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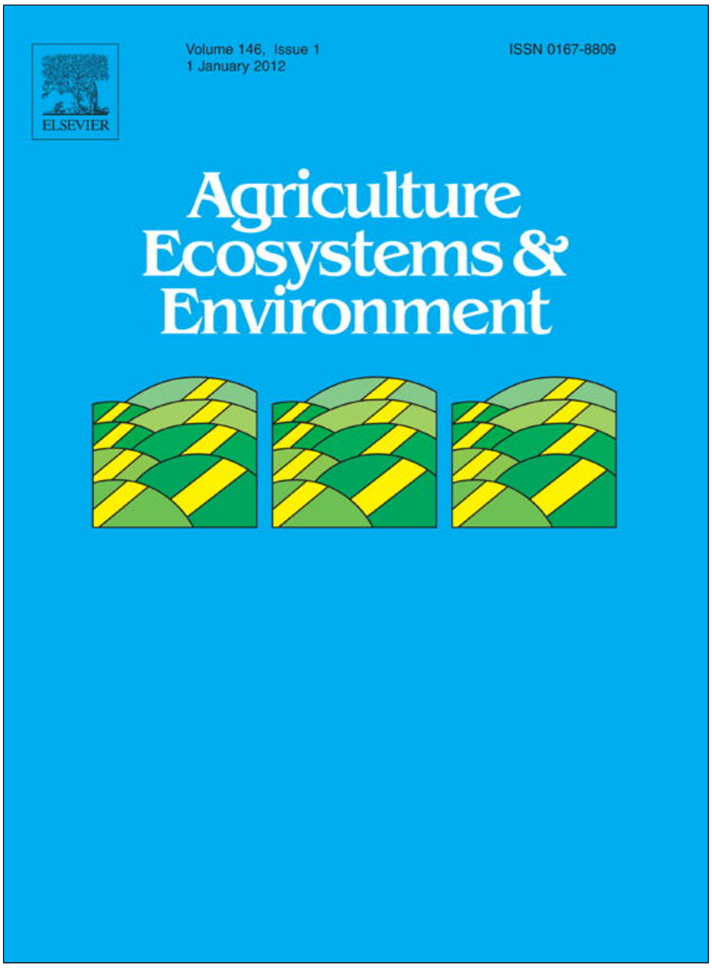

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Agriculture, Ecosystems and Environment 159 (2012) 49–54

Contents lists available at SciVerse ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Short communication

Preliminary study on parasitism of aphids (Hemiptera: Aphididae) in relation to characteristics of alfalfa fields (*Medicago sativa* L.) in the Argentine Pampas

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ARTICLE INFO

Article history:
Received 18 July 2011
Received in revised form 13 June 2012
Accepted 15 June 2012

Keywords:
Conservation biological control
Non-crop vegetation
Natural borders
Crop age
Aphid density

ABSTRACT

Alfalfa is a perennial crop and is one of the most relevant forage resources for cattle in the Argentine Pampas, with aphids (Hemiptera Aphididae), one of the main pest insects in alfalfa, being frequently attacked by a rich fauna of aphidiinae parasitoids (Hymenoptera: Braconidae). The aim of this study was to identify the possible field characteristics that influence the parasitism rates of aphids in alfalfa, in order to recommend simple methods of environmental manipulation to enhance the action of parasitoids. The abundance of aphids and their parasitism were estimated fortnightly, over a period of seven months in fourteen alfalfa fields located near the city of Rafaela, Santa Fe, through the collection of stem cuttings. The influence of field size, age of crop, and percentage of borders with spontaneous vegetation, on aphid abundance and their parasitism rates, were assessed through general lineal models with repeated measures. Greater aphid populations were observed in fields with a low percentage of natural borders, whereas the impact by parasitoids was higher in older crops and in those fields with a high percentage of natural borders. The relative importance of the characteristics of fields on parasitism of aphids is discussed, bearing in mind that conservation is the strategy of biological control recommended for developed countries given its low cost and potential sustainability.

