

# Relationships between Aphids and Ladybeetles on Alfalfa Stands in Northeastern Spain <sup>†</sup>

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**Abstract:** Aphids are one of the main pests of alfalfa in Spain. The relationship between aphids and ladybeetles has been determined. Alfalfa was sampled by sweep-netting along its productive period in several commercial stands each year between 2010–2020. *Acyrtosiphum pisum* and *Therioaphis trifolii* were the most abundant aphid species, peaking in the 2<sup>nd</sup> and 4<sup>th</sup> intercut, respectively. *Coccinella septempunctata* and *Hippodamia variegata* were by far the coccinellid prevalent species. There were positive correlations between the abundance of *C. septempunctata* and *A. pisum* at the 2<sup>nd</sup> intercut; *H. variegata* and *T. trifolii* at the 4<sup>th</sup> intercut; and *H. variegata* and the total number of aphids in the 5<sup>th</sup> intercut.

**Keywords:** *Acyrtosiphum pisum*; *Therioaphis trifolii*; *Aphis craccivora*; *Coccinella septempunctata*; *Hippodamia variegata*; population dynamics; numerical responses

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## 1. Introduction

Alfalfa, *Medicago sativa* L., is the world's most valuable cultivated forage crop [1]. In Spain, it is a traditional component of crop rotations. Stands usually remain in the field for 3–6 years, being 4 the usual time. It covers more than 250,000 ha, which represents around the 20 % of the alfalfa surface in Europe [2]. Spain is the main European country exporting alfalfa (dehydrated or pellets), especially to the Middle East countries and China [3]. Its management in Spain consists in regular cuttings during its growing season (usually 5 from the end of April to the end of September at intervals of 30–40 days).

Several pests can economically damage the crop: *Hypera postica* (Col., Curculionidae), *Colaspidea barbarum* (Col., Chrysomelidae); *Holotrichapion pisi* (Col., Curculionidae), lepidopteran leaf feeders (several species) and aphids (Hem., Aphididae). Aphids species occurring in Spanish alfalfa stands are *Acyrtosiphon pisum* (Harris), *Aphis craccivora* Koch and *Therioaphis trifolii* (Monell). Their seasonal occurrence, phenology and population dynamics have been previously reported [4–6]. Although the number of insecticide treatments against aphids has been reduced during the last few years, some sprayings are still applied. In order to develop more sustainable aphid control strategies, it is necessary to know which are the natural enemies associated to aphids and their relationships. In this sense, studies on the occurrence and abundance of natural enemies of the alfalfa aphids in northern Catalonia were developed and the relationships between aphids and parasitoids [6,7] and some heteropteran predators [5] were reported.

Relationships between aphids and ladybeetles (Col.; Coccinellidae) were also investigated in Pons et al. [5] reporting that significant numerical relationships only occurred during the growing alfalfa period between the first and the second cutting. The fact that only two growing seasons were considered in the study and data came from a reduced area from northern Catalonia, close to the Pyrenees, could have mediated the results.

Therefore, more information from a wider crop cultivation area is needed in order to determine the ladybeetle species complex, their numerical relationships with the main alfalfa aphid species and their potential role as control agents.

The aims of the present work were: 1) to identify which coccinellid species were present in alfalfa stands; 2) to determine their relative occurrence; 3) to analyse the aphid-coccinellid species associations and 4) to define numerical relationships between the most abundant coccinellids and the aphid complex species in each of the five growing alfalfa periods between cuttings (intercuts in advance; see [5,7]).

## 2. Material and Methods

The study was conducted along the Ebro Valley region, in the NE of Spain, where 60 % and 20 % of the Spanish and European alfalfa surface, respectively, is cultivated.

A total of 93 untreated commercial alfalfa stands were sampled within the period 2010 to 2020 in each of the five intercuts of the growing alfalfa period (from March to September). As the study was part of a more extensive project dealing with the integrated pest management of alfalfa in the region, unequal number of fields were sampled in each of those intercuts, and more data were obtained from the first and the second intercut, when the most damaging pests occurs, *H. postica* and *C. barbarum* [8].

