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Spatio-temporal variation of predatory hoverflies (Diptera: Syrphidae) and their relationship with aphids in organic horticultural crops in La Plata, Buenos Aires

DIAZ LUCAS, María F.[1], PASSARELI, Lilián M.[2], MAZA, Noelia[5], AQUINO, Daniel A.[1,3,4], GRECO, Nancy M.[1] & ROCCA, Margarita[1,*]

[1] CEPAVE (CONICET – UNLP). La Plata, Argentina. *E-mail: mrocca@cepave.edu.ar

[2] Laboratorio de Estudios de Anatomía Vegetal Evolutiva y Sistemática (LEAVES), Facultad de Ciencias Naturales y Museo de La Plata. La Plata, Argentina.

[3] Museo de Ciencias Naturales de La Plata. La Plata, Argentina.

[4] Zoología Agrícola, Centro de Investigación en Sanidad Vegetal, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata. La Plata, Buenos Aires, Argentina.

[5] Facultad de Agronomía y Zootecnia, Universidad Nacional de Tucumán. San Miguel de Tucumán, Argentina.

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Variación espacio-temporal de sírfidos depredadores (Diptera: Syrphidae) y su asociación con áfidos en cultivos hortícolas orgánicos de La Plata, Buenos Aires

RESUMEN. Las variaciones poblacionales de sírfidos depredadores en los agroecosistemas dependen principalmente de los recursos ofrecidos por los cultivos y la vegetación silvestre, así como de la mortalidad causada por sus enemigos naturales. En este trabajo identificamos I) las especies de sírfidos afidófagos en lechuga y brócoli, II) las variaciones estacionales de los estados inmaduros y el parasitismo larval, y III) las plantas aledañas a los cultivos más visitadas. Se registraron los estados inmaduros de los sírfidos y los áfidos colectando quincenalmente 30 hojas al azar en cada cultivo durante 2018-2019. Los adultos de sírfidos registrados por observación directa durante 10 minutos en parches de vegetación silvestre, fueron colectados manualmente y se determinaron las plantas visitadas. Los sírfidos fueron *Allograpta exotica* Wiedemann y *Toxomerus duplicatus* Wiedemann; solamente la primera especie fue registrada en los cultivos. Los áfidos más abundantes fueron *Myzus persicae* (Sulzer) en lechuga y *Brevycorine brassicae* (L.) en brócoli. El parasitismo larval varió entre 8 y 100%, registrándose *Diplazon laetatorius* (Fabricius) en ambos cultivos, y *Pachyneuron aff. nelsoni* solo en brócoli. *Galinsoga parviflora* Cav. y *Matricaria chamomilla* L. fueron las plantas silvestres más visitadas. Estos conocimientos son relevantes para el control biológico de áfidos por sírfidos en el marco del control biológico por conservación.

PALABRAS CLAVE. *Allograpta exotica*. Brócoli. Control biológico por conservación. Lechuga. *Toxomerus duplicatus*.

ABSTRACT. Population variations of predatory hoverflies in agroecosystems depend mainly on the resources that crops and wild vegetation provides them as well as death caused by natural enemies. We identified I) aphidophagous hoverfly species in lettuce and broccoli crops in Buenos Aires, II) the seasonal variations of the immature stages and their larval parasitism, and III) the wild plants, surrounding the crops, visited by adults. Fortnightly, 30 leaves were randomly selected in both crops during 2018-2019 and immature stages of syrphids and aphids *per* leaf were recorded. Adults were manually collected in patches of the

wild plants (during 10 min of direct observations), and plants visited by adults were determined. The hoverflies were *Allograpta exotica* Wiedemann and *Toxomerus duplicatus* Wiedemann. Only *A. exotica* was recorded in crops. The dominant aphids were *Myzus persicae* (Sulzer) in lettuce and *Brevycorine brassicae* (L.) in broccoli. Parasitism rates ranged between 8 and 100% and the species were *Diplazon laetatorius* (Fabricius), in both crops, and *Pachyneuron aff. nelsoni* only in broccoli. *Galinsoga parviflora* Cav. and *Matricaria chamomilla* L. were the wild plants most often visited by hoverflies. This work provides basic information for the control of aphids by hoverflies in the framework of conservation biological control.