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1. Introduction

In agricultural settings, non-crop habitats adjacent to crop fields provide the natural enemies of insect pests with food, shelter, favorable microclimates, alternative hosts, or a combination of these resources (Costamagna et al., 2004; Thomson and Hoffmann, 2010), thus increasing their populations and generally resulting in improved pest control (Bianchi et al., 2006; Bale et al., 2008). There is extensive theoretical literature predicting that plant biodiversity at field margins could enhance natural enemies and suppress pests (Tylianakis et al., 2004; Landis et al., 2005; Norris and Kogan, 2005; Griffiths et al., 2008; Pisani-Gareau and Shennan, 2010; Letourneau et al., 2011), with several hypotheses having been postulated to explain how vegetation diversity outside fields can directly affect crop pests (see review in Poveda et al., 2008). In fact, many pest populations can be managed by enhancing the efficiency and local abundance of the existing community of natural enemies through modification of the environment, a practice known as conservation biological control (Barbosa, 1998). In order to improve ecosystem services mediated by arthropods, it is necessary to characterize the manipulation of agricultural landscapes that support beneficial arthropods (Isaacs et al., 2009). Increasing landscape heterogeneity is generally associated with a rise in natural enemy abundance, and positive relationships between environmental complexity and rates of parasitism have been documented in many systems (Chaplin-Kramer et al., 2011). However, in spite of evidence published on this topic, general conclusions concerning the relationship between agrobiodiversity and the natural pest control function are still unclear, and need more scientific support before being accepted as a basic principle by farmers (Bianchi et al., 2006).

Perennial crop systems are potentially more favorable to conservation biological control than ephemeral annual systems are, because they are subject to lower levels of disturbance, and as a result, resident populations of natural enemies may persist from year to year (Landis et al., 2000). In the case of alfalfa (*Medicago sativa* L.), it is one of the most well-known and widely used perennial forage crops in the world, with Argentina being the second largest producer of alfalfa in the world, where this crop constitutes one of the most relevant forage resources for cattle (Basigalup and Ustarroz, 2007). More than six million sown hectares are devoted to

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http://dx.doi.org/10.1016/j.agee.2012.06.019

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alfalfa, which are mostly concentrated in the Pampa Region (Central Argentina).

One of the most important pest insects in alfalfa are aphids (Hempitera, Aphididae). Of these, *Therioaphis trifolii* (Monell 1882) and *Acyrthosiphon pisum* (Harris 1776) have been major pests of alfalfa for a long time worldwide, and in recent decades *Acyrthosiphon kondoi* (Shinji 1938) has occupied first place as being the most harmful (Takahashi, 1996). To control aphids, parasitoids of the subfamily Aphidiinae (Hymenoptera: Braconidae) are the most widely used biological agents. These are relative specialized, attacking only a few species of aphids which are taxonomically and biologically related (Tizado Morales et al., 1992). Alfalfa yield has been inversely related to aphid density and also positively with the abundance of natural enemies (Cardinale et al., 2003).

The aim of this study was to analyze the relationship between age of crop, field size and percentage of natural borders with aphid abundance and their parasitism on alfalfa crops. The percentage of parasitism in herbivores is a good estimator for the outcome of biocontrol (Hawkins and Gross, 1992; Hawkins et al., 1993). To evaluate this factor in our study, neither aphids nor parasitoids were discriminated in species, in order to obtain the general levels of parasitism of aphids in an ecologically and taxonomically homogeneous insect community, irrespective of the identity of the occurring species or the strength of particular interactions.

Our strong expectation, based on the "resource concentration hypothesis" (initially proposed by Root, 1973, also see Hambäck and Englund, 2005; Malézieux et al., 2009), was to encounter larger aphid populations in fields with smaller percentages of non-crop plants. In our study site, the alfalfa fields adjoining other alfalfa fields may function as very big monocultures, with the probability of a higher aphid colonization rate and a faster reproduction. Within the framework of the same hypothesis, larger fields were expected to sustain greater aphid populations than smaller ones (Connor et al., 2000).

