

## Heteropterans as aphid predators in inter-mountain alfalfa

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**Abstract.** The relationships between predatory Heteroptera and their potential prey species were investigated during two crop seasons in an inter-mountain area close to the Pyrenees (Iberian Peninsula). Regression was used to analyze the potential numerical responses shown by heteropterans to aphids and other potential prey during alfalfa intercrops (the plant growth period between cuts) of high aphid occurrence. The most abundant heteropterans were *Orius* spp., *Nabis* spp. and mirids, and all were present in alfalfa stands throughout the season. *Acyrtosiphon pisum* was the prevalent species during the 2<sup>nd</sup> intercrop, *Therioaphis trifolii* during the 4<sup>th</sup> and *Aphis craccivora* during the 5<sup>th</sup>. We performed simple regression analysis between the *Orius* sp., *Nabis* sp. and mirids and the prevalent aphid species, forward multiple regression analysis between heteropterans and all possible soft-bodied prey species; and both analyses for coccinellids, as relative aphid predator specialists. The heteropterans responded numerically to *A. pisum* but not to *T. trifolii* or *A. craccivora*. Heteropterans also showed numerical responses to other potential prey (leafhoppers, thrips, and other soft bodied prey) that remained at low densities throughout the season. All these preys may contribute to the presence of heteropterans in alfalfa stands throughout the season. The results suggest that heteropterans may contribute to reduce *A. pisum*, particularly at the beginning of the growing season, and on other potential pests when they invade or recolonize alfalfa, and may help to delay or prevent the build-up of these pest populations.

### INTRODUCTION

Alfalfa is the most important fodder crop in Spain and one of the most common crops participating in arable crop rotation, especially in the northeast, where winter cereals and maize are the other main crops.

To determine the relationships between pests and natural enemies in alfalfa fields in Spain, several studies have been carried out since 1994 (e.g. Núñez, 2002; Pons et al., 2005). These studies have dealt with major pest and predator occurrence and seasonal abundance, the role of alfalfa as a reservoir of natural enemies and more specifically the relationships between aphids and natural enemies. Potential insect pests include *Colaspidema atrum* Olivier (Coleoptera: Chrysomelidae), *Hypera postica* Gyllenhal (Coleoptera: Curculionidae), lepidopteran larvae (especially *Helicoverpa armigera* Hübner) and aphids [*Acyrtosiphon pisum* Harris, *Aphis craccivora* Koch and *Therioaphis trifolii* (Monell)]. Some of these pests may cause economic losses and on average 3–4 sprays of non-selective insecticides per season are applied. This extended control strategy can seriously affect natural enemies, leading to an increase in the pest incidence year after year and limiting the role of alfalfa as natural enemy reservoir for field crops. Under low or no insecticide treatment regimes, there are a great variety of natural enemies in the study area and more than 100 predatory species of Heteroptera (Anthocoridae, Nabidae, Miridae, Lygaeidae), Thysanoptera, Neuroptera (Chrysopidae, Hemerobiidae), Coleoptera (Coccinellidae, Carabidae, Staphylinidae, Cantharidae), Diptera (Syrphi-

dae), and several species of spiders and other Arachnida have been recorded (Núñez, 2002; Pons et al., 2005).

Heteropteran predators are characterized as generalist (Schaefer & Panizzi 2000). They are a major component of the arthropod fauna in both natural and managed habitats (Coll & Ruberson, 1998) and they are regarded as relevant natural enemies of pests in agricultural systems (Ruberson & Coll, 1998). Heteropteran predators have been identified among the primary insect predators in alfalfa fields of New York, and in California they constituted about 70% of all heteropterans found in alfalfa (Yeagan, 1998). In Spain, they represent about 50–60% of the predators recorded in alfalfa stands (Pons et al., 2005).

Heteropterans have been successfully used as agents of biological control of pests such as whiteflies, thrips and mites (Albajes & Alomar, 1999; Xu et al., 2006; Arnó et al., 2008), but their role as aphid predators is not clear. Most heteropteran predators are limited in their rate of population growth by relatively long generation times compared with aphids, and there is little evidence that they aggregate in high-density aphid patches. These biological trends hinder the capacity of heteropterans to keep aphid populations in check (Yeagan, 1998). However, Desneux et al. (2006) reported *Orius insidiosus* to be a key predator for suppression of aphids in soybeans. Because heteropterans are very abundant in alfalfa fields and remain there during most of the year (Pons et al., 2005), they may play a role in the regulation of aphid populations. However, there is very little literature dealing with this subject.

This study was carried out in an area which is being transformed to a more intensified agricultural system: permanent pastures are being replaced by crop rotations of arable crops and alfalfa under no-till and integrated pest management systems. This crop system allowed us to investigate the effects that this transformation may have on the relationships between pests and natural enemies. In this paper we examine the relationship between heteropterans and aphids on alfalfa by analysing numerical responses. Because aphids are not the only prey for heteropterans, we also examine predator numerical response to other potential prey species. The fact that no pesticides have been applied to alfalfa since it has been extensively cultivated in the area provides a suitable environment for studying the influence of heteropterans on their potential prey.

## MATERIAL AND METHODS

The study was carried out in an inter-mountain area near the Pyrenees (the Alt Urgell county, Catalonia, Spain). The alfalfa fields were at 700–800 m altitude, with an average rainfall of 650 mm per year and a mean temperature during the alfalfa-growing season of about 17°C. In the region, alfalfa has been extensively cultivated since 2002, when it was introduced as an alternative to natural pastures in the no-till crop rotation that included winter cereal and silage maize (Eizaguirre et al., 2005).

Four and eight fields sown with the cultivar Aragon were monitored in 2006 and 2007, respectively. Crop age ranged from 1 to 3 years, the area averaged 5 ha (range 2–12 ha) and all fields were sprinkler-irrigated. The growing season was from April to mid October and all fields underwent 5 cuttings each year, performed on the same day for all fields each year (Table 1). The fields were free of insecticides during the study years and had not been sprayed previously.

