SHORT COMMUNICATION

Foliar and ground-foraging predators of aphids associated with alfalfa crops in Chile: Are they good or bad partners?

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Abstract
Carabids and coccinellids are the most abundant aphid predators in alfalfa. Depending on the amount of prey consumed, the impact of their combined effect can be additive, antagonistic, or synergistic. Laboratory trials demonstrated that a higher proportion of *Aphis craccivora* Koch dropped from the alfalfa plants in the presence of coccinellids, and that the interaction between these predators was additive or synergistic, but never antagonistic, suggesting that they might be good partners in controlling aphids.

Keywords: *Aphid consumption, carabids, coccinellids, biological control, predator–predator interactions*

In alfalfa crops (*Medicago sativa* L.), coccinellids and carabids are the most abundant aphid predators, representing important beneficial organisms in agricultural systems (Obrycki & Kring 1998; Kromp 1999; Symondson et al. 2002). They increase in alfalfa under certain cropping conditions, such as a crop distributed in small patches (Grez et al. 2004a, b; Zaviezo et al. 2006). This concomitant increase in the abundance of both kinds of predators may alter their interactions, changing the amount of aphid consumed and potentially affecting the efficiency of biological control.

The impact of the combined effect of several natural enemies can generate three different outcomes: additive, antagonistic, or synergistic (Lucas 2005). If two species of natural enemies do not interact, then their combined effect on the prey population will be additive and equal the sum of their individual impacts. If two species of natural enemies compete for the same prey and one of them also feeds upon its competitor ("intraguild predation", Polis et al. 1989) or interferes with its foraging behavior, the

interaction between natural enemies will be antagonistic and fewer prey than expected will be killed by their combined action. If the foraging activity of one species of natural enemy alters the behavior or feeding niche of the prey, making it more susceptible to attack by another species, more prey will be killed when both species are present than the sum of their individual impacts, a phenomenon named "predator facilitation" (Soluk 1993).

Intraguild predation has been frequently observed among aphidophagous coccinellid species, and it has been suggested as an important force in structuring these guilds (Yasuda & Shinya 1997; Sato & Dixon 2004). But also, predatory aphidophagous guilds may interact synergistically. This occurs when aphids escape from foliar-foraging predators, dropping from their host plant to the ground, where they become more susceptible to attack by ground-foraging predators, resulting in higher consumption rates than when no foliar-foraging predators are present. This phenomenon has been reported particularly for coccinellids (that forage mostly on the foliage), and carabids (which forage on the ground), consuming aphids on alfalfa plants (Losey & Denno 1998a, b, c, 1999). The realism of this phenomenon has been questioned, because this kind of behavior would be maladaptive to aphids under natural conditions (Dixon 2000).

In central Chile, around ten species of coccinellids, both natives and introduced, are commonly found associated with alfalfa crops. Among them, *Eriopis connexa* (Germ), *Adalia bipunctata* (Linnaeus) and *Hippodamia variegata* (Goeze) are the more abundant (Zaviezo et al. 2004, 2006). Carabids are also present throughout the growing season, and the more abundant species in Chile belong to the genus *Agonum*, *Notiobia*, *Crossonychus*, *Feroniomorpha* and *Metius* (Zaviezo et al. 2004).

Because most ground-foraging predators rarely climb on plants and most foliar-foraging predators only occasionally forage on the ground, an antagonistic relationship such as intraguild predation or interference among predators is unlikely (Losey & Denno 1999). Nevertheless, such antagonistic interactions cannot be completely ruled out, because under certain situations coccinellids fall to the ground and carabids forage on plants, allowing physical contact between these predators to occur (Honek 1985; Snyder & Ives 2001).

In the present study, using some of the most common coccinellid and carabid species found in alfalfa crops in Chile, the dropping behavior of *Aphis craccivora* Koch in the presence of foliar-foraging predators, aphid consumption by different predators, and the combined impact of foliar and ground-foraging predators on aphid population, were determined. These laboratory experiments are part of a larger study examining predator guild interactions in alfalfa crops, in order to understand how alfalfa crops can be managed to favor biological control.

The study was conducted in the laboratory with the aphid *Aphis craccivora*, the foliar-foraging predators (Coccinellidae) *Adalia bipunctata*, *Eriopis connexa* and *Hippodamia variegata*, and the ground-foraging predators (Carabidae) *Notiobia cupripennis* (Germ.) and *Metius flavipes* Dej. These are the more abundant species of aphids and predators in alfalfa crops in central Chile, and coexist throughout the growing season (Grez 1997; Grez et al. 2004b; Zaviezo et al. 2004). All predators used in the experiments were adults. They were collected in alfalfa crops near the laboratory, and kept in an insect growth chamber without food for 24 h before the experiments.