Each field was divided in four sectors and three samples per sector were collected following the central part of one of the main diagonals and at least 25 m apart (following [5]). Samples were taken with a 38 cm diameter sweep net sweeping it from side to side five times in a 180° arc. Samples were placed in an icebox and transported to the laboratory, where they were frozen and stored until aphid and ladybeetle individuals were counted and identified to species level. For each aphid species, all morphs and instars were considered as a whole, whereas larvae and adults of ladybeetles were distinguished. One sample per intercut was obtained. The 1<sup>st</sup> intercut corresponded to the period from the start of the growing season to the 1<sup>st</sup> cutting, the 2<sup>nd</sup> intercut to the period from 1<sup>st</sup> to the 2<sup>nd</sup> cutting, and so on.

### 2.1. Data Analysis

The total number of ladybeetles of each species (adults, larvae and total) and aphids (all stages together) recorded in each sampling point of one field were averaged and the field was considered as a replication. Because alfalfa cuttings involve a temporary but drastic change to the system, the five intercuts were considered as separate units.

The numerical relationship between ladybeetle and aphid species in each intercut were determined by correlation analysis. Shapiro Wilk test was used to check for normality before and after transforming data as  $(\log(x+1))$ . As in any case data followed a normal distribution, the Spearman correlation test was used. No correlation analyses were performed for the 3<sup>rd</sup> intercut, as the aphid abundances were very low.

Statistical analyses were performed by R Core Team (2020).

## 3. Results

### 3.1. Ladybeetle Species and Relative Abundance

Thirteen coccinellid species were recorded: *Coccinella septempunctata* L., *Hippodamia variegata* Goeze, *Propylea quatuordecimpunctata* L., *Scymnus* spp., *Coccinula quatuordecimpustulata* L., *Exochomus nigromaculatus* (Goeze), *Tytthaspis sedecimpunctata* L., *Hyperaspis* sp., *Adalia bipunctata* L., *Subcoccinella vigintiquattuorpunctata* L., *Chilochorus bipustulatus* L., *Psyllobora vigintiduopunctata* L. and *Stethorus punctillum* (Weise). Whereas *C. septempunctata*, *H. variegata*, *P. quatuordecimpunctata*, *C. quatuordecimpustulata*, *Scymnus* sp. and *E. nigromaculatus* are aphidophagous predators, *Hyperaspis* sp. and *C. bipustulatus* are mainly coccidophagous, but have aphids as a second-

ary prey, *T. sedecimpunctata* and *P. vigintiduopunctata* eat on fungus, especially powdery mildew and *S. vigintiquattuor punctata* is herbivorous. *Stethorus punctillum* is a mite predator.

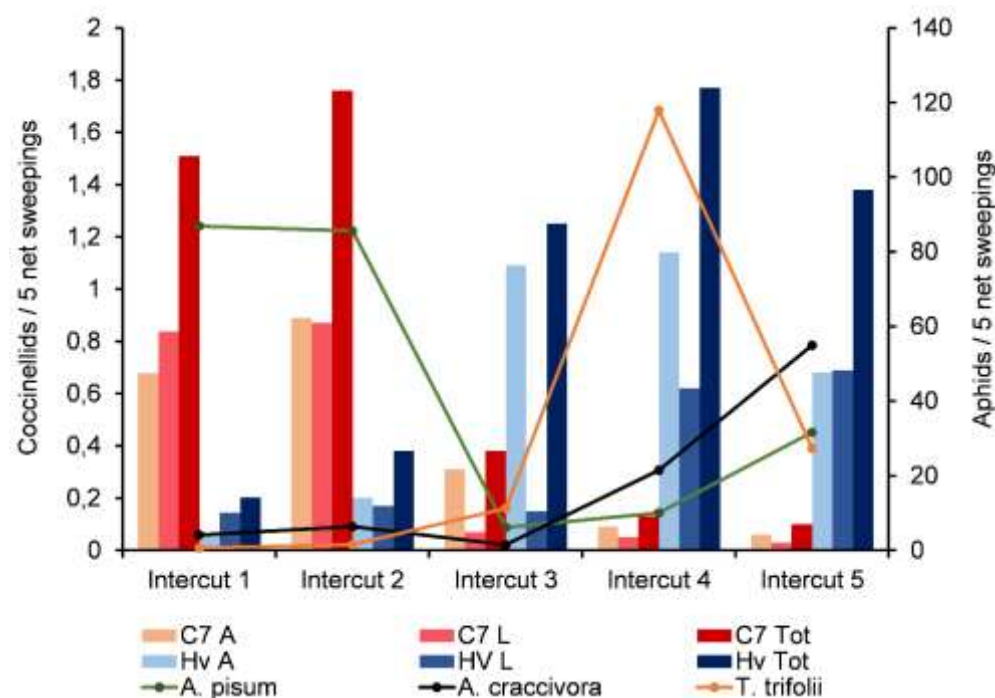
A total of 7428 ladybeetles were collected along the ten-year study. The most abundant species were *C. septempunctata* and *H. variegata*, both species accounting for more than 90 % of the total every year and more than 95 % in the whole study. *Propylea quatuordecimpunctata* and *Scymnus* sp. were also common species. Adults of these four main species were found over the alfalfa growing season, but the presence of *P. quatuordecimpunctata* and *Scymnus* sp. larvae was very scarce. The occurrence of the other species was only sporadic.