From April/May to September sweep-net samples were collected 2 or 3 times between cuttings (Table 1). Each sample consisted of 5 sweeps of 180° made with a sweep-net of 38 cm diameter. Each field was divided into 4 sectors and 3 samples per sector were collected. Samples were stored in an icebox and transported to the laboratory, where pests and predators were identified, distinguishing nymphs or larvae from adults when possible, and their numbers recorded. In the two years the fields were monitored on the same sampling dates.

Because alfalfa cuttings involve a temporary, but drastic, change to the system, we considered the periods of alfalfa growth between cuts (intercut hereafter) to be separate units: 1<sup>st</sup> intercut corresponding to the period from starting growing season to the 1<sup>st</sup> cutting, 2<sup>nd</sup> intercut to the growing period from 1<sup>st</sup> to the 2<sup>nd</sup> cutting, the 3<sup>rd</sup> intercut from the 2<sup>nd</sup> to the 3<sup>rd</sup> cutting and so on. Relationships between heteropterans and aphids were studied through regression analysis shown by these predators to aphids and other potential prey during intercrops of high aphid occurrence. Correlation studies do not give cause-effect relationships but allow the association between predators and prey to be inferred (Naranjo & Hagler, 1998), and this is an essential step towards more precise studies for estimating and quantifying predator-prey effects. Several components of the predator-prey relationships (numerical and aggregative responses, prey specificity, or even functional response components) may lead to significant immediate or lag correlations (Kidd & Jervis, 1996). To determine whether the response was immediate or delayed, we performed the analysis with no lag and with predator abundance one sampling date lagged throughout.

TABLE 1. Sampling dates and dates when the alfalfa was cut. Within the year all the fields were cut on the same day.

2006		2007	
Sampling dates	Days of cut	Sampling dates	Days of cut
	18 April	19 April	
4 May			24 April
16 May		7 May	
26 May		15 May	
	4 June	30 May	
16 June			19 June
26 June		29 June	
6 July		11 July	
	9 July		17 July
20 July		23 July	
31 July		1 August	
10 August		10 August	
	16 August		21 August
4 September		30 August	
18 September		12 September	
	4 October	27 September	
			9 October

(1) Simple regression analysis between the most abundant heteropterans and the prevalent aphid species.

(2) Forward multiple regression analysis between those heteropterans and all the recorded soft-bodied prey: prevalent and other aphid species, thrips, leafhoppers, larvae of alfalfa weevil *H. postica*, and other soft-bodied prey (lepidopteran larvae, collembolla and planthoppers). Because nabids can also prey on mirids (Lattin, 1989; Braman, 2000), mirids were included in the analysis of nabid numerical response (nymphs and adults for *Nabis* adults and nymphs only for *Nabis* nymphs). The significance level for inclusion of a predictor variable was set at  $\alpha = 0.05$ .

(3) Simple and multiple analyses as above for coccinellids, as relative aphid specialists, in order to compare them with the generalist heteropterans.

All statistical analyses were made using PROC REG (SAS Institute, 2000). Data for statistical analysis were transformed to  $\text{sqr}(x+0.5)$  according to Little & Hills (1972). Simple regression analyses were performed separately each year. In multiple regression analyses the year was included as a covariate and when it was not significant ( $P > 0.05$ ) the analysis was performed again by pooling data from 2006 and 2007. As the catches of nymphs of *Orius* sp. were low (less than 0.5 individuals per 5 sweeps), they were not included in the analysis. Mirids were analyzed as a family because no separation by species or genus was made.

## RESULTS

### Abundance of aphids and other potential prey

Aphids were the most abundant herbivore during the study with percentages of abundance usually higher than 80%, except for the 2<sup>nd</sup> intercut period in 2007 (Table 2). Alfalfa weevil was also common during the 2<sup>nd</sup> intercut and larvae of this species represented nearly 9% and more than 25% of total herbivores collected at that time in 2006 and 2007, respectively. Population densities of the other potential pests were much lower.

Three aphid species were recorded: *Acyrtosiphon pisum*, *Aphis craccivora* and *Therioaphis trifolii*. In 2006, *A. pisum* and *T. trifolii* were the prevalent species,

TABLE 2. Mean (x, in insects\*5 sweeps) and relative (% in percentage) abundance of herbivorous insects during the 2<sup>nd</sup>, 4<sup>th</sup> and 5<sup>th</sup> intercuts of alfalfa and during the whole alfalfa growing season in 2006 and 2007.

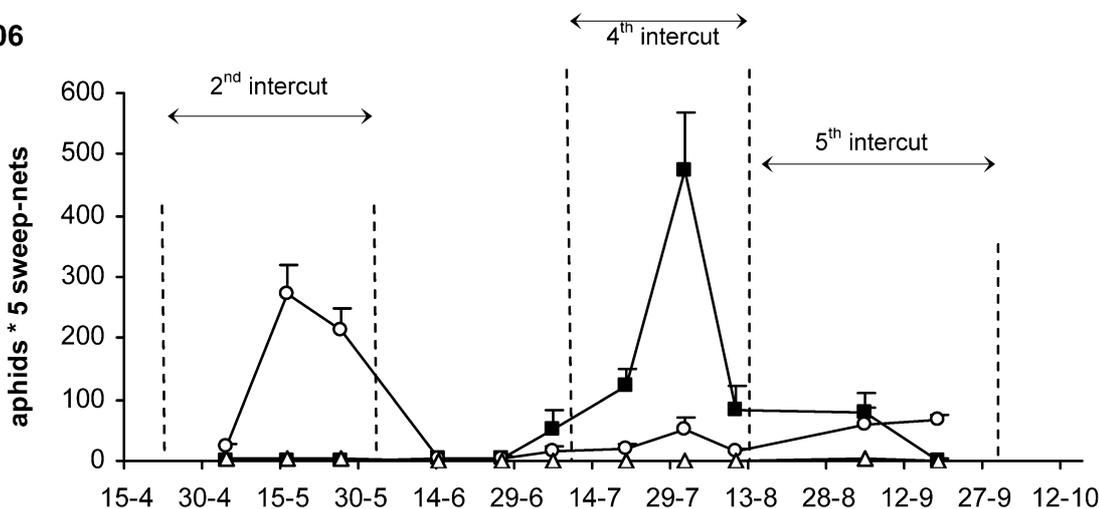
Herbivore	2006 <sup>c</sup>								2007 <sup>d</sup>							
	2 <sup>nd</sup>		4 <sup>th</sup>		5 <sup>th</sup>		Seasonal		2 <sup>nd</sup>		4 <sup>th</sup>		5 <sup>th</sup>		Seasonal	
	x	%	x	%	x	%	x	%	x	%	x	%	x	%	x	%
Aphids	174.6	83.2	255.8	94.8	106.6	86.0	144.9	86.3	38.4	51.1	260.6	91.9	164.6	87.6	122.7	78.4
Alfalfa weevil larvae	18.2	8.7	2.4	0.9	0.28	0.2	6.8	4.0	19.9	26.5	0.17	<0.1	0.1	<0.1	18.6	11.5
Thrips	6.2	2.9	2.3	0.8	1.2	0.9	5.8	3.5	6.0	8.0	7.4	2.6	5.7	3.0	7.6	4.7
Leafhoppers	3.1	1.5	4.5	1.7	5.7	4.6	4.1	2.4	1.2	1.6	6.4	2.3	11.5	6.1	5.5	3.4
Soft-bodied <sup>a</sup>	1.7	0.8	1.7	0.6	3.7	2.9	2.5	1.5	1.2	2.0	2.5	0.9	2.4	1.3	2.8	1.7
Others <sup>b</sup>	6.0	2.9	3.1	1.1	6.6	5.3	3.8	2.3	8.1	10.8	6.4	2.3	3.6	1.9	5.4	3.3

