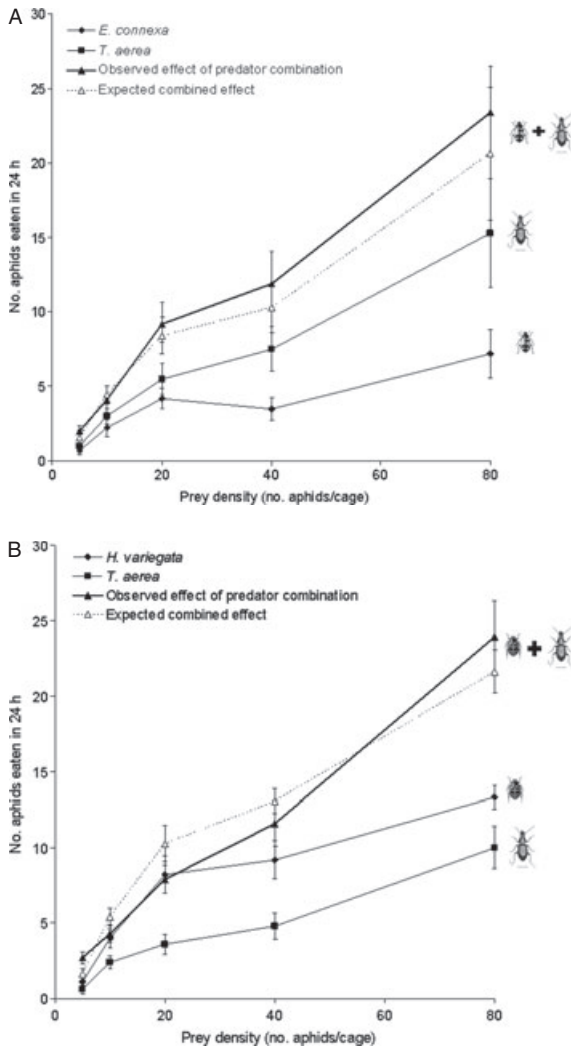
$P < 0.001$ ), aphid density ( $F_{3,180} = 98.2$ ,  $P < 0.001$ ), and their interaction ( $F_{12,180} = 4.7$ ,  $P < 0.001$ ; Figure 2). On average, the species that ate significantly more aphids were *T. striatula*, *T. aerea*, and *C. vagans* (ca. 15 aphids in 24 h), whereas *T. viridis* and *N. cupripennis* ate ca. six aphids, even at high prey densities (Figure 2). *Trirammatus striatula*, *T. aerea*, and *C. vagans* increased their consumption when aphid density increased, up to 25–30 prey items at 40 aphids offered. *Notiobia cupripennis* consumption was highest at a density of 20 aphids per cage. Consumption by *T. viridis* was similar across all prey densities (Figure 2).

#### Effect of prey density on intraguild interactions among foliar- and ground-foraging aphidophagous predators

Aphid consumption was significantly affected by prey density and predator treatment, when both *E. connexa* and *H. variegata* were the foliar predators (Table 1, Figure 3).

**Table 1** Analysis of variance results for the effects of aphid density (5, 10, 20, 40, and 80 individuals per cage), predator treatment, and their interaction on daily aphid predation rate, when *Eriopis connexa* or *Hippodamia variegata* was the foliar predator

Source of variation	d.f.	<i>E. connexa</i>			<i>H. variegata</i>		
		MS	F	P	MS	F	P
Aphid density	4	553.402	2.955	<0.001	643.665	5.311	<0.001
Predator	2	3527.615	18.835	<0.001	3766.152	31.073	<0.001
Density*predator	8	67.113	0.358	0.941	349.123	2.880	<0.001
Error	135	187.289			121.203		



**Figure 3** Mean ( $\pm$  SEM) number of aphids eaten in 24 h by foliar and ground predators under different predator treatments (foliar predator alone, ground predator alone, and both predators in combination) at different *Acyrtosiphon pisum* densities (5, 10, 20, 40, and 80 aphids per cage). Also shown is the expected combined effect of predators based on equation 1. (A) *Trirammatus aerea* with *Eriopis connexa*, (B) *T. aerea* with *Hippodamia variegata*.