### 3.2. Ladybeetle and Aphid Seasonal Occurrence

Based on the relative abundance of the coccinellid species, we restrict the results to *C. septempunctata* and *H. variegata*.

There was a seasonal succession between the occurrence of the two most abundant ladybeetle species (Figure 1). *Coccinella septempunctata* was abundant, mainly during the 1<sup>st</sup> and 2<sup>nd</sup> intercut. Later, the density of adults and larvae decreased considerably. On the contrary, *H. variegata* mainly occurred during the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> intercut. Number of larvae of this species was low in the 3<sup>rd</sup> intercut but it increased considerably during the 4<sup>th</sup> and 5<sup>th</sup> intercut.

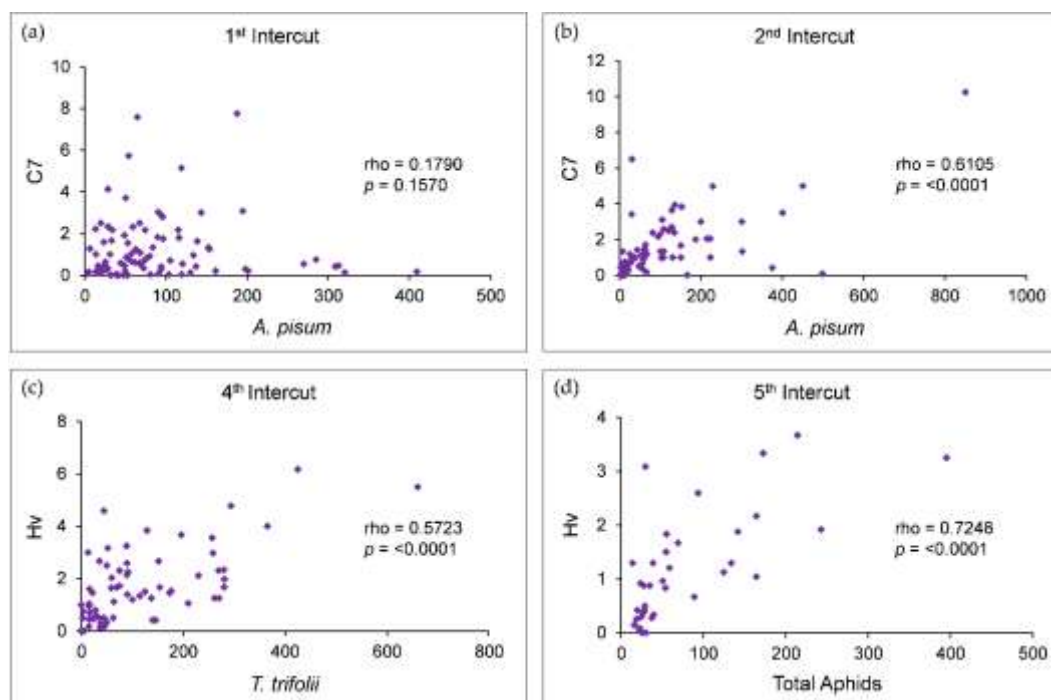
Relatively to the aphid species, the abundance of *A. pisum* reached its highest values during the 1<sup>st</sup> and 2<sup>nd</sup> intercut and coincided quite well with those of *C. septempunctata*. *Therioaphis trifolii* peaked in the 4<sup>th</sup> intercut, just when more *H. variegata* occurred. However, a drastic decrease of its populations was recorded during the 5<sup>th</sup> intercut.