<sup>a</sup>Other soft-bodied insects: planthoppers + Collembola + lepidopteran larvae; <sup>b</sup>alfalfa weevil adults, *Apion* sp., *Sitona* sp., adults of Lepidoptera and other non-soft-bodied insects; <sup>c</sup>values of the 2<sup>nd</sup>, 4<sup>th</sup> and 5<sup>th</sup> intercuts come from the average of 144 samples (3 sampling dates \* 4 fields \* 4 sectors/field \* 3 samples/sector). Seasonal value comes from the average of 528 samples (11 sampling dates); <sup>d</sup>Values of the 2<sup>nd</sup>, 4<sup>th</sup> and 5<sup>th</sup> intercuts come from the average of 288 samples (3 sampling dates \* 8 fields \* 4 sectors/field \* 3 samples/sector). Seasonal value comes from the average of 1152 samples (12 sampling dates).

whereas *A. craccivora* remained at very low levels (Fig. 1). Seasonal abundance of aphids showed 2 peaks: the first one, composed of *A. pisum*, occurred during the second intercut and the second one, formed mainly by *T.*

*trifolii*, occurred during the fourth intercut. In 2007, the pattern of the aphid seasonal abundance was similar until September, when a peak of *A. craccivora* was recorded (Fig. 1).

### A 2006



### B 2007

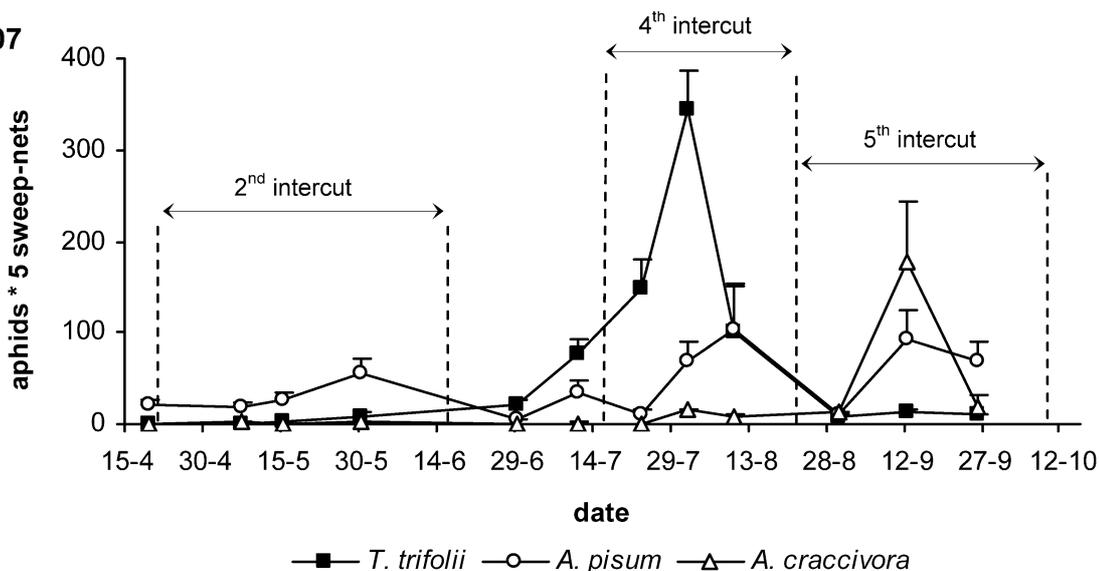
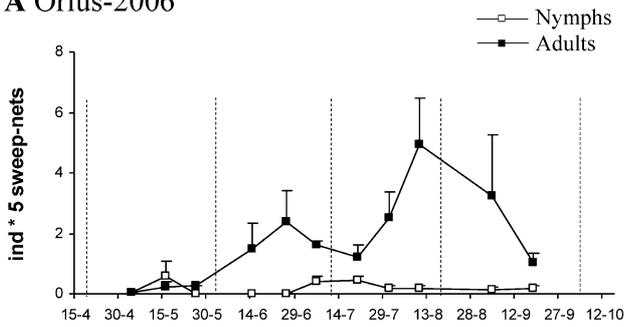
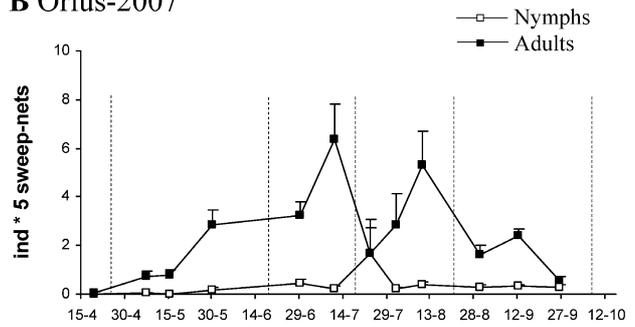


Fig. 1. Seasonal abundance of the prevalent aphid species during the alfalfa growing seasons of 2006 (A) and 2007 (B). Values are means (+ SE). Dashed vertical lines indicate the dates of alfalfa cutting.