"Natural enemies hypothesis" (Root, 1973) predicts that in fields with a higher proportion of non-crop plants dispersed among the cultivated ones, the parasitoid populations would be greater. Thus, in fields with a larger percentage of natural borders the impact of parasitoids on aphids would be expected to be higher. Regarding the age of the crops, a higher parasitism is predicted in older ones, since greater stability and lower disturbance levels are known to promote host–parasitoid interactions (Landis et al., 2000).

2. Material and methods

The fourteen studied alfalfa fields were located at INTA (Instituto Nacional de Tecnología Agropecuaria, Rafaela Experiment Station, 31°11′S; 61°29′W), in the west of the province of Santa Fe in the Argentine Pampa region (Table 1). Fields were separated by a minimum of 0.14 and a maximum of 3.93 km. The study site has a typic Argiudoll soil (Soil Survey Staff, 1999; Giorgi et al., 2008), being deep and moderately well drained. The zone is characterized by plains and extended landscapes. The annual average rainfall is 1050 mm (variation WE = 125 mm), distributed with an isohigro regime, with 70% of the rainfall in spring–summer, 23% during autumn and just 7% in winter. The annual mean temperature is 18.0 °C (variation NS = 1.0 °C), with 26.0 °C and 12.7 °C being the means in January and July, respectively, at the hottest and coldest months of the year and with an average thermal amplitude of 13.3 °C (Panigatti, 1980; Panigatti et al., 1982).

In all the plots, the traditional management practice used depended on the growth stage of the alfalfa: grazing (rotary strip up to a total density of 1.5 total cows per ha) on flowering buds, or being cut for hay or silage when 10–20% of flowering was reached (recommended practice for dairy cows) (Comeron and Romero, 2007). LPS 9500, Wl 903, Monarca and GAPP 969 were the varieties used, and no insecticides were sprayed on alfalfa plants during the sampling period.

Table 1
Geographic location of fourteen studied alfalfa fields, indicating their size, age and percentage of borders with natural vegetation. The category in which the fields were assigned, according to their age (Y = young, O = old) and percentage of natural borders (L = low, H = high), is indicated in brackets.

Latitude S	Longitude W	Size (ha)	Age	Natural border (%)
31°11′11.50″	61°30′11.22″	5.8 B	3 (O)	37 (L)
31°11′03.83″	61°30′57.87″	2.1 S	4 (O)	50 (H)
31°12′07.59″	61°30′41.01″	5.0 B	1 (Y)	25 (L)
31°12′17.90″	61°30′34.03″	5.8 B	1 (Y)	25 (L)
31°12′22.19″	61°30′16.10″	2.5 S	1 (Y)	50 (H)
31°12′51.20″	61°29′54.24″	2.3 S	1 (Y)	50 (H)
31°12′15.66″	61°30′44.03″	5.5 B	1 (Y)	0 (L)
31°12′25.20″	61°30′28.04″	6.0 B	5 (O)	25 (L)
31°12′26.96″	61°30′11.47″	2.2 S	2 (O)	25 (L)
31°12′28.68″	61°30′12.09″	2.5 S	4 (O)	50 (H)
31°12′29.58″	61°29′53.56″	2.3 S	4 (O)	50 (H)
31°12′32.68″	61°29′56.26″	3.1 S	3 (O)	50 (H)
31°13′00.15″	61°29′53.45″	3.1 S	1 (Y)	50 (H)
31°13′01.53″	61°30′00.99″	8.0 B	1 (Y)	0 (L)

Every two weeks, from November 2006 to May 2007, 15 alfalfa stems (from 30 to 50 cm each) were collected along both diagonals of each field. Stems sustaining aphids were placed in plastic bags, labeled and transported to the laboratory, where aphids and mummies were then counted. Aphid abundance was calculated as the total number of aphids per stem (irrespective of their parasitization), whereas the percentage of parasitism was calculated from the same samples as the number of mummified aphids per total number of aphids (Chen and Hopper, 1997).

In order to discard spatial effects of the field location on the aphid abundance and parasitism rates, the Mantel Test was performed based on total aphid abundance and parasitism of each field. The matrices of the Euclidean distances of both variables were compared with the matrix of geographic distances of the fields, expressed as the latitude and longitude of the field center (Table 1). These calculations were performed using the program past 2.04.