**Figure 1.** Abundance of the two main ladybeetle species (bars), *Coccinella septempunctata* (C7) and *Hippodamia variegata* (Hv) and of the three alfalfa aphid species (lines), *Acyrtosiphum pisum*, *Therioaphis trifolii* and *Aphis craccivora* in each of the 5 intercuts of the growing season. A: adult; L: larvae; Tot: total.

### 3.3. Numerical Relationship between Aphid and Ladybeetle Species

The numerical relationships between the abundance of ladybeetles and aphids was demonstrated with the correlation analysis in three out of the five intercuts. No significant correlations between *C. septempunctata* and *A. pisum* were found in the 1<sup>st</sup> intercut (Figure 2a, Table A1). Conversely, these two variables were positively correlated during the 2<sup>nd</sup> intercut (Figure 2b, Table A1). Similarly, positive correlations between *H. variegata* and *T. trifolii* were found in the 4<sup>th</sup> intercut (Figure 2c, Table A1). Moreover, in this intercut the abundance of *H. variegata* was also positively correlated with the total number of aphids (which includes *A. craccivora* and *A. pisum*). Finally, in the 5<sup>th</sup> intercut, *H. variegata* was positively correlated with *A. craccivora* and again, with the total number of aphids (Figure 2d, Table A1).



**Figure 2.** More representative correlation analyses: (a) *A. pisum* – *C. septempunctata* (1<sup>st</sup> intercut); (b) *A. pisum* – *C. septempunctata* (2<sup>nd</sup> intercut); (c) *T. trifolii* – *H. variegata* (4<sup>th</sup> intercut); (d) Total aphids – *H. variegata* (5<sup>th</sup> intercut). C7: *C. septempunctata*; Hv: *H. variegata*.

## 4. Discussion

Alfalfa is known to be an important reservoir of natural enemies [4,7]. However, studies dealing with the predator/parasitoid-prey relationships of this crop are scarce in Europe. Increasing our knowledge on this area is crucial for the proper management of the agricultural ecosystems. In this ten-year-long study, valuable information of coccinellid-aphid relationships is provided. Thirteen ladybeetle different species were recorded. Within them, *C. septempunctata* and *H. variegata* were the prevalent species. This results concur with those of other studies, which also reported these two species as dominant coccinellids on the alfalfa stands in Europe [4,9]. Regarding their seasonal occurrence, our results show a clear transition between both species. *Coccinella septempunctata* was present on high abundances during the 1<sup>st</sup> and 2<sup>nd</sup> intercut, but then, its number considerably decreased in the 3<sup>rd</sup> intercut, when *A. pisum* populations declined. Ricci et al. [10] reported that, when aphids are scarce, coccinellid adults leave crops searching for new aphid food, which can be a plausible justification for this drop. This is reinforced by Madeira et al. [11] and di Lascio et al. [12], who observed that, other common crops in the Ebro Valley area, such as maize, can have a “sink” effect during its vegetative growing

period (coinciding with the alfalfa 3<sup>rd</sup> intercut) over the alfalfa *C. septempunctata* populations. Summer diapause has also been described as a common trait of Mediterranean *C. septempunctata* populations [13], so it should also be considered as a possible cause for this decrease. More studies are needed in order to elucidate the phenology of this species in the Ebro Valley area.