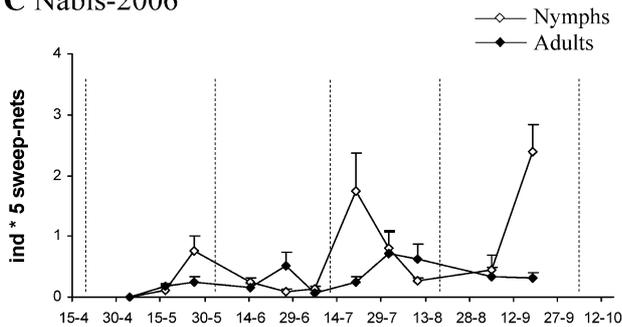
**A Orius-2006**



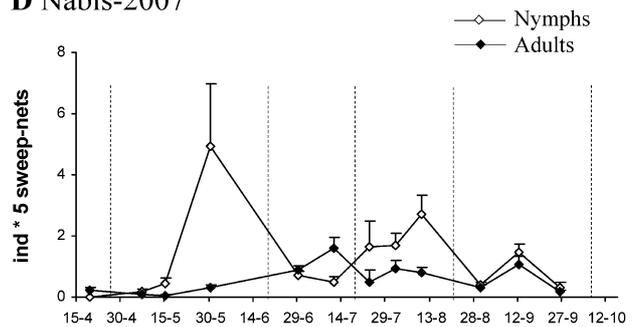
**B Orius-2007**



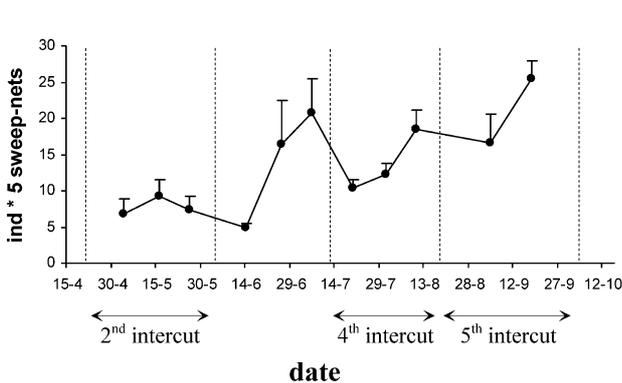
**C Nabis-2006**



**D Nabis-2007**



**E Mirids-2006**



**F Mirids-2007**

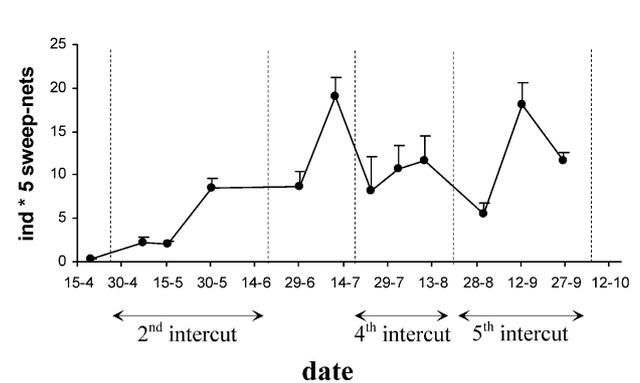


Fig. 2. Seasonal occurrence of Heteroptera predators (*Orius* sp., *Nabis* sp., and Mirids) during the alfalfa growing seasons of 2006 (A, C, E) and 2007 (B, D, F). Values are means (+ SE). Dashed vertical lines indicate the dates of alfalfa cutting. As mirids were not distinguished by species, only the seasonal abundance of all species together is presented.

**Predator abundance**

Heteroptera were the most abundant predatory group and represented nearly 45% of the total predators collected in 2006 and 2007 (Table 3). Predatory thrips were also very abundant, followed by Arachnida and Coleoptera. Among Heteroptera, *Orius* sp. (mainly *Orius niger* Wolf and *Orius majusculus* Reuter), *Nabis* sp. (mainly *Nabis provencalis* Remane) and Miridae were predominant. Coccinellidae were the prevalent coleopteran predators. *Coccinella septempunctata* L. was the most common species in spring, whereas in summer it was *Hippodamia variegata* Goeze. *Sphaerophoria scripta* L. was the most abundant hoverfly species and its abundance peaked in

summer. Several undetermined species of spiders were present during the growing season.

Seasonal abundance of heteropterans shows that they were present throughout the growing season (Fig. 2). Adults of *Orius* spp. were first recorded in mid-April and reached their highest abundance in mid-July and August (during the 3<sup>rd</sup> or 4<sup>th</sup> intercut). However, the number of nymphs recorded was very low, probably because the sweep-net is not an efficient method for *Orius* sp. nymphs. Nevertheless, the results suggested that at least 3 generations of *Orius* spp. occurred. Adults of *Nabis* spp. were first recorded in mid-April and nymphs appeared later. Nymphs peaked three times during the season, first at the end of May (during the 2<sup>nd</sup> intercut), later in mid-summer (during the 4<sup>th</sup> intercut) and finally in September

TABLE 3. Mean (x, in insects\*5 sweeps) and relative (% , in percentage) abundance of predatory insects during the 2<sup>nd</sup>, 4<sup>th</sup> and 5<sup>th</sup> intercuts of alfalfa and during the whole alfalfa growing season in 2006 and 2007.