Three features of alfalfa fields were studied for analyses: age of crop, percentage of borders with spontaneous vegetation, and field size (Table 1). The first two were treated as factors for statistical analysis due to their low variability, whilst field size was treated as a continuous variable (Table 1).

The percentage of borders with vegetation was measured as the percentage of the field perimeter which was limited by strips (4–6 m wide) with spontaneously growing plants. Field bounds without natural vegetation consisted of dirt roads between fields. Alfalfa was the only crop grown. Given the particularities of the system, the higher the percentage of natural borders, the lower the percentage of borders limiting with other alfalfa fields, with the correlation between the two variables (measured as continuous ones) being near 90% (Pearson $r = -0.87$, $P < 0.0001$). Moreover, when these were treated as two level factors, they were completely inversed.

All plant species occurring in the borders of alfalfa fields were identified by comparison with voucher specimens in collaboration with specialists at INTA (see Acknowledgments).

A general linear model with repeated measures was used to investigate the variability of aphid abundance and parasitism rates. In both models, crop age and percentage of natural borders were used as within-subject factors, with field size being included as a continuous variable. The sampling date represented a between-subject factor, and interactions were also investigated. In order to meet the requirements of the model regarding the relation between the number of experimental units and temporal measurements (Tabachnick and Fidell, 2007), only the last ten out of fourteen

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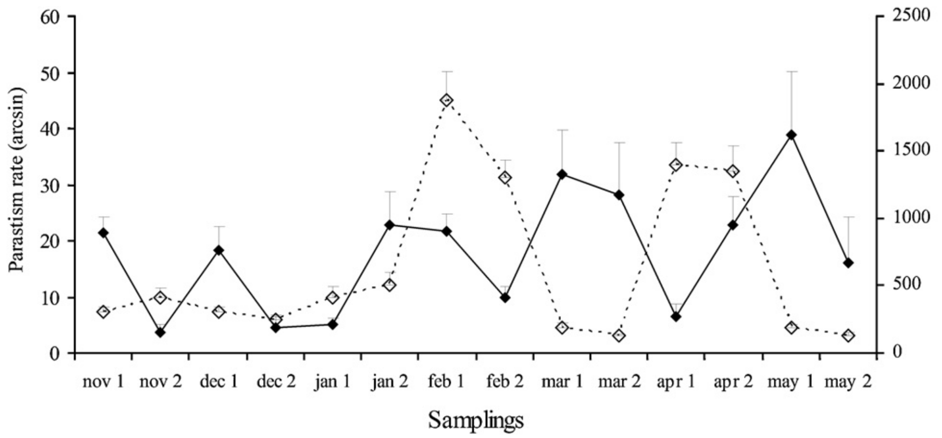

Fig. 1. Average aphid abundance (empty symbols), parasitism rates (filled symbols) and standard errors (vertical bars) observed in 14 alfalfa fields during the sampling period.

sampling dates (January–May) were included. In the parasitism rate model, aphid abundance in the field (average of 14 monthly measures made during the sampling period) was also included as a covariate. However, interactions between within-subject factors were not considered given the low number of fields included in the model. The aphid abundance data were log transformed whereas parasitism rates were arcsin transformed before performing analysis. Both data sets satisfied the assumptions of sphericity (Mauchly Test) and homogeneity of variances (Levene Test) (Potvin et al., 1990), thus the multivariate approach of the analysis was used in both models (Tabachnick and Fidell, 2007). Differences of $P < 0.05$ were considered as significant. Graphs were made from untransformed data to give a better visualization of results. Analyses were performed in SPSS for Windows 17.0.

3. Results

In all, a total of 110,781 aphids and 13,478 parasitoids was observed. Thus, on average, a 12% parasitism was detected on aphids of alfalfa. Five species of aphids, which are commonly associated with alfalfa in different parts of the world (Rakhshani et al., 2009), were observed on alfalfa during the study period: *A. pisum* Harris ("pea aphid"), *A. kondoi* Shinji ("blue-green aphid), *Aphis craccivora* Koch ("cowpea aphid"), *T. trifolii* Monel ("yellow clover aphid") and *Myzus persicae* Sulzer ("green peach aphid"), with the two first mentioned being the most abundant.