*Hippodamia variegata* highest abundances were shown during the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> intercut. Its low occurrence during the 1<sup>st</sup> and 2<sup>nd</sup> intercut could be related to its better adaptation for reproduction during summer [14,15]. Moreover, the high abundances of *C. septempunctata*, whose dominance over other coccinellid species has been reported on several studies [16,17], may also have contributed to it. Rondoni et al. [18] recorded intra-guild predation between *C. septempunctata* and *H. variegata*, being asymmetric for the former. Such interactions, could have postponed the proper establishment of *H. variegata* in alfalfa until *C. septempunctata* departure. Although the aphid abundance recorded during the 1<sup>st</sup> and 2<sup>nd</sup> intercut was relatively high, intra-guild predation involving coccinellids has been reported as common even at high extraguild prey densities [19].

Results showed positive correlations between *C. septempunctata* and *A. pisum* during the 2<sup>nd</sup> intercut, which concurs with those of Pons et al. [5]. However, no correlations were found during the 1<sup>st</sup> intercut, when *A. pisum* was equally abundant. Coccinellid aggregative and numerical responses to aphids can be influenced by several factors [20,21], such as the presence of alternative prey [22]. *Hypera postica* is the main pest attacking alfalfa during this intercut [8] and it has been cited several times as an alternative prey for *C. septempunctata* [23,24]. As no significant correlations between *C. septempunctata* and *A. pisum* were found, and because we had data on the *H. postica* different larval instar abundances for the sampled period, some Spearman correlation tests were run, taking into account this data. We found significant positive correlations between the number of *C. septempunctata* adults and that obtained after the addition of *A. pisum* and *H. postica* 4<sup>th</sup> instar larvae abundances.

The positive correlations recorded for *H. variegata* in the 4<sup>th</sup> and 5<sup>th</sup> intercut partially differ from the results of Pons et al. [5], who only reported a positive correlation between this coccinellid species and *A. craccivora* in the 5<sup>th</sup> intercut. In our study, the abundant presence of *T. trifolii* during the 4<sup>th</sup> intercut, may have acted as an attracting and arrestant stimulus for *H. variegata* individuals [25], and thus, led to their positive correlation. During the 5<sup>th</sup> intercut, the dominance of *A. craccivora*, which has been already described as a very suitable prey for *H. variegata* [26], may explain its positive correlation with this aphid species, and thus, with the total number of aphids. The sharp decrease of *T. trifolii* abundance recorded in this intercut could be due to its own phenology but the predation of *H. variegata* and perhaps other specific natural enemies as parasitoids may also have contributed to it.

Our study provides previously unreported coccinellid-aphid positive correlations, such as *H. variegata*-*T. trifolii*, besides new information about the coccinellid complex of alfalfa in the Ebro Valley region. More studies are needed in order to increase the knowledge on the predator-prey interactions of this crop in Europe.

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**Conflicts of Interest:** The authors declare no conflict of interest.

### Appendix A

**Table A1.** Spearman’s correlation coefficients (rho), t –test and p-values between the most abundant aphid species and ladybeetles in four of the five intercuts of the alfalfa growing season (the 3<sup>rd</sup> intercut has not been included due to the low aphid abundance). C7: *C. septempunctata*; Hv : *H. variegata*; A = Adults; L = Larvae.

	<i>A. pisum</i>			<i>T. trifolii</i>			<i>A. craccivora</i>			Total aphids		
	rho	t-test	p-value	rho	t-test	p-value	rho	t-test	p-value	rho	t-test	p-value
1 <sup>st</sup> intercut												
C7 A	0.1889	1.51	0.1349	--	--	--	--	--	--	--	--	--
C7 L	0.1667	1.33	0.1882	--	--	--	--	--	--	--	--	--
C7 (L+A)	0.1790	1.43	0.1570	--	--	--	--	--	--	--	--	--
2 <sup>nd</sup> intercut												
C7 A	0.5648	5.56	<0.0001	--	--	--	--	--	--	--	--	--
C7 L	0.5325	5.11	<0.0001	--	--	--	--	--	--	--	--	--
C7 (L+A)	0.6105	6.26	<0.0001	--	--	--	--	--	--	--	--	--
4 <sup>th</sup> intercut												
Hv A	-0.2031	-1.65	0.1046	0.4093	3.56	0.0007	-0.366	-0.29	0.7762	0.3684	3.15	0.0025
Hv L	0.5260	4.91	<0.0001	0.4654	4.17	<0.0001	0.6036	6.01	<0.0001	0.4858	4.41	<0.0001
Hv (L+A)	0.1004	0.80	0.4263	0.5723	5.54	<0.0001	0,3745	3.21	0.0021	0.5901	5.80	<0.0001
5 <sup>th</sup> intercut												
Hv A	0.3395	2.10	0.0427	0.3818	2.41	0.0216	0.4780	3.09	0.0040	0.5541	3.88	0.0004
Hv L	0.4398	2.86	0.0073	0.0111	0.07	0.9460	0.6518	5.15	<0.0001	0.6310	4.74	<0.0001
Hv (L+A)	0.4433	2.88	0.0068	0.1900	1.13	0.2671	0.7298	6.22	<0.0001	0.7248	6.13	<0.0001