KEYWORDS. *Allograpta exotica*. Broccoli. Conservation biological control. Lettuce. *Toxomerus duplicatus*.

INTRODUCTION

Predatory hoverflies are relevant in agricultural systems because they provide two very important ecosystem services. Adults are pollinators, feeding on nectar and pollen, meanwhile syrphid larvae are biological control agents of agricultural pests. As such, hoverflies provide significant pollination services to wild flowers and crops (Dunn et al., 2020; Rodríguez-Gasol et al., 2020).

Regarding their role as biological control agents, the syrphid larvae, important natural enemies in agriculture, are largely found in the subfamily Syrphinae which has more than 1,800 species (Rojo et al., 2003). They feed on soft-bodied Hemiptera, mainly aphids, although some species can also feed on other arthropods such as thrips, whiteflies mites, young lepidopteran larvae, and psyllids (Rojo et al., 2003, Villa et al., 2016). Aphidophagous larvae are considered good biological control agents in several crops because of their voracity (Nelson et al., 2012; van Lenteren, 2012; Gomes Fidelis et al., 2018).

In Argentina, there are studies of taxonomy and biology of several species, mostly from Mendoza, Tucumán, Santa Fe and Entre Ríos (Greco, 1995; Driutti, 1999; Bertolaccini et al., 2008) and recently, more attention about their role as biological control agents has increased (López García & Maza, 2013; Maza et al., 2016; Díaz & Maza, 2017). In the province of Buenos Aires, Greco (1995) studied the phenology and habitat selection of 6 aphidophagous hoverflies species and found that they were present throughout the year, and distributed along an environmental mosaic with different crops and natural vegetation.

Plant diversity management is a technique of conservation biological control which consists of environmental manipulation to improve the effectiveness of established natural enemies (Barbosa, 1998). In addition to this, habitat management can be considered as a conservation biological control method that alters habitats to improve availability of the resources required by natural enemies for optimal performance, conserving and enhancing favorable conditions or reducing unfavorable ones (Landis et al., 2000). Adult hoverflies need pollen, rich in protein, to mature the reproductive system in both females and males, and also females use pollen for eggs development (Gilbert, 1981; Haslett, 1989). In turn, flower nectar is an important energy resource both for flight and for the survival of adult hoverflies (Haslett, 1989). Floral resources and wild plants, that provide food and refugia to the hoverflies both within and around crops, are essential for their role as biological control agents (Pineda, 2011; van Rijn et al., 2013).

Moreover, one negative aspect for biological control is the presence of natural enemies of the control agents (Mills, 2006). Aphidophagous syrphids larvae are parasitized by Hymenoptera species of the superfamilies Ichneumonoidea and Chalcidoidea. Parasitoid pressure can decrease hoverfly larval rate of predation on prey, and consequently could limit the biological control provided by them (Tinkeu & Hance, 1997; Hazell et al., 2005). However, the impact of parasitoids on hoverfly populations has not been widely studied, and in general, is highly variable ranging from nil to more than 50% of parasitization rates (Rodríguez-Gasol et al., 2020).

Aphids are a common pest in horticultural crops (van Emden & Harrington, 2017). Aphid predators and parasitoids are used as biological control agents through different strategies worldwide to control aphid populations. In horticultural systems, hoverflies are used through augmentative and conservation biological control, mainly in Europe (van Lenteren, 2012; van Lenteren et al., 2018). In Argentina, the knowledge of the biology and ecology of the most frequent species in this system is still incipient (Maza et al., 2016; Díaz & Maza, 2017; Díaz et al., 2020).

The aims of the current study were: a) to determine the most common predatory hoverfly species in organic crops of lettuce and broccoli in the horticultural belt of La Plata, b) to describe the numerical variations of immature hoverflies throughout the year and their relationship with the aphids' abundance, c) to analyze the percentage of larval parasitism, and d) to identify wild plants species near to these crops that are frequently visited by hoverflies.