Predator	2006 <sup>a</sup>								2007 <sup>b</sup>							
	2 <sup>nd</sup>		4 <sup>th</sup>		5 <sup>th</sup>		Seasonal		2 <sup>nd</sup>		4 <sup>th</sup>		5 <sup>th</sup>		Seasonal	
	x	%	x	%	x	%	x	%	x	%	x	%	x	%	x	%
Heteroptera	9.7	48.8	19.1	44.9	20.9	51.7	15.4	46.7	7.1	58.2	12.3	35.5	12.7	49.0	11.3	44.1
<i>Orius</i> sp.	0.4	1.9	3.2	7.5	3.1	7.5	1.9	5.7	1.5	12.5	3.3	9.7	1.8	6.9	2.6	10.1
<i>Nabis</i> sp.	0.4	2.1	1.5	3.5	1.4	3.6	0.8	2.4	2.0	16.5	2.5	7.4	1.3	4.8	1.9	7.1
Miridae	7.8	38.9	13.7	32.2	15.6	38.6	11.8	35.8	3.1	25.7	5.4	15.7	10.0	38.5	6.5	24.8
Others	1.2	5.8	0.7	1.7	0.8	2.0	0.9	2.9	0.4	3.5	1.0	2.8	0.2	0.9	0.5	2.0
Thysanoptera	3.5	17.6	14.2	33.4	9.2	22.7	9.6	29.2	1.7	13.6	10.6	30.6	5.9	22.6	6.7	25.6
Aeolothripidae	3.5	17.6	14.2	33.4	9.2	22.7	9.6	29.2	1.7	13.6	10.6	30.6	5.9	22.6	6.7	25.6
Neuroptera	<0.1	0.3	0.5	1.2	0.4	0.9	0.3	0.8	<0.1	0.1	0.2	0.5	<0.1	0.2	0.1	0.4
Chrysopidae	<0.1	0.3	0.5	1.1	0.4	0.9	0.2	0.8	<0.1	0.1	0.2	0.5	<0.1	0.2	0.1	0.4
Hemeroibiidae	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Coleoptera	4.6	22.9	3.0	7.2	3.3	8.2	3.1	9.5	2.2	17.7	1.9	5.5	2.8	10.9	2.3	8.7
Coccinellidae	4.1	20.5	2.9	6.8	3.1	7.7	2.9	8.6	1.5	12.2	1.9	5.4	2.7	10.3	1.9	7.4
<i>H. variegata</i>	0.2	0.8	1.6	3.8	1.9	4.6	0.9	2.7	0.3	2.3	0.8	2.2	0.7	2.5	0.6	2.2
<i>C. septempunctata</i>	1.2	6.1	0.1	0.2	0.1	0.2	0.5	1.5	0.5	3.7	<0.1	<0.1	<0.1	<0.1	0.2	0.8
Larvae	2.7	13.3	1.1	2.5	1.1	2.6	1.4	4.2	0.7	5.5	0.9	2.7	2.0	7.5	1.0	4.0
Others	<0.1	0.3	0.1	0.3	0.1	0.3	0.1	0.2	0.1	0.8	0.2	0.5	0.1	0.2	0.1	0.4
Cantharidae	0.1	0.6	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	<0.1	<0.1	<0.1	0.1	0.1	0.3	<0.1	<0.1
Staphylinidae	0.3	1.3	<0.1	0.1	0.1	0.2	0.1	0.4	0.6	4.6	<0.1	<0.1	0.1	0.3	0.3	1.0
Carabidae	0.1	0.6	0.1	0.2	0.1	0.2	0.1	0.3	0.1	0.5	<0.1	<0.1	0.1	0.3	<0.1	0.2
Diptera	0.7	3.5	2.9	6.9	4.1	10.2	1.3	4.0	0.2	1.6	5.0	14.5	0.9	3.3	1.5	5.6
Syrphidae	0.7	3.5	2.9	6.9	4.1	10.2	1.3	4.0	0.2	1.6	5.0	14.5	0.9	3.3	1.5	5.6
Arachnida	1.4	6.9	2.8	6.5	2.6	6.4	3.3	9.9	1.1	8.8	4.7	13.5	3.6	14.0	4.0	15.6
Araneae	1.3	6.4	2.8	6.5	2.5	6.0	3.2	9.8	1.1	8.8	4.5	12.8	3.5	13.4	3.9	15.2
Others	0.1	0.5	0.0	0.0	0.1	0.4	<0.1	0.1	0.0	0.0	0.2	0.7	0.1	0.6	0.1	0.4

<sup>a</sup>Values of the 2<sup>nd</sup>, 4<sup>th</sup> and 5<sup>th</sup> intercuts come from the average of 144 samples (3 sampling dates \* 4 fields \* 4 sectors/field \* 3 samples/sector). Seasonal value comes from the average of 528 samples (11 sampling dates). <sup>b</sup>Values of the 2<sup>nd</sup>, 4<sup>th</sup> and 5<sup>th</sup> intercuts come from the average of 288 samples (3 sampling dates \* 8 fields \* 4 sectors/field \* 3 samples/sector). Seasonal value comes from the average of 1152 samples (12 sampling dates).

(during the 5<sup>th</sup> intercut); similarly, three peaks of adults were also evident. These results suggest that *Nabis* spp. had three generations during the growing season. Two main peaks of mirid populations occurred during the 3<sup>rd</sup> and the 5<sup>th</sup> intercuts but other lower peaks were also recorded during the 2<sup>nd</sup> and the 4<sup>th</sup> intercuts.

### Predator-aphid relationships

These relationships were studied during the 2<sup>nd</sup>, 4<sup>th</sup> and 5<sup>th</sup> intercuts, when the aphid populations peaked. Relationships with no or one date lag were studied through the 2<sup>nd</sup> and 4<sup>th</sup> intercuts in 2006 and 2007. For the 5<sup>th</sup> intercut, predator-prey relationships were analyzed only in 2007 since in 2006 no aphid species clearly peaked at that time.

#### Analysis with no lag

In the 2<sup>nd</sup> intercut in 2007, all the heteropteran predators showed positive correlations with the prevalent aphid species, *A. pisum*, but no correlation was observed in 2006 (Table 4a). In 2007, correlations accounted for less than 50% of the observed variation, that of Miridae being the greatest. When the multiple regression analysis was performed, taking into account other prey in addition to *A. pisum*, there was also an effect of year, but different predator-prey relationships appeared and a greater per-

centage of the observed variation was explained (Table 5a). Adults of *Orius* sp. did not correlate with any potential prey in 2006 but in 2007 their numbers increased with numbers of leafhoppers and alfalfa weevil larvae ( $R^2 = 0.65$ ), whereas inclusion of *A. pisum* did not significantly improve the explained variation. Quite a similar pattern was observed with adults of *Nabis* sp.; in this case, the numbers of *Nabis* sp. adults increased with the numbers of leafhoppers and mirid adults ( $R^2 = 0.67$ ). Nymphs of *Nabis* sp. were correlated with thrips in 2006 ( $R^2 = 0.35$ ) but in 2007 showed a good correlation with the other aphid species (*A. craccivora* + *T. trifolii*) in addition to *A. pisum* ( $R^2 = 0.73$ ). No effect of the year was found for Miridae and the numbers of this predatory group increased with those of *A. pisum* and leafhoppers ( $R^2 = 0.59$ ) when data for both years were pooled.