## References

1. Orloff, S.B. Introduction. In *Intermountain alfalfa management*; Orloff, S., Carlson, H., L.R. T., Eds.; University of California. Division of Agriculture and National Resources: Oakland, 1997; pp. 2–3.
2. Delgado, I.; Lloveras, J. Historia y distribución de la alfalfa. In *La alfalfa: agronomía y utilización*; Lloveras, J., Delgado, I., Chocarro, C., Eds.; Edicions de la Universitat de Lleida: Lleida, Spain, 2020; pp. 17–32.
3. Capistrós, J. Comercialización. In *La alfalfa: Agronomía y utilización*; Lloveras, J., Delgado, I., Chocarro, C., Eds.; Edicions de la Universitat de Lleida: Lleida, Spain, 2020; pp. 343–364.
4. Pons, X.; Núñez, E.; Lumbierres, B.; Albajes, R. Epigeal aphidophagous predators and the role of alfalfa as a reservoir of aphid predators for arable crops. *Eur. J. Entomol.* **2005**, *102*, 519–525, doi:10.14411/eje.2005.074.
5. Pons, X.; Lumbierres, B.; Albajes, R. Heteropterans as aphid predators in inter-mountain alfalfa. *Eur. J. Entomol.* **2009**, *106*, 369–378, doi:10.14411/eje.2009.047.
6. Pons, X.; Lumbierres, B.; Comas, J.; Madeira, F.; Starý, P. Effects of surrounding landscape on parasitism of alfalfa aphids in an IPM crop system in northern Catalonia. *BioControl* **2013**, *58*, 733–744, doi:10.1007/s10526-013-9534-y.
7. Pons, X.; Lumbierres, B.; Antoni, R.; Starý, P. Parasitoid complex of alfalfa aphids in an IPM intensive crop system in northern Catalonia. *J. Pest Sci. (2004)*. **2011**, *84*, 437–445, doi:10.1007/s10340-011-0383-0.
8. Pons, X.; Núñez, E. Plagas de la alfalfa: Importancia, daños y estrategias de control. In *La alfalfa: Agronomía y utilización*; Lloveras, J., Delgado, I., Chocarro, C., Eds.; Edicions de la Universitat de Lleida: Lleida, Spain, 2020; pp. 167–202.
9. Jovičić, I.; Radonjić, A.; Petrović-Obradović, O. Aphids (Hemiptera: Aphididae) on alfalfa and their coccinellid predators in Serbia: Seasonal abundance. *Acta Zool. Bulg.* **2016**, *68*, 581–587.
10. Ricci, C.; Ponti, L.; Pires, A. Migratory flight and pre-diapause feeding of *Coccinella septempunctata* (Coleoptera) adults in agricultural and mountain ecosystems of Central Italy. *Eur. J. Entomol.* **2005**, *102*, 531–538, doi:10.14411/eje.2005.076.
11. Madeira, F.; di Lascio, A.; Carlino, P.; Costantini, M.L.; Rossi, L.; Pons, X. Stable carbon and nitrogen isotope signatures to determine predator dispersal between alfalfa and maize. *Biol. Control* **2014**, *77*, 66–75, doi:10.1016/j.biocontrol.2014.06.009.
12. di Lascio, A.; Madeira, F.; Costantini, M.L.; Rossi, L.; Pons, X. Movement of three aphidophagous ladybird species between alfalfa and maize revealed by carbon and nitrogen stable isotope analysis. *BioControl* **2016**, *61*, 35–46, doi:10.1007/s10526-015-9697-9.
13. Katsoyannos, P.; Kontodimas, D.C.; Stathas, G.J. Summer diapause and winter quiescence of *Coccinella septempunctata* (Col. Coccinellidae) in Central Greece. **1997**, *42*, 483–491, doi:10.1007/BF02769808.