MATERIAL AND METHODS

This study was developed in one commercial horticultural farm located in Etcheverry, locality belonging to the horticultural belt of La Plata, Buenos Aires, Argentina (35°01'21.3" S; 58°03'25.5" W). Horticultural farms of the region have several seasonal crops (tomato, sweet pepper, eggplant, artichoke, leaf vegetables, and strawberry) that are cultivated throughout the year under open fiel or greenhouse conditions. A random sampling design was used in lettuce and broccoli crops, which had approximately 300 plants each. Samples were taken fortnightly, between March 2019 and March 2020, and samples consisted of 30 sampling units (one leaf *per* plant) of each crop.

Lettuce and broccoli crops

The collected leaves were analyzed under a stereoscopic microscope in the laboratory. Aphids and immature stages of syrphids (larvae and pupae) were identified following Malais & Ravensberg (2004) and Maza (2018), respectively, and the number of individuals of each species was recorded. The syrphids were placed individually in Petri dishes and conditioned in rearing chambers (25 °C, 55-65% RH and 14:10 L:D) until the emergence of adults or parasitoids. The pupae from which no insects emerged were kept for one month and then they were dissected to detect the presence of developing parasitoids. Adults of syrphids identification were carried out following the specific keys of Thompson et al. (2010), and parasitoids identification with the keys of Fitton & Rotheray (1982) and Gibson (2001). The percentage of parasitism was estimated as the number of parasitoids in relation to the total number of larvae and pupae collected. The number of parasitoids was estimated as the number of emerged adults plus the number of individuals of the parasitoid that did not complete their development.

Wild plants

The wild plants present in one or two patches adjacent to each crop, depending on the time of the year and the cultural management of the field were surveyed. The coverage of each wild plant species was evaluated in order to consider the most abundant species. The coverage estimation was made in a rough way and those species that visually covered more than 30% of the total patch area were considered abundant. Two whole plants of each abundant species were taken for their identification at the laboratory. The plants were botanized and deposited in the Laboratory of Evolutionary and Systematic Plant Anatomy Studies (LEAVES) of the Faculty of Natural Sciences and Museum of La Plata.

At the same time, three points (around 1 m diameter) from each patch were randomly selected, and 10 minutes observations were made in each point to collect adults of syrphid using plastic boxes. The plant on which they were, as well as whether the plants were in bloom, were recorded by direct observation. The specific identification of syrphids was carried out, in the laboratory, following Thompson et al. (2010). The frequency of syrphid adults of each species in the different seasons of the year was compared separately using one-way ANOVA or ANOVA with permutation test when normality and homoscedasticity assumptions were not fulfilled. Analyses were performed using the software R, version 3.5.1 (R Core Team, 2018).

RESULTS

The hoverfly adults recorded in the wild plants in this study were *Allograpta exotica* Wiedemann, *Toxomerus duplicatus* Wiedemann and *Syritta flaviventris* Macquart (Diptera: Syrphidae). The last one belongs to the subfamily Eristalinae and it has saprophagous habits, whereas *A. exotica* and *T. duplicatus* belong to the subfamily Syrphinae and their larvae have predatory habits, feeding mainly on aphids.

Lettuce crop

In this crop immature stages (eggs, larvae and pupae) were found from August, all of them belonging to *A. exotica*. The greatest abundance was recorded in spring, coinciding with the greatest abundance of aphids (Fig. 1a).

Three aphid species were recorded throughout the study period, *Myzus persicae* (Sulzer), *Macrosiphum euphorbiae* (Thomas) and *Nasonovia ribis-nigri* (Mosley). *Myzus persicae* was the most abundant, in the autumn, winter and spring, followed by *M. euphorbiae* which had higher relative abundance in the summer (Fig. 2a).

In lettuce crop, the species *Diplazon laetatorius* (Fabricius) (Hymenoptera: Ichneumonidae), a larval parasitoid of *A. exotica*, was recorded in October and February, causing 33% and 100% of parasitization, respectively (Fig. 3a).

Broccoli crop

The presence of immature stages of syrphids were recorded from October to February and, as in the lettuce crop, all belonged to *A. exotica*. In the spring and summer, two increases in the abundance of *A. exotica* were observed and coincided with the greatest abundance of aphids in the crop (Fig. 1b). The species of aphids recorded in this crop were *M. persicae, M. euphorbiae* and *Brevicoryne brassicae* (L.), the latter being predominant followed by *M. persicae* (Fig. 2b).