Higher correlations and more consistent annual relationships were found for the specialist aphid predators *C. septempunctata* and for larvae of Coccinellidae (Table 4a). Adults of *C. septempunctata* showed a numerical relationship only with *A. pisum* and not with other potential prey, whereas numbers of coccinellid larvae increased with numbers of *A. pisum* and other soft-bodied prey, but

TABLE 4. Values of  $R^2$  and  $P$  of the no-lag and one sampling date lag regression between the most abundant Heteroptera or Coccinellidae and the prevalent aphid species during the 2<sup>nd</sup> (*A. pisum*), 4<sup>th</sup> (*T. trifolii*) and 5<sup>th</sup> (*A. craccivora*) alfalfa intercrops in 2006 and 2007.

Intercrop	2 <sup>nd</sup>		4 <sup>th</sup>		5 <sup>th</sup>
	<i>A. pisum</i> <sup>a</sup>		<i>T. trifolii</i> <sup>b</sup>		<i>A. craccivora</i> <sup>c</sup>
Prevalent aphid species	2006	2007	2006	2007	2007
Regression parameters	$R^2$ ( $P$ ) <sup>d</sup>	$R^2$ ( $P$ )	$R^2$ ( $P$ )	$R^2$ ( $P$ )	$R^2$ ( $P$ )
(a) No lag					
Heteroptera					
<i>Orius</i> sp. adults	0.05 (NS)	0.29 (<0.01)	0.03 (NS)	<0.01 (NS)	0.10 (NS)
<i>Nabis</i> sp. adults	0.26 (NS)	0.38 (<0.01)	<0.01 (NS)	0.02 (NS)	0.14 (NS)
<i>Nabis</i> sp. nymphs	0.28 (NS)	0.33 (<0.01)	<0.01 (NS)	0.03 (NS)	0.12 (NS)
Mirids Total	0.31 (NS)	0.46 (<0.01)	0.04 (NS)	<0.01 (NS)	0.08 (NS)
Coccinellidae					
<i>C. septempunctata</i>	0.56 (<0.01)	0.55 (<0.01)	–	–	–
Larvae	0.38 (0.03)	0.65 (<0.01)	0.05 (NS)	0.12 (NS)	0.05 (NS)
<i>H. variegata</i>	– <sup>e</sup>	–	0.09 (NS)	0.05 (NS)	0.32 (<0.01)
(b) One sampling date lag					
Heteroptera					
<i>Orius</i> sp. adults	0.03 (NS)	0.02 (NS)	<0.01 (NS)	0.02 (NS)	0.20 (NS)
<i>Nabis</i> sp. adults	0.03 (NS)	0.01 (NS)	<0.01 (NS)	0.03 (NS)	0.21 (NS)
<i>Nabis</i> sp. nymphs	0.71 (<0.01)	<0.01 (NS)	0.44 (NS)	<0.01 (NS)	0.50 (<0.01)
Mirids Total	0.01 (NS)	0.04 (NS)	0.06 (NS)	0.11 (NS)	0.23 (NS)
Coccinellidae					
<i>C. septempunctata</i>	0.07 (NS)	0.12 (NS)	–	–	–
Larvae	0.72 (<0.01)	0.13 (NS)	0.18 (NS)	0.03 (NS)	0.10 (NS)
<i>H. variegata</i>	–	–	0.38 (NS)	0.13 (NS)	0.04 (NS)

<sup>a</sup>2006: no lag, n = 12, one sampling date lag, n = 8; 2007: lag, n = 24, one sampling date lag, n = 16; <sup>b</sup>2006: no lag, n = 12, one sampling date lag, n = 8; 2007: lag, n = 20, one sampling date lag, n = 13; <sup>c</sup>2007: no lag, n = 21; one sampling date lag, n = 14; <sup>d</sup>NS =  $P > 0.05$ ; <sup>e</sup>– = not included in the analysis.

were negatively correlated with numbers of alfalfa weevil larvae.

In contrast, when correlations of Heteroptera with the prevalent aphid species during the 4<sup>th</sup> intercrop, *T. trifolii*, were calculated, no response of any heteropteran or coccinellid to *T. trifolii* were observed (Table 4a). Multiple regression analysis performed, taking into account other potential prey in addition to *T. trifolii*, showed that heteropterans mainly had relationships with prey other than aphids (Table 5b). Adults of *Orius* sp. and nymphs of *Nabis* sp. were numerically related to other soft-bodied potential preys, whereas adults of nabids were related to nymphs of mirids. In all these cases  $R^2$  values were below 0.30 (Table 5b). The numerical response of mirids depended on the year, no response was observed in 2006, but in 2007 numbers of mirids increased with those of thrips and other soft-bodied prey. No numerical relationship between *H. variegata*, the most abundant coccinellid during the 4<sup>th</sup> intercrop, and any of the preys considered could be found. Ladybird larvae were numerically related to leafhoppers but not to aphids.

As in the 4<sup>th</sup> intercrop, heteropterans did not increase their numbers in concert with the prevalent aphid species during the 5<sup>th</sup> intercrop (Table 4a). The multiple regression analysis (Table 5c) showed that numbers of nabid adults increased with mirid nymphs, whereas nabid nymphs were numerically related to leafhoppers and to aphids other than *A. craccivora*, with these two prey groups

explaining a high percentage of the observed variation. Thrips were strongly related to mirids.

*H. variegata*, the most abundant coccinellid during the 5<sup>th</sup> intercrop, increased its population in concert with *A. craccivora* (Table 4a). However, the multiple regression analysis showed that the abundance of *H. variegata* was more related to non-prevalent aphid species (*A. pisum* mainly) and to other soft-bodied herbivores since the inclusion of *A. craccivora* (the prevalent species) in the analysis did not explain any additional variation. Larvae of coccinellids also showed a numerical relationship with thrips and aphids other than the prevalent species (Table 5c).