For all predators and combinations, aphid consumption increased continuously with aphid density.

In both experiments, predator combination affected aphid consumption (Table 1), with more prey eaten when a foliar- and a ground-foraging predator were combined, than when either predator was alone (Figure 3). When predators were alone, *E. connexa* consumed fewer aphids than *T. aerea* (mean  $\pm$  SEM number of aphids eaten across densities was  $3.6 \pm 0.8$  vs.  $6.5 \pm 1.4$ , respectively; Figure 3A), but *H. variegata* ate more prey than *T. aerea*

( $7.2 \pm 0.8$  vs.  $4.3 \pm 0.7$ , respectively; Figure 3B; Tukey's HSD:  $P < 0.05$  for both). For *H. variegata*, there was a significant density\*predator interaction (Table 1), consuming the same number of prey as when both predators were combined at densities of 10 and 20 aphids per cage. This did not occur when *E. connexa* was used as the foliar predator (Figure 3).

Foliar- and ground-foraging predators interacted in an additive way at all prey densities, because the observed aphid predation when predators were combined was statistically similar to the expected consumption, for experiments with either *E. connexa* or *H. variegata* as the foliar predator (Table 2, Figure 3).

## Discussion

Coccinellids induced aphid dropping, but this behaviour varied according to aphid and coccinellid species, which agrees with previous studies (e.g., Nelson & Rosenheim, 2006). The different aphid dropping behaviour may reflect alternative evolutionary strategies for avoiding predator-induced mortality. On the one hand, species with frequent dropping, such as *T. trifolii* and *A. pisum*, avoid direct contact and mortality from foliar-foraging predators (Francke et al., 2008), although this defensive behaviour has costs (Roitberg & Myers, 1979; Losey & Denno, 1998b; Nelson & Rosenheim, 2006; Nelson, 2007). In species with less frequent dropping, like *A. craccivora* and *Aphis gossypii* Glover (Nelson & Rosenheim, 2006; Grez et al., 2007), aggregation might be a successful strategy to counteract predation from foliar-foraging predators.

Aphid dropping behaviour was altered in the presence of coccinellids, suggesting that this behaviour is a response to predator cues. When no coccinellids were added to the plants, all aphid species presented very infrequent dropping, but when coccinellids were present, *T. trifolii* and *A. pisum* drop more. When pooling the data for all aphid species, a higher dropping behaviour was found in the presence of *H. variegata*, which was more mobile than *E. connexa*. Francke et al. (2008) also observed that more aphids dropped when the coccinellid *Harmonia axyridis* Pallas increased its movement on the plant. The higher dropping in the presence of *H. variegata* may result not only in an increase in predation probability from ground-foraging predators but also in a higher exposure to other negative effects like lower nutrient intake and increased mortality caused by adverse abiotic ground conditions (Roitberg & Myers, 1979; Losey & Denno, 1998b; Nelson & Rosenheim, 2006; Nelson, 2007). This suggests that interaction between carabids and coccinellids should be more intense when *H. variegata* is the foliar-foraging predator. But not all carabid species would represent the



**Table 2** Results of comparisons between observed and expected mean ( $\pm$  SEM) consumption of aphids by *Trirammatus aerea* combined with either *Eriopis connexa* or *Hippodamia variegata* at different densities of *Acyrtosiphon pisum*. All treatments were replicated 10 times. Expected values were obtained from equation 1