Two species of syrphid parasitoids, *D. laetatorius* and *Pachyneuron aff. nelsoni* (Hymenoptera: Pteromalidae), were recorded during the spring and summer. *Diplazon*

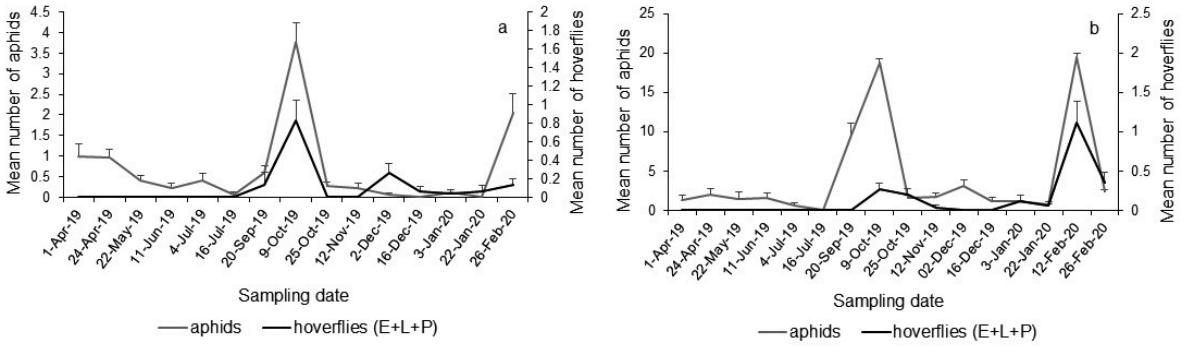

Fig. 1. Mean number of the hoverfly *Allograpta exotica* and aphids *per* leaf in the (a) lettuce crop and (b) broccoli crop. E: eggs, L: larvae, P: pupae. Brackets indicate SE.

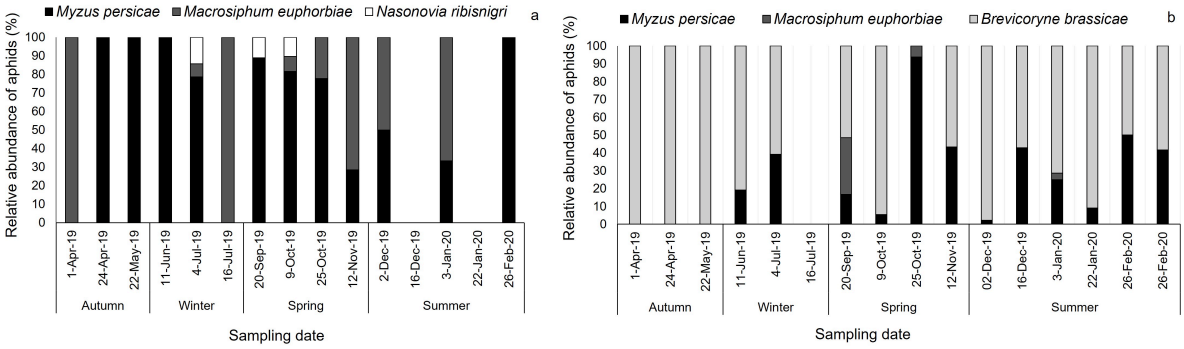

Fig. 2. Relative abundance of aphids in the (a) lettuce crop and (b) broccoli crop.