Experiments were done following Losey and Denno (1998a, b). Alfalfa plants (15 cm in height) contained in 10-cm diameter black plastic bags, were surrounded by

plastic transparent cylinders (20×15 cm), that were pushed into the ground, sealed along the edges with masking-tape and on the top covered with a fine organdy-mesh, to avoid insect escape. Soil surface was covered with moist clay to facilitate aphid encounter after the experiments.

Thirty aphids (fourth and fifth instar nymphs) were carefully added to the foliage of the alfalfa plant and kept inside a clip cage for 12 h before adding the predators, in order to allow their settlement on the plant. Thereafter, the clip cages were removed and 24 h starved adult predators were added. For the six predator combinations, each run of an experiment consisted of four different treatments run simultaneously, following an additive series design (Soluk & Collins 1988): (1) control, no predator, (2) one coccinellid, (3) one carabid, and (4) one coccinellid and one carabid.

After adding the predators, the experimental plants were put in an insect growth chamber simulating natural conditions (a 12:12 L:D cycle, and 14 and 21°C as minimum and maximum temperatures), for 24 h. After that, predators were removed and the remaining aphids, on the plant and on the soil surface, were counted.

Most of the experiments were run between September and December 2005, with the exception of four experiments run in April 2006, for the *A. bipunctata–A. cupripennis* combination. They were blocked by date, according to the abundance of the coccinellid species in alfalfa crops. Experiments with *A. bipunctata* were carried out over 7 days, from 21 September to 11 April; those with *H. variegata* were run for 10 days, from 4 October to 13 December, and experiments with *E. connexa* were run for 6 days, between 13 December and 28 December, with several replicates per date. In total 10–12 replicates were done for each species combination.

The proportion of aphids that dropped to the ground (no. aphids on the ground/no. aphids on the ground+no. aphids on the plant) was compared between the control and the treatment having only the foliar-foraging predator. The effect of foliar-predator species, foliar-predator presence, and their interaction on aphids dropping from alfalfa plants was analyzed using analysis of variance, with time as block. Data were arcsine-square root transformed prior to analysis.

Aphid consumption was estimated by subtracting the initial and final numbers of live aphids in each experimental plant. The number of aphids remaining in the predation treatments was corrected for lost individuals by its corresponding control, using Abbott's formulae (Abbott 1925). The effect of species combination, predator treatment, and their interaction, on total aphid consumption was analyzed using analysis of variance. As mentioned before, time was considered as a block. Normality was analyzed through the Shapiro–Wilks test (Zar 1996) and treatment means were compared with Tukey tests (Zar 1996).

Finally, to determine if the interaction among predators was antagonistic, additive or synergistic, we compared the observed predation rate for both predators combined with the expected one. Following Losey and Denno (1998a), expected values for each experiment was calculated using the equation (Soluk 1993):

$$C_{fs} = N_{aph} \left(P_{coc} + P_{car} - P_{coc} \times P_{car} \right) \qquad (1)$$

where C_{fs} is the expected combined consumption, N_{aph} is the initial aphid density, and P_{coc} and P_{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 density). This equation assumes lack of independence in capture probabilities (i.e. capture of prey by either predator lowers capture probability by

the other), which is a more realistic scenario than assuming complete independence (Soluk 1993). Because we did not have any previous information on how these species of coccinellids and carabids would interact (synergistically or antagonistically), expected and observed values were compared with a two-tailed paired *t*-test. Additionally, we also observed if intraguild predation occurred. All statistical analyses were made in InfoStat (2004).

Independent of the foliar-foraging predator species, a significantly higher proportion of *A. craccivora* dropped from alfalfa plants when coccinellids were present than when absent ($F_{1,114} = 109.05$, $P < 0.001$), though the proportion that dropped in both cases was very low (mean ± 1 SE, 0.073 ± 0.007 and 0.007 ± 0.002, respectively).

Aphid consumption varied between foliar and ground foraging predators. Coccinellids consumed ~ 12 aphids per day (mean ± 1 SE, *E. connexa*: 11.96 ± 0.80, *H. variegata*: 12.04 ± 0.97, *A. bipunctata*: 11.5 ± 0.82, *M. flavipes*: 5.65 ± 0.53).