Aphid abundance was high in all fields and on each sampling date. This fluctuated between 31 and 62 aphids per stem (mean = 41.70 SE = 2.80 n = 14 fields), with maximum values found in February and April, and minimum ones in March (Fig. 1). The parasitism rates fluctuated between 4% and 25% in the 14 studied alfalfa fields (mean = 11% SE = 2%), with the two maximum average values of parasitism being observed in the first fortnights of March and May (mean = 29% SE = 4 and mean = 28% SE = 5 respectively, n = 14) (Fig. 1).

The Mantel test discarded any effect of spatial location of fields on total aphid abundance (p = 0.70, R = 0.087) or total parasitism rates (p = 0.36, R = 0.041), suggesting that the fields did not represent a single landscape with interacting insect populations.

The repeated measures model indicated that the only factor which significantly affected the abundance of aphids was "natural border", with fields with lower percentages of natural borders showing significantly higher aphid populations (Table 2, Fig. 2). The parasitism rates were higher in fields with a high percentage of natural borders and in crops at least 2 years old (Table 2, Fig. 3). However, field size neither affected aphid abundance nor parasitism rates, and average aphid abundance (treated as covariate) had no effect on the parasitism model (Table 2).

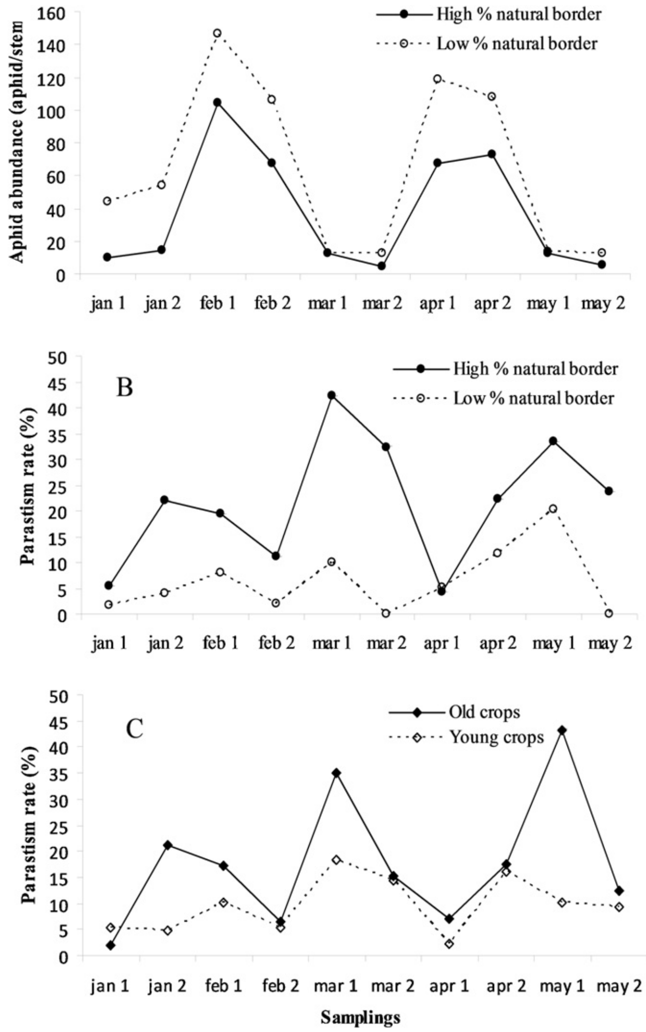

Fig. 2. Estimated means of aphid abundance (A) and parasitism rates (B) in fields with low and high percentages of natural borders; parasitism rates in young and old crops (C). In all cases, according to the general linear model with repeated measures.