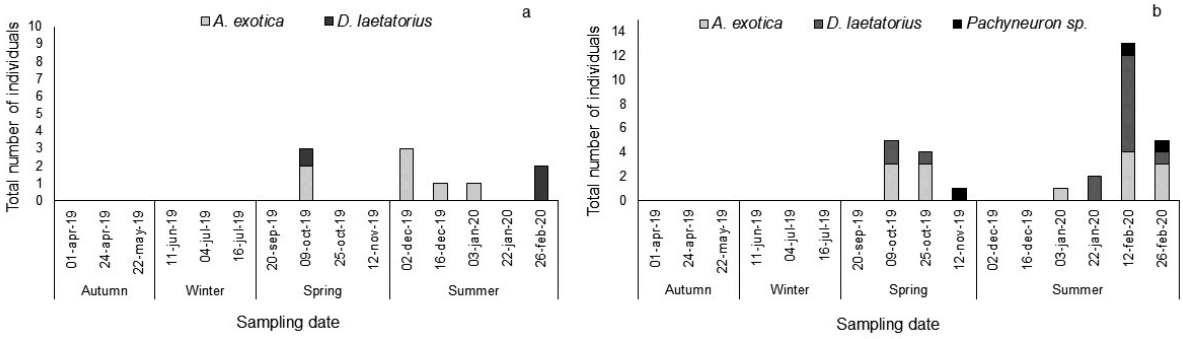

Fig. 3. Total number of healthy immature stages of *A. exotica* and parasitized by *D. laetatorius* and *Pachyneuron* sp. in the (a) lettuce crop and (b) broccoli crop.

laetatorius caused a parasitism that ranged between 20% and 100%, and the parasitism by *P. aff. nelsoni* ranged between 8 and 100% (Fig. 3b).

Wild plants

Hoverfly adults were recorded on five wild plants in vegetation patches adjacent to lettuce and broccoli crops, all of them Asteraceae, and the most frequent throughout the year were *Galinsoga parviflora* Cav. "wild basil" and *Matricaria chamomilla* L. "chamomile". A seasonal alternating occurrence of these plants (*i.e.* some replacement of these species over time), which were visited in their flowering period by both species of predatory syrphids, was observed (Fig. 4). Adults of *A. exotica* on wild plants were more abundant in the winter ($F = 8.35$; d.f. = 3, 11; $P = 0.003$) meanwhile *T.*

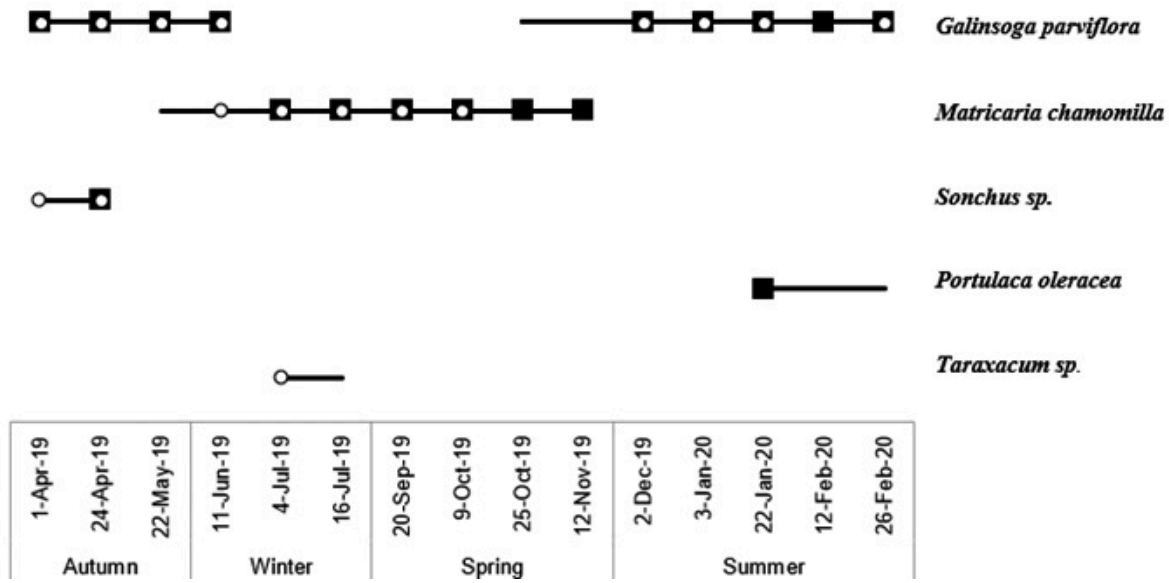

Fig. 4. Presence of *Allograpta exotica* (black squares) and *Toxomerus duplicatus* (white circles) on wild plants in bloom (black lines) adjacent to the lettuce and broccoli crops throughout the year in one horticultural farm in La Plata, Buenos Aires, Argentina.