For all species combinations, there was a significant effect of predator treatment on daily aphid consumption ($F_{2,172} = 186.42$, $P < 0.001$). Aphid consumption was higher when foliar-foraging predators and ground-foraging predators were combined than when each one was alone. When combined, the daily aphid consumption was 1.3–1.7 times higher than when the foliar-foraging predator was alone, and 2.3–3.8 times higher than when the ground-foraging predator was alone (Figure 1).

The interaction between predators was additive or synergistic, but never antagonistic. When both types of predators were combined, the observed aphid consumption was always similar or higher to the expected if they were acting additively (i.e. no interaction between predators, Figure 1). In four out of six species combinations the interaction was additive given there were no statistical differences between the observed and the expected aphid consumption (paired *t*-test, $P > 0.05$, Figure 1). In the other two combinations the interaction was synergistic, because the observed combined consumption was significantly higher than expected ($P < 0.05$, Figure 1).

No intraguild predation was observed in treatments with both predators, although carabids were found on plants in 10 of the 140 experimental units that included them. On the other hand, coccinellids were never found on the soil.

Dropping behavior of phytophagous insects in the presence of foliar-foraging predators or parasitoids is a well-documented behavioral defense strategy (Gross 1993; Losey & Denno 1998b, c). In our study, *A. craccivora* dropped significantly more when coccinellids were present, independent of the species. Nevertheless, the proportion of falling aphids detected was very low: 7% in presence of coccinellids and less than 1% in their absence. These low percentages contrast with those of Losey and Denno (1998b), where 73% of *Acyrthosiphon pissum* Harris and 42% of *Acyrthosiphon kondoi* Shinji dropped from the plant in the presence of *Coccinella septempunctata* Linnaeus, and less than 3% in their absence. Thus, dropping behavior in *A. craccivora* probably is not as important as for the species studied by Losey and Denno (1998b). Nevertheless, we might have underestimated dropping behavior in this species because we checked this response after 24 h, while Losey and Denno (1998b) did after 2 h. Therefore, aphids may have dropped from plants and then climbed back up, without noticing.

Coccinellids ate two times more aphids than carabids, suggesting that they would be more efficient in controlling aphid populations. This is not an unexpected result, since adult coccinellids, though having a wide dietary range, feed mainly on aphids (Dixon 2000), whereas carabids are typically polyphagous. Also carabids may feed on a variety
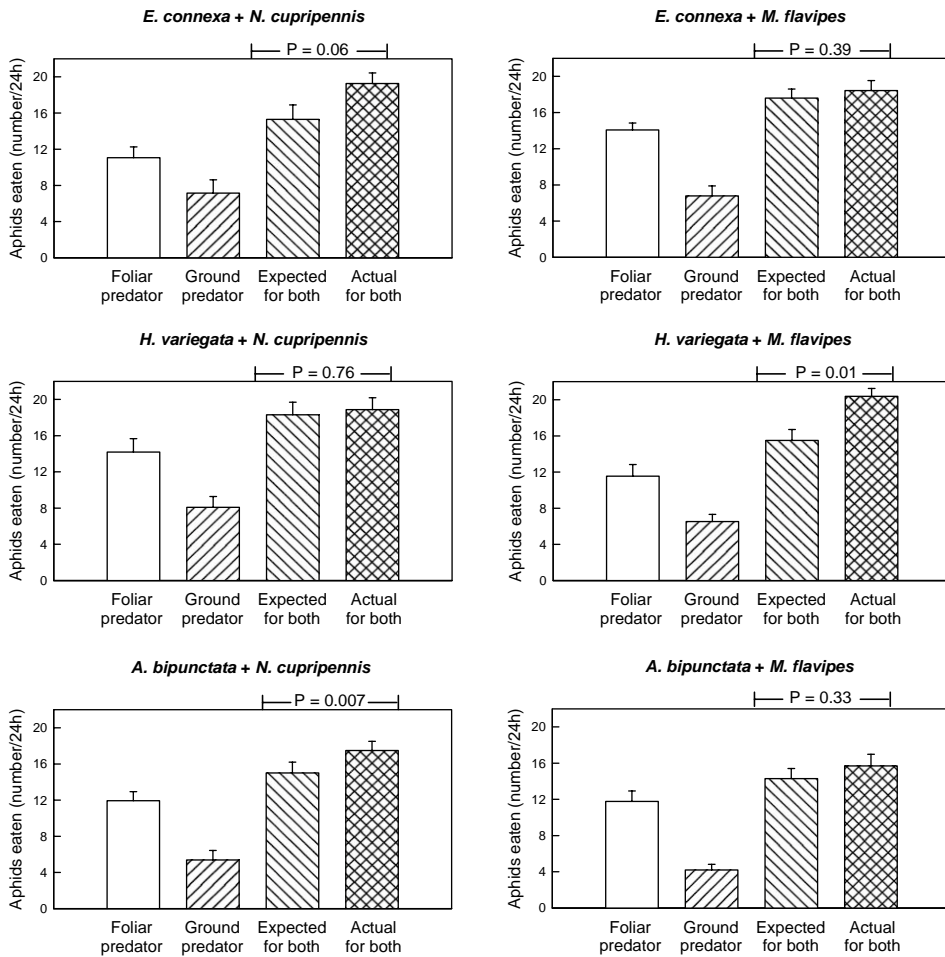
Figure 1. Daily aphid (*A. craccivora*) consumption by foliar-foraging predators (*E. connexa*, *H. variegata* and *A. bipunctata*) and ground-foraging predators (*N. cupripennis* and *M. flavipes*), when acting alone or in combination (expected and actual values). Data are mean±SE, corrected by Abbott (1925). Probabilities resulting from the paired *t*-tests for the difference between expected and actual aphid consumption are shown over the bars.