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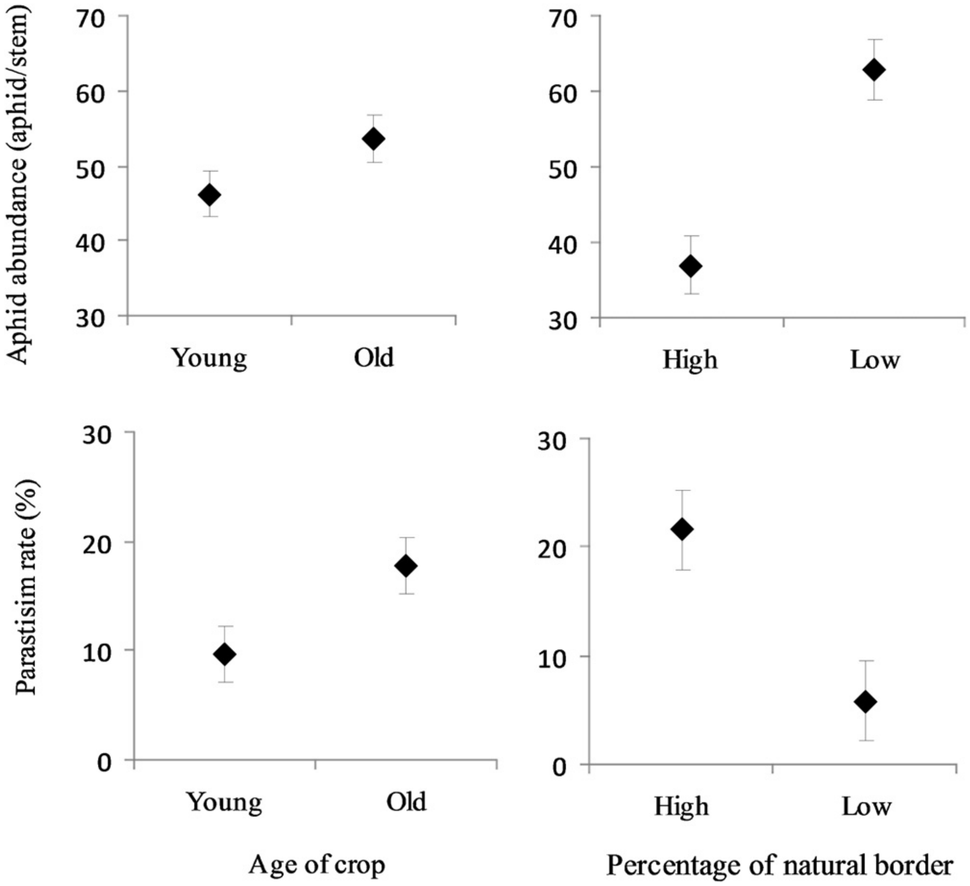

Fig. 3. Estimated means of aphid abundance (aphid/stem) and parasitism rates (%) for the factors studied (field age and percentage of natural borders), according to the general linear model with repeated measures. Aphid abundance was significantly affected by the percentage of natural borders whereas parasitism rates were affected by the percentage of natural borders and crop age (Table 2).

4. Discussion

This study evaluated the influence of different features of alfalfa fields on aphid abundance and their parasitism rates. We chose characteristics of the fields which could be easily changed, as a simple practice of parasitoid conservation.

Aphids were abundant throughout the sampling period, with maximum values occurring in February and April and minimum ones in March. Previous evidence suggests that the aphid population in the field is affected by both climatic conditions (Berberet et al., 1983; Carvalho et al., 1996; Silva et al., 2007) and density-dependent factors such as crowding (Dimeanon, 1977). In our study, an intense rainfall in March may have been the reason for the observed decline in aphid populations. Nevertheless, irrespective of temporal fluctuations during the sampling period, the aphid abundance was significantly higher in fields with low levels of natural borders. As mentioned above, low levels of natural borders were equivalent to high levels of borders adjoining other alfalfa fields. In agreement with the resource concentration hypothesis (which states that herbivorous insects are more likely to be found and to remain in denser and less diverse patches of their host plants, according to Root, 1973, but see also Hambäck and Englund, 2005), the greater abundance of aphids in fields with low percentages of borders and with spontaneous vegetation may be explained by the few limitations imposed on the aphid movement at these sites. In addition, theory predicts that herbivorous insects would be more likely to be found as they would remain in large monospecific stands of their host plants rather than in small or heterogeneous patches (Connor et al., 2000). In our data analysis, the effect of field size "per se" on aphid density was effectively discarded, arguably due to the absence of natural borders in the fields, which by erasing limits between them, allowed fields to function as big monocultures. At our study site, aphid colonization and reproduction may have been facilitated by these large extensions of alfalfa. Roschewitz et al. (2005), in a study of aphids on wheat, observed a similar pattern, with an inverse relationship

Table 2
Repeated-measures GLM model for the effects of sampling date and treatment (field features), and their interactions on aphid abundance and parasitism rates.