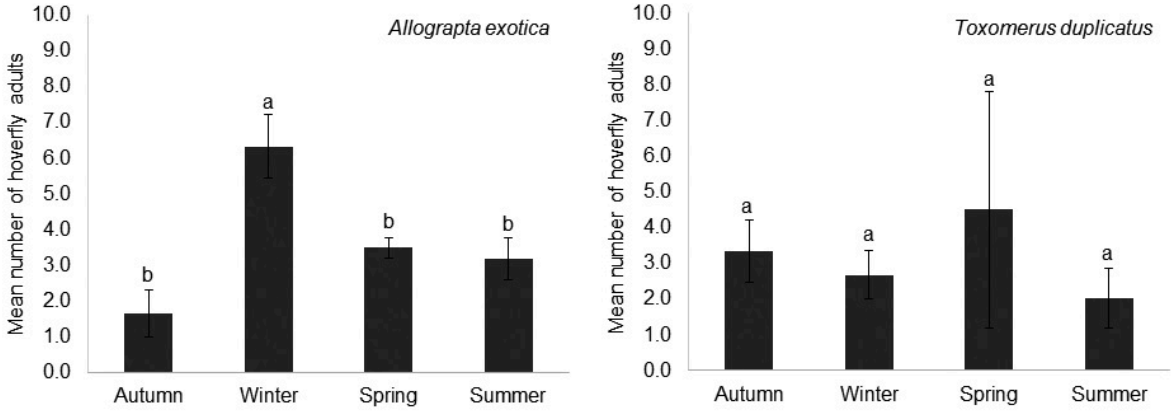

Fig. 5. Mean number of *Allograpta exotica* (left) and *Toxomerus duplicatus* (right) adults throughout the year in the wild vegetation near the lettuce and broccoli crops. Brackets indicate the SE.

duplicatus abundance was similar in every season (F = 0.35; d.f. = 3, 11; P = 0.83) (Fig. 5).

DISCUSSION

Allograpta exotica and *T. duplicatus* are predatory hoverflies that were found on wild plants and in the lettuce and broccoli crops. Both *A. exotica* y *T. duplicatus* are widely distributed in the Neotropical region, from Mexico to southern Chile and Argentina. In Argentina, these species have been recorded from Jujuy to Río Negro provinces in many agricultural and natural systems (Maza, 2018). In horticultural crops of Entre Ríos province, Díaz & Maza (2017) found *T. duplicatus* with *T. watsoni* (Curran) in lettuce crop as well as adults of the same species and *A. exotica* in alyssum strips *Lobularia maritima* (L.) Desv. (Brassicales: Brassicaceae), added to enhance the biological control by conservation.

The predominant aphids associated with *Toxomerus*

species in Argentina have been *Uroleucon sonchi* (L.) and *M. euphorbiae* (Díaz & Maza, 2017), while in our study, the most abundant aphid associated with *A. exotica* was *M. persicae*, followed by *M. euphorbiae*. On the other hand, the lettuce aphid *N. ribisnigri*, that is predominant in North America, Europe, New Zealand and Australia (Díaz & Fereres, 2005) was recorded, in Argentina, as an important species only in greenhouse lettuce crop in La Pampa (Baudino et al., 2007). The relative abundance of *N. ribisnigri* in our study system was low and there are no records of its population density in Buenos Aires crops.

In relation to the crucifer crops in Argentina, syrphid larvae have been found associated with *B. brassicae* in cabbage (Dubrovsky Berensztein et al., 2017), since this species is the most frequent and abundant in plants belonging to that group. Gomes Fidelis et al. (2018) found that one of the main causes of *B. brassicae* mortality in Brazil was the predation of nymphs and adults by larvae of *A. exotica*. Larvae of other syrphid species are known to be effective predators of *B. brassicae* and could be considered an important factor to reduce their population growth (Jankowska, 2005).