14. Ipert, G. The choice of oviposition sites in aphidophagous Coccinellidae. In *Ecology of Aphidophagous Insects*; Hodek, I., Ed.; Academia: Prague, Czech Republic, 1966; pp. 121–122.
15. Mandour, N.S.; Sarhan, A.A.; El-Basha, N.A.; Abdel-Motaal, D.S. Effect of different temperature regimes on the biology, reproduction and predation of *Hippodamia variegata* (Goeze) (Coleoptera: Coccinellidae). *Egypt. J. Biol. Pest Control* **2011**, *21*, 305–312.
16. Obrycki, J.J.; Kring, T.J. Predaceous coccinellidae in biological control. *Annu. Rev. Entomol.* **1998**, *43*, 295–321, doi:10.1146/annurev.ento.43.1.295.
17. Snyder, W.E.; Clevenger, G.M.; Eigenbrode, S.D. Intraguild predation and successful invasion by introduced ladybird beetles. *Oecologia* **2004**, *140*, 559–565, doi:10.1007/s00442-004-1612-5.
18. Rondoni, G.; Ielo, F.; Ricci, C.; Conti, E. Intraguild predation responses in two aphidophagous coccinellids identify differences among juvenile stages and aphid densities. *Insects* **2014**, *5*, 974–983, doi:10.3390/insects5040974.
19. Gardiner, M.M.; Landis, D.A. Impact of intraguild predation by adult *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Aphis glycines* (Hemiptera: Aphididae) biological control in cage studies. *Biol. Control* **2007**, *40*, 386–395, doi:10.1016/j.biocontrol.2006.11.005.
20. Hemptinne, J.L.; Dixon, A.F.G.; Coffin, J. Attack strategy of ladybird beetles (Coccinellidae): factors shaping their numerical response. *Oecologia* **1992**, *90*, 238–245, doi:10.1007/BF00317181.
21. Tulli, M.C.; Carmona, D.M.; Vincini, A.M. Influence of plant diversity on the numerical response of *Eriopis connexa* (Coleoptera: Coccinellidae) to changes in cereal aphid density in wheat crops. *Int. J. Ecol.* **2013**, *2013*, doi:10.1155/2013/789532.
22. Evans, E.W.; Toler, T.R. Aggregation of polyphagous predators in response to multiple prey: ladybirds (Coleoptera: Coccinellidae) foraging in alfalfa. *Popul. Ecol.* **2007**, *49*, 29–36, doi:10.1007/s10144-006-0022-4.
23. Kalaskar, A.; Evans, E.W. Larval responses of aphidophagous lady beetles (Coleoptera: Coccinellidae) to weevil larvae versus aphids as prey. *Ann. Entomol. Soc. Am.* **2001**, *94*, 76–81, doi:10.1603/0013-8746(2001)094[0076:LROALB]2.0.CO;2.
24. Richards, D.R.; Evans, E.W. Reproductive responses of aphidophagous lady beetles (Coleoptera: Coccinellidae) to nonaphid diets: an example from alfalfa. *Ann. Entomol. Soc. Am.* **1998**, *91*, 632–640, doi:10.1093/aesa/91.5.632.
25. Honek, A. Distribution and habitats. In *Ecology and behaviour of the ladybird beetles (Coccinellidae)*; Hodek, I., Van Emden, H., Honek, A., Eds.; Blackwell: Chichester, UK, 2012; pp. 110–140.
26. Pervez, A.; de Holanda Nunes Maia, A.; Bozdoğan, H. Reproduction and demography of an aphidophagous ladybird, *Hippodamia variegata* on six aphid species. *Int. J. Trop. Insect Sci.* **2020**, *541*–548, doi:10.1007/s42690-020-00101-2.