	Aphid abundance			Parasitism rates		
	F	df	P	F	df	P
Within-subject effects						
Sampling date	2.11	9,90	0.36	1.43	9,90	0.57
Age × sample date	1.07	9,90	0.57	1.64	9,90	0.54
Size × sample date	0.62	9,90	0.74	0.88	9,90	0.68
Natural border × sample date	0.80	9,90	0.66	0.24	9,90	0.92
Average aphid abundance × sample date	–	–	–	1.53	9,90	0.55
Between-subject effects						
Age	2.56	1,10	0.14	3.14	1,9	0.04
Size	0.51	1,10	0.49	2.49	1,9	0.14
Natural border	20.50	1,10	0.001	11.63	1,9	0.02
Average aphid abundance	–	–	–	2.05	1,9	0.19

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being found between cover of arable weeds in the habitat and aphid colonization.

Aphids were parasitized in every month from December to May, with the peak parasitism rates being observed in March and May. The parasitoids frequently associated with alfalfa aphids in the study region belong to the genera *Lysiphlebus* Förster and *Aphidius* Nees (Hymenoptera: Braconidae, subfamily Aphidiinae) (Zumoffen unpublished data).

Parasitism rates were higher in the fields with a high percentage of natural borders and in older crops. The beneficial effect of these factors on the impact caused by parasitoids on hosts seemed to be direct, instead of being a consequence of an inverse densodependent relationship with aphid abundance, since when host density was included as a covariate, no effect on parasitism was observed. The enemies hypothesis of Root (1973) suggests that increased plant diversity can benefit natural enemies by providing them with shelter (Thomas et al., 1992; Hossain et al., 2002), alternative hosts or prey (Matthews et al., 2004), or with plant-based foods such as nectar and pollen (Wäckers et al., 2007). In this context, the management of weed strips has been advocated for organic crops (Nentwig, 1998). Model simulations of the attractiveness and nectar availability of flowering field margins suggest that the aggregation of parasitoids on flower strips are caused by the prolonged longevity of parasitoids feeding on floral nectar, as well as by the attraction of parasitoids from the surrounding area (Tylianakis et al., 2004; Bianchi and Wäckers, 2008; Vollhardt et al., 2010; Chaplin-Kramer et al., 2011). In the present study, it was found that borders with spontaneous vegetation were composed of a rich community of flowering forbs Appendix A, with previous investigations in the region confirming that no plant species occurring on the borders of alfalfa fields harbored pestiferous aphids of alfalfa (except for one, *Capsella bursa-pastoris*, Zumoffen, unpublished data). In contrast, some species have already been mentioned as hosting innocuous aphids, being alternative hosts for aphidiinae parasitoids. In Spain, *Verbascum* sp. and *Foeniculi vulgare* have been reported to be reservoirs of the parasitoid *L. testaceipes* (Michelena et al., 2004), whereas in our study region, *Ammis majus* harbors alternative prey and hosts for aphid predators and parasitoids (Salto et al., 1993). Finally, the role of non-crop plant species at the borders of alfalfa fields as food or shelter for parasitoids is still not eludicated in the region.

As expected, given the greater stability offered by a resource available over a longer time (Landis et al., 2000), fields aged two years or more displayed higher parasitism in comparison to younger fields. In a similar study dealing with the entomofauna on alfalfa fields, Farigh and Jonsen (1998) observed that age of the crop significantly affected the diversity of species, which was higher in fields of two years than in younger fields. However, as far as we know, there is no data related to aphid parasitism as a function of crop age in the literature.