Analysis with a lag of one sampling date in predator abundance

No significant lag correlations were found between adults of *Orius* sp. and *Nabis* sp. or mirids and *A. pisum* in the 2<sup>nd</sup> intercrop but this kind of relationship appeared for nymphs of *Nabis* sp. in 2006 (Table 4b). When the multiple regression analysis was performed, the year was not significant and the increase in numbers of *Nabis* sp. could not be associated with any potential prey, whereas adults of *Orius* sp. were associated with leafhoppers ( $R^2 = 0.31$ ,  $P = 0.0047$ ) and mirids with larvae of alfalfa weevil ( $R^2 = 0.30$ ,  $P = 0.0060$ ). On the other hand, no lag correlations were found between *C. septempunctata* and aphids but they were found between larvae of Coccinellidae and *A. pisum* ( $R^2 = 0.73$ ,  $P < 0.0001$ ) independently of the year (Table 4b).

TABLE 5. Forward multiple regression analysis for aphid predator abundance. Prey variables are listed in decreasing order of their contribution to the model  $R^2$ . (a) 2<sup>nd</sup> intercut, *A. pisum* was the prevalent aphid species; (b) 4<sup>th</sup> intercut, *T. trifolii* was the prevalent aphid species; (c) 5<sup>th</sup> intercut, *A. craccivora* was the prevalent aphid species.

Dependent variable (Predator)	Independent variables (Prey) included	$R^2$	$P$
(a) 2 <sup>nd</sup> intercut			
Heteroptera			
<i>Orius</i> sp. Adults	Year	0.2630	0.0014
2006	– <sup>c</sup>	–	–
2007	Leafhoppers + alfalfa weevil larvae	0.6466	<0.0001
<i>Nabis</i> sp. Adults	Year	0.1397	0.0078
2006	– <sup>c</sup>	–	–
2007	Leafhoppers + mirid Adults	0.6653	<0.0001
<i>Nabis</i> sp. Nymphs	Year	0.0563	0.0436
2006	Thrips	0.3536	0.0414
2007	Other aphids <sup>a</sup> + <i>A. pisum</i>	0.7286	<0.0001
Mirids Total	<i>A. pisum</i> + leafhoppers	0.5932	<0.0001
Coccinellidae			
<i>C. septempunctata</i>	<i>A. pisum</i>	0.6231	<0.0001
Larvae	<i>A. pisum</i> – alfalfa weevil larvae + soft-bodied <sup>b</sup>	0.7012	<0.0001
(b) 4 <sup>th</sup> intercut			
Heteroptera			
<i>Orius</i> sp. adults	Soft-bodied <sup>b</sup>	0.2737	0.0021
<i>Nabis</i> sp. adults	Mirid nymphs	0.2749	0.0021
<i>Nabis</i> sp. nymphs	Soft-bodied <sup>b</sup>	0.2192	0.0069
Mirids Total	Year	0.2275	0.0058
2006	– <sup>c</sup>	–	–
2007	Thrips + soft-bodied <sup>b</sup>	0.7737	<0.0001
Coccinellidae			
<i>H. variegata</i>	Year	0.1631	0.0219
2006	–	–	–
2007	–	–	–
Larvae	Leafhoppers	0.1994	0.0104
(c) 5 <sup>th</sup> intercut (only 2007)			
Heteroptera			
<i>Orius</i> sp. adults	– <sup>c</sup>	–	–
<i>Nabis</i> sp. adults	Mirid nymphs	0.2528	0.0202
<i>Nabis</i> sp. nymphs	Leafhoppers + other aphids <sup>d</sup>	0.7959	<0.0001
Mirids Total	Thrips	0.6839	<0.0001
Coccinellidae			
<i>H. variegata</i>	Other aphids <sup>d</sup> + soft-bodied <sup>b</sup>	0.6553	<0.0001
Larvae	Thrips + other aphids <sup>d</sup>	0.4460	0.0049

<sup>a</sup>Other aphids: (*A. craccivora* + *T. trifolii*); <sup>b</sup>soft-bodied: planthoppers + Collembola + lepidopteran larvae; <sup>c</sup> – no related prey variables were found; <sup>d</sup>other aphids: (*A. pisum* + *T. trifolii*).

Heteropterans did not show lag correlations with *T. trifolii* during the 4<sup>th</sup> intercut, nor did coccinellids (Table 4b). Multiple regression revealed correlations between adults of *Orius* sp. and leafhoppers ( $R^2 = 0.27$ ,  $P = 0.0155$ ), between nabid nymphs and mirid nymphs in 2007 ( $R^2 = 0.34$ ,  $P = 0.0354$ ), and between mirids and leafhoppers in 2007 ( $R^2 = 0.52$ ,  $P = 0.0056$ ). The coccinellid *H. variegata* was found to be correlated with thrips ( $R^2 = 0.21$ ,  $P = 0.0361$ ) and ladybird larvae were not correlated with any potential prey.

During the 5<sup>th</sup> intercut, adults of *Orius* sp., nabid adults and mirids did not show lagged numerical responses to *A. craccivora* and nabid nymphs showed a negative response (Table 4b). Multiple regression analysis indicated that *Orius* sp., nabid adults and mirids were related to thrips and soft-bodied preys ( $R^2 = 0.60$ ,  $P = 0.0067$ ,  $R^2 = 0.62$ ,  $P = 0.0055$  and  $R^2 = 0.76$ ,  $P = 0.0004$ , respectively) and nabid nymphs to other aphids ( $R^2 = 0.65$ ,  $P =$

0.0005). Coccinellids did not show lag relationships with any potential prey.

## DISCUSSION

Composition and seasonal abundance of aphid species observed during this study closely agree with previous reports from Spain (Pons et al., 2005), although our records come from an inter-mountain area where alfalfa is a relatively recent crop. Two population peaks characterize the aphid seasonal abundance. The first peak occurs in spring during the 2<sup>nd</sup> intercut with *A. pisum* as the dominant species and the second peak occurs during the 4<sup>th</sup> intercut with *T. trifolii* as the dominant species. Aphid densities in these peaks were below the economic thresholds for this region (Pons, 2002). The extremely low densities of *A. craccivora* in 2006 do not allow us to make any inference with respect to the importance of this species as a pest in the study area.