of plant material (Kromp 1999 and Refs. therein). *Notiobia cupripennis*, one of the species studied here, also consumes weed seeds (Lietti et al. 2000).

In nature, antagonistic interaction between coccinellids and carabids may occur. Even when carabids forage most of the time on the ground, they eventually climb to alfalfa stems where they may interact directly with coccinellids. Also coccinellids, during part of the day move from the foliage down to the ground, depending on the temperature and sunlight intensity (Honek 1985). Also, Snyder and Ives (2001) demonstrated that carabids are able to climb into short alfalfa plants and interact with aphids. In our experiment, that used 15-cm tall plants, on 10 occasions carabids were observed on plants, and this behavior may explain their aphid consumption in the absence of coccinellids. These up and down movement of coccinellids and carabids may have also enhanced competitive interference or intraguild predation. Nevertheless,

during the course of experiments, these activities were never registered, nor did we observe lower aphid consumption when both predators were combined. Instead, for all species combinations, when both predators were present, aphid consumption was higher than when each predator was alone. Moreover, in all the species combinations, predators engaged in additive or synergistic, but never antagonistic, interactions. As Losey and Denno (1998a) pointed out, if predator–predator interactions in a community are largely additive or synergistic, predator complexes would promote stability and deter outbreaks of prey populations. Thus, based on present findings under laboratory conditions, the species of coccinellids and carabids are potentially good partners for the biological control of *A. craccivora* in alfalfa.

The precise mechanism underlying additive/synergistic interactions between these predators remains to be unraveled. Because of our experimental design, we were unable to identify if the higher aphid consumption when both predators were combined was due to an increase in the consumption by coccinellids, carabids or both. Nevertheless, because the increase in aphid consumption when both predators were together was small, but similar to the proportion of aphids dropping to the ground, it seems that dropping behavior could be one of the mechanisms determining the synergistic interactions observed for *H. variegata–M. flavipes* and *A. bipunctata–N. cupripennis*.

Antagonistic interactions may appear at low prey densities (Lucas et al. 1998; Obrycki et al. 1998; Jakobsen et al. 2004), while synergism frequently occurs at higher prey densities (Losey & Denno 1998a; Chang & Eigenbrode 2004). For example, Losey and Denno (1998a) found that at initial densities lower than 40 aphids, like the ones used in our study, 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. Therefore, it is necessary to carry out similar experiments at higher and lower *A. craccivora* densities to test if the type of interaction found here and its strength changes with aphid density.

In the field there are many other factors that may modify interactions among predators, like plant characteristics, habitat configuration or surrounding habitat (Chang & Eigenbrode 2004; Grez et al. 2004a, b; Zaviezo et al. 2006). In experimentally highly fragmented alfalfa crops, coccinellids and carabids are more concentrated in small fragments, which may force them to interact (Grez et al. 2004a; Zaviezo et al. 2006). Therefore, there is a need to test these interactions and their consequences on prey populations in the field, under a more complex but real scenario.

Acknowledgements

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