Our results suggest, at least in the studied landscape, that the increase of natural vegetation at the borders of the fields could be a simple practice to implement in alfalfa fields. Although, in this preliminary study, we did not investigate the mechanisms by which these strips of natural vegetation help to decrease aphid populations and to enhance the parasitism rates, it is probable that borders provide feeding resources (hosts and floral products) and also act as barriers (Holland and Fahrig, 2001; Steingröver et al., 2010), thus avoiding the free colonization by aphids between fields. Crops of different ages harbored similar aphid densities, but significantly higher parasitism rates were observed in older crops. Therefore, keeping the alfalfa plants for a longer period than a year, may also be a recommendable measure to enhance the action of aphid parasitoids on their hosts.

Further research, including the study of particular plant-aphid-parasitoid interactions in this system, will be necessary to improve decision making regarding the design of field margins with spontaneous vegetation in order to provide natural enemies as tools to control crop pests, thereby minimizing insecticide applications, favoring species richness and promoting the impact of enemies on pests.

Acknowledgments

The authors thank Lic. Romina Manfrino, Prof. Silvia Luiselli, Claire Oĭ Riordan and Dr. Paul Hobson, native speaker, for the revision of the manuscript; Lic. Julieta Merke for the identification of plant species; Dr. Miguel Angel Delfino for the identification of aphids; Marcos Gerding and Ana Figueroa (INIA Quilamapu) for their teaching and dedication. Salvo and Zumoffen belong to CONICET.

Appendix A. Plant species in the spontaneous vegetation of alfalfa field borders, indicating their taxonomic families and their most common names.

Plant family	Species	Common names
ACANTACEAE	*Dicliptera tweediana* (Nees)	Coral del monte
ALISMATACEAE	*Sagittaria montevidensis* (Cham. Et Schlecht)	Sagitaria, arrowhead
APIACEAE	*Ammi majus* (L.)	Apio cimarrón, bishopĭs weed
	Ammi visnaga (L.) *Lam.*	Bisnaga
	Foeniculum vulgare (Miller)	Hinojo, common fennel
	Lamiun amplemeanicaule (L.)	Ortiga mansa, henbit
ASTERACEAE	*Baccharis* sp.	Chilca,
	Cirsium vulgare (Savi Ten.)	Cardo negro, bull thistle
	Sonchus oleraceus (L.)	Cerraja, common sowthistle
	Tarameanacum officinale (F.H. Wigg)	Diente de león, dandelion
	Bidens subalternans (D.C.)	Amor seco, greater beggar's-ticks
	Bidens pilosa (L.)	Saetilla, hairy beggarticks
	Matricaria chamomilla (L.)	Manzanilla, chamomilla
	Eupatorium sp.	
	Cichorium intybus (L.)	chicory
	Solidago chilensis Meyen	Vara de oro
	Helianthus petiolaris (Nutt.)	Girasol silvestre, prairie sunflower
BRASSICACEAE	*Brassica* sp.	Nabo, field mustard
	Raphanus sativus (L.)	Nabón, radish
	Capsella bursa-pastoris (L. Medik.)	Bolsa de pastor, shepherd's-purse
BORAGINACEAE	*Echium plantagineum* (L.)	Flor morada, viborera, Paterson's curse
CONVOLVULACEAE	*Ipomoea L. spp*	Campanilla, morning glories
ESCROPHULARIACEAE	*Veronica persica* Poiret	Canchalagua, birdseye speedwell
	Verbascum virgatum Stokes	Polillera
FUMARIACEAE	*Fumaria officinalis* (L.)	Flor de pajarito, fumitory
MALVACEAE	*Sida* L. sp.	Afata, teaweeds
POACEAE	*Zea mays* (L.)	Maiz, corn
POLYGONACEAE	*Muehlenbeckia sagittifolia* (Ortega) (Meisn)	Zarzaparrilla colorada
RANUNCULACEAE	*Clematis dioica* (L.)	Barba de viejo, cabello de angel
VERBENACEAE	*Glandularia peruviana* (L. Druce)	Margarita punzó, peruvian mock vervain, red vervain
	Verbena gracilescens (Cham. Herter)	Verbena, vervain
	Lantana cámara (L.)	Lantana, red sage

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