Predator combination	Aphid density	Observed	Expected	t	P (two-tailed)
<i>T. aerea</i> – <i>E. connexa</i>	5	2.0 $\pm$ 0.4	1.6 $\pm$ 0.5	0.97	0.36
	10	4.1 $\pm$ 0.9	4.4 $\pm$ 0.6	–0.32	0.76
	20	9.2 $\pm$ 1.5	8.4 $\pm$ 1.2	0.59	0.57
	40	11.9 $\pm$ 2.2	10.3 $\pm$ 1.6	0.64	0.54
	80	23.4 $\pm$ 3.1	20.6 $\pm$ 4.5	0.80	0.45
<i>T. aerea</i> – <i>H. variegata</i>	5	2.7 $\pm$ 0.4	1.6 $\pm$ 0.3	2.05	0.07
	10	4.3 $\pm$ 0.5	5.4 $\pm$ 0.5	–1.34	0.21
	20	7.9 $\pm$ 0.9	10.2 $\pm$ 1.2	–1.88	0.09
	40	11.6 $\pm$ 1.5	13.0 $\pm$ 0.8	–1.00	0.34
	80	23.9 $\pm$ 2.4	21.6 $\pm$ 1.3	0.97	0.35

same risk for aphids. In our study, the number of aphids consumed by carabids varied greatly among species, with *T. striatula*, *T. aerea*, and *C. vagans* consuming significantly more than *T. viridis* and *N. cupripennis*. In general, larger species ate more aphids than smaller species, but this was not always the case. For example, *N. cupripennis* is a large carabid, but had one of the lowest consumption rates. Furthermore, the functional response of carabids to aphid density varied among species, with the three more voracious species increasing their consumption as prey density increased, whereas the other two species showed some increase in consumption only up to 20 aphids.

In the experiments with alfalfa stems, when aphids were presented to the predators alone or in combination, consumption increased with aphid density in all cases, with both coccinellids and the carabid showing a type II functional response. Consumption of aphids when presented on alfalfa stems to predators alone varied according to predator species, independent of aphid density. In the experiment with *T. aerea* and *E. connexa*, run in spring-summer, the carabid ate more aphids than the coccinellid, whereas in the experiment with *T. aerea* and *H. variegata*, in the fall, the coccinellid ate more aphids than the carabid. In the latter experiment, *H. variegata* showed high consumption rates, but also *T. aerea* lowered its consumption when compared with the spring experiment. The different consumption rates of *T. aerea* in the two experiments may be attributed to different physiological states and activities between the times of year. It is also interesting to note that the high aphid consumption by *H. variegata* occurred in spite of aphid dropping behaviour, which if precluded could be even higher, as it has been experimentally demonstrated for *H. axyridis* (Francke et al., 2008). This suggests that *H. variegata* might be efficient in controlling *A. pisum*, but this needs to be demonstrated under field conditions where other factors may modulate its efficiency.

When foliar- and ground-foraging predators were combined, more aphids were consumed than when either of the coccinellid species were alone, which agrees with previous studies with coccinellids and carabids (Losey & Denno, 1998a,b, 1999; Grez et al., 2007). Nevertheless, in these experiments, observed and expected aphid consumption rates (Soluk, 1993) were similar, implying that the interaction between these predators was additive. This contrasts with the observations of Losey & Denno (1998a,b, 1999) who found a synergistic interaction between the coccinellid *Coccinella septempunctata* L. and the carabid *Harpalus pennsylvanicus* DeGeer, using *A. pisum* as a prey. Furthermore, present results partially agree with those of a similar experiment, combining three coccinellid species (*E. connexa*, *H. variegata*, and *A. bipunctata*) with two carabid species (*N. cupripennis* and *M. flavipes*), using *A. craccivora* as a prey (Grez et al., 2007). These authors found that in most combinations, interactions among these predators were additive, and only in two synergistic. All these results suggest that in alfalfa, coccinellids and carabids do not interact negatively, and thus, their combination might not be detrimental for aphid biological control, although this needs to be proven under controlled field experiments.

We predicted that relationships between predators could be mediated by prey density, being antagonistic at low densities, because of competition or intraguild predation, and synergistic as prey density increases. Nevertheless, in this study, the additive relationship found between coccinellids and carabids was not affected by aphid density, even though it varied greatly (between 5 and 80 aphids). This could be explained in part by the low frequency of intraguild predation among the species studied, where only on two occasions, the carabid was found preying on the coccinellids on the ground (once with each species), and it was observed at intermediate prey densities (20 and 40 aphids). Probably, intraguild predation was rare

because these carabids do not fly and were never found foraging on plants, even when it has been described that carabids may occasionally climb into short alfalfa plants (Snyder & Ives, 2001). Additionally, coccinellids were rarely found on the ground, lowering the probability of direct contact with carabids.