The predator complexes also parallel previous findings in other areas of NE Spain (Pons et al., 2005), where heteropterans are the most abundant predators, especially *Orius* spp., *Nabis* spp. and mirids. In our study, these heteropterans were present in alfalfa stands throughout the season and had at least 3 generations. Alfalfa cutting does not seem to affect them negatively and populations increase or recover quickly after cutting (Fig. 2). This capacity to remain or re-colonize the crop after “catastrophic” agricultural practices, such as cutting, is important for generalist predators (Symondson et al., 2002) and a desirable characteristic for biological control agents in ephemeral crops (Wiedenmann & Smith, 1997).

Density-dependent processes, including the functional and numerical responses, are determinant factors for predator efficacy in controlling pests (Coll & Ridgway, 1995). Whereas several heteropteran predators show type II functional responses to aphids (Foglar et al., 1990; Alvarado et al., 1997; Rutledge & O’Neil, 2005), numerical responses are less well known. Weak numerical responses of heteropteran predators to alfalfa aphids in field studies have been reported by both Elliot et al. (2002) in the USA and Pons et al. (2005) in Spain. Whereas Elliot et al. (2002) described the numerical response of adults of *Nabis americanoferus* Carayon, Pons et al. (2005) did so for adults and nymphs of *N. provençalis* and for adults of *Orius* sp. However, in these reports the whole season and all aphid species were considered together rather than separately for periods in which different aphid species predominated, as done in the present study.

Although significant coefficients of correlation between populations densities do not necessarily imply cause-effect relationships, significant field correlations between predators and prey numbers may give a preliminary indication of a potential impact of the predator on prey (Naranjo & Hagler, 1988). Therefore, our analyses are exploratory and present as a first step towards determining cause-effect relationships.

The results presented here show that heteropterans respond to aphids only in some cases and that the response is immediate rather than lagged. There was no immediate or lagged numerical response of heteropterans to the prevalent aphid species during the 4<sup>th</sup> and the 5<sup>th</sup> intercut. These results agree with a large body of literature on generalist predators, that suggest they are unlikely to impact pests once these increase exponentially (Murdoch et al., 1985; Symondson et al., 2002; Harwood & Obrycky, 2005), and suggest the same is true for heteropterans (Yeagan, 1998; Harwood et al., 2007). However, there was a no-lag positive response for *Orius* sp., *Nabis* sp. and mirids in 2007 and a lag response of *Nabis* sp. nymphs in 2006 to *A. pisum*, the prevalent aphid species at the beginning of the alfalfa growing season, indicating that heteropterans track aphid populations at the beginning of the season when aphid populations are low. This was also recorded by Desneux et al. (2006) and Harwood et al. (2007), who found *O. insidiosus* targeting *A. glycines* early in the season when aphid density was

extremely low and concluded that *O. insidiosus* was a valuable natural enemy of *A. glycines* in soybean agroecosystems. On the other hand, *O. majusculus* and *Orius laevigatus* (Fieber) have been proposed for biological control of *Aphis gossypii* Glover and *Macrosiphum euphorbiae* (Thomas) in cucumbers (Alvarado et al., 1997). However, results of the multiple regression analysis suggest that adults of *Orius* sp. may have a preference for leafhoppers or alfalfa weevil larvae rather than aphids in alfalfa. A similar pattern may be applied for adults of *Nabis* sp. On the other hand, nymphs of *Nabis* sp. showed numerical responses to *A. pisum* in the multiple regression analysis, not only during the second intercut but also during the 5<sup>th</sup>, when it became the main component of the other aphid species complex (Fig. 1). Moreover, there was also a positive correlation with aphids other than *A. pisum* during the 2<sup>nd</sup> intercut. These results emphasize the role of nabids as aphid predators in alfalfa, reported by Elliot et al. (2002) and Pons et al. (2005), especially at low aphid densities. Mirid numbers increased with those of *A. pisum* in the 2<sup>nd</sup> intercut, reflecting mirid aggregation in fields where *A. pisum* occurred. Although the dietary heterogeneity of the mirids in the study may mask the role of these predators in aphid control they should not be discounted as potentially good predators of alfalfa aphids at the beginning of the growing season.

Our results show that heteropteran predators responded numerically to several non-aphid prey, all of which remained in low densities. There were aggregative responses with no lag and a lag of one sampling date of *Orius* sp., *Nabis* sp. and mirids to leafhoppers, thrips and other soft-bodied herbivores. These responses to densities of non-aphid prey favour the continued presence of predators in the crop (Evans & Toler, 2007) in addition to their ability for using some plant food resources. Moreover, there were aggregative responses of adults of *Nabis* sp. to mirids in the three intercrops considered. Because nabids are known to prey on mirids (Lattin, 1989; Braman, 2000), our results suggest that mirids may be an important prey for adults of nabids in alfalfa, and that intraguild predation occurs.

Although numerical responses of coccinellids to aphid density have been reported in alfalfa (Neuenschwander et al., 1975; Frazer et al., 1981; Elliot et al., 2002; Evans & Toler, 2007), we only found it at low aphid densities. Aphids interact with several predatory species, including heteropterans and coccinellids, but also with parasitoids and fungal pathogens which may have great influence in regulating aphid populations in alfalfa when densities are high, as reported by Gutierrez et al. (2008) in California.

In summary, in our study heteropterans were present in alfalfa stands throughout the season, responded numerically to aphids when these were at low density and also responded to several other prey species that remained in low densities throughout the season, alone or together with aphids. These characteristics suggest that heteropterans: (1) May help to delay and prevent the build-up of aphids – particularly *A. pisum* at the beginning of the crop

growing season – and that of other potential pest populations. (2) Respond to the varying local densities of several prey species, which help them to persist in the crop even in periods with no aphids. (3) Are able to remain on alfalfa in spite of cutting, or re-colonize it soon after cutting, and to aggregate on potential pests when they colonize the crop again – a desirable characteristic for biological control in ephemeral crops.

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