Regarding larval-pupal parasitism, of the two *A. exotica* parasitoid species identified *D. laetatorius* was the predominant species in both lettuce and broccoli crops. This is a solitary parasitoid with a worldwide distribution (Bordera et al., 2001). The association of this parasitoid with *A. exotica* has been mentioned by Korytkowski (1967) and Greco (1997) in Peru and Argentina, respectively, and *A. exotica* is regarded as one of its main hosts. The other parasitoid recorded in our study is gregarious and belongs to the genus *Pachyneuron*, although it has not been identified to species level it was possible to determine that it is closely related to *P. nelsoni* (Díaz Lucas et al., 2019). *Pachyneuron* is a cosmopolitan genus that includes about 60 species distributed mainly in the Palaearctic region (Gibson, 2001; Noyes, 2020), and its position in the trophic network is very wide. Most species of Pachyneuron are hyperparasitoids of Aphididae or other phytophagous Hemiptera (Coccoidea, Psylloidea) through their Braconidae (Ichneumonoidea), Aphelinidae, and Encyrtidae (Chalcidoidea) primary parasitoids. Furthermore, they can be primary parasitoids or hyperparasitoids of predators belonging to different taxonomic groups (Diptera: Syrphidae, Chamaemyiidae; Coleoptera: Coccinellidae; Neuroptera: Chrysopidae) (Noyes, 2020). *Pachyneuron nelsoni* is a widely distributed species that has not been recorded yet in the Americas and is generally known as a parasitoid of syrphids (Gibson, 2001; Noyes, 2020). The absence of taxonomic specific keys for the Chalcidoidea of the Neotropical region makes the determination of the species found in Argentina difficult. The right identification of the species found in this study and further investigation to know its biology will allow us to understand its position in the trophic network and its potential effect on the biological control of aphids. Therefore, if it were a primary syrphid parasitoid its effect would be negative, while if it were a hyperparasitoid -*i.e.* parasitoid of a syrphid parasitoid-, its presence in the system could be positive for biological control.

The two more frequent plant species, *M. chamomilla* and *G. parviflora*, in the patches of wild plants adjacent to the lettuce and broccoli crops, have annual growth. *Matricaria chamomilla* is a plant native to Europe and Iran, introduced in our country in 1916. In the studied area, the observed flowering period -from winter to spring- differs from that recorded by Wojciechowicz-Żytko & Jankowska (2017), who mention that the flowering period in the northern hemisphere is from early summer to early autumn. Several authors found that *M. chamomilla* flowers were more attractive to different species of syrphids than other wild, aromatic, and flowering plants in several countries (Sadeghi, 2008; Wojciechowicz-Żytko & Jankowska, 2017). *Galinsoga parviflora* grows in temperate and subtropical zones, is native to Central and South America and was later introduced to Europe where it is now widely distributed. It is usually found in gardens and agricultural areas associated with various crops such as tomatoes, cabbage, potatoes, strawberries and corn. The flowering period occurs in the warm months of the year (Damalas, 2008), as was observed in this study.

The flowering plants are essential to provide pollen, nectar and refuge to pollinators and biological control agents; however, it is necessary to evaluate eventual negative aspects of these plants, mainly as host potential pests. It is known that colonies of the aphid *Aphis fabae* Scopoli are frequently associated with *M. chamomilla* (Wojciechowicz-Żytko & Jankowska, 2017), as well as other aphids and cicadellids are associated with *G. galinsoga* (Batra, 1979).

The temporal occurrence of the adults throughout the year and the immature stages of hoverflies only in the warmest months observed in this study is concordant with the finding of Villa et al. (2016) and Greco (1995), and could be due to facultative reproductive diapause of females in the winter, as was observed in some species (Rodríguez Gasol et al., 2020). The wild plants surrounding the crops will probably favor overwintering hoverflies by providing both undisturbed habitat and more overwintering sites (Rodríguez Gasol et al., 2020). Therefore, the temporal alternation of the flowering between *M. chamomilla* and *G. galinsoga* would offer resources to hoverfly adults to remain in this horticultural system throughout the year.

The results of this study contribute to the identification of the wild plants adjacent to agricultural crops frequently visited by syrphids. We also present new information on the parasitoid species attacking syrphid larvae within lettuce and broccoli crops. Wild flowering plants and parasitoids can be viewed as positive and negative factors for Syrphids, respectively and should be considered to design aphid biological control strategies in these crops.

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