In summary, dropping behaviour depended on the aphid and foliar-foraging species, and this behaviour was more frequently observed in the presence of more mobile coccinellid. When foliar- and ground-foraging predators were combined, the interaction was additive at all aphid densities, suggesting that the coexistence of these species might not hinder aphid biological control in alfalfa.

### Acknowledgements

We thank Elizabeth Gazzano, Bárbara Viera, and Francisca Morales for their help in collecting and maintaining the insects in the laboratory, and two anonymous referees who made significant contributions to this paper. This study was financially supported by FONDECYT 1070412.

### References

- Chang GC (1996) Comparison of single versus multiple species of generalist predators for biological control. *Environmental Entomology* 25: 207–212.
- Chang GC & Eigenbrode SD (2004) Delineating the effects of a plant trait on interactions among associated insects. *Oecologia* 139: 123–130.
- Francke DL, Harmon JP, Harvey CT & Ives AR (2008) Pea aphid dropping behaviour diminishes foraging efficiency of a predatory ladybeetle. *Entomologia Experimentalis et Applicata* 127: 118–127.
- Grez AA, Rivera P & Zaviezo T (2007) Foliar and ground-foraging predators of aphids associated with alfalfa crops in Chile: are they good or bad partners? *Biocontrol Science and Technology* 17: 1071–1077.
- Jakobsen L, Enkegaard A & Brødsgaard HF (2004) Interactions between two polyphagous predators, *Orius majusculus* (Hemiptera: Anthocoridae) and *Macrolophus caliginosus* (Heteroptera: Miridae). *Biocontrol Science and Technology* 14: 17–24.
- Losey JE & Denno RF (1998a) Positive predator-predator interactions: enhanced predation rates and synergistic suppression of aphid populations. *Ecology* 79: 2143–2152.
- Losey JE & Denno RF (1998b) Interspecific variation in the escape responses of aphids: effect on risk of predation from foliar-foraging and ground-foraging predators. *Oecologia* 115: 245–252.
- Losey JE & Denno RF (1998c) The escape response of pea aphids to foliar-foraging predators: factors affecting dropping behaviour. *Ecological Entomology* 23: 53–61.
- Losey JE & Denno RF (1999) Factors facilitating synergistic predation: the central role of synchrony. *Ecological Applications* 9: 378–386.
- Lucas E (2005) Intraguild predation among aphidophagous predators. *European Journal of Entomology* 102: 351–364.
- Lucas E, Coderre D & Brodeur J (1998) Intraguild predation among aphid predators: characterization and influence of extraguild prey density. *Ecology* 79: 1084–1092.
- MINITAB (2000) Statistical Software Release 13.3. Minitab, State College, PA, USA.
- Nelson EH (2007) Predator avoidance behaviour in the pea aphid: costs, frequency, and population consequences. *Oecologia* 151: 22–32.
- Nelson EH & Rosenheim JA (2006) Encounters between aphids and their predators: the relative frequencies of disturbance and consumption. *Entomologia Experimentalis et Applicata* 118: 211–219.
- Roitberg BD & Myers JH (1979) Behavioural and physiological adaptations of pea aphids (Homoptera: Aphididae) to high ground temperatures and predator disturbance. *Canadian Entomology* 111: 515–519.
- Rosenheim JA, Wilhoit LR & Armer CA (1993) Influence of intraguild predation among generalist insect predators on the suppression of an herbivore population. *Oecologia* 96: 439–449.
- Snyder WE & Ives AR (2001) Generalist predators disrupt biological control by specialist parasitoid. *Ecology* 82: 705–716.
- Soluk DA (1993) Multiple predator effects: predicting combined functional response of stream fish and invertebrate predators. *Ecology* 74: 219–225.
- StatSoft (2001) STATISTICA: Data Analysis Software System. StatSoft, Tulsa, OK, USA.