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Alien Species Numerically Dominate Natural Enemy Communities in Urban Habitats: A Preliminary Study

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ABSTRACT

Urbanization generally leads to reduced biodiversity of insects, mainly native species. Knowing the structure of natural enemy communities is important because of the significant role they play as biological control agents of herbivores. Here, we evaluated the influence of urbanization on the complex of natural enemies of the aphid *Aphis gossypii* (Hemiptera Aphididae), which feeds on the flowers of jacaranda (*Jacaranda mimosifolia*, Bignoniaceae). Seven sites with different urbanization levels were selected in the city of Córdoba (central Argentina) and three jacaranda trees with aphids were sampled in each site. Diversity, α and relative contribution of native and alien species were estimated. In general, the results indicate that urbanization did not have a significant effect on the community of natural enemies, with species richness showing a marginally significant increase with increasing urbanization levels. Although a similar richness of native and alien species was recorded across sites, 80% of natural enemy abundance belonged to alien species. Aphid populations were found to increase with increasing level of urbanization, but this variable did not have an influence on natural enemies. The dominance of alien and/or generalist species resistant to disturbances might account for the lack of relationship between urbanization and structure of natural enemy communities.

Key words: Aphids, parasitoids, predators, hyperparasitoids, urbanization.

INTRODUCTION

Urban environments are complex heterogeneous and disturbed systems, characterized by reduced and isolated natural habitats, high pollution levels, elevated temperatures, compacted soils and great amounts of concrete, among other features (Pickett *et al.,* 2011; Jones and Leather, 2012). In general, these conditions have negative effects on different groups of terrestrial animals (McKinney, 2008), including insects (Kotze *et al.,* 2011; Jones and Leather, 2012), causing declines in abundance and species richness and producing changes in community structure, such as increased dominance of alien (McKinney, 2002; McDonald *et al.,* 2013), opportunistic and generalist species (Faeth *et al.,* 2005). Alien species sometimes replace native ones faster than they are lost (McKinney, 2008); hence, urbanization may favor an increase in biodiversity at the local scale (Frankie and Ehler, 1978; Niemelä, 1999). The process of replacement of local biotas by nonindigenous and locally expanding species that can co-exist with humans is known as biotic homogenization (McKinney and Lockwood, 1999). This process promotes the geographical expansion of some species, the “winners”, and the geographical reduction of others, the “losers” (Baskin, 1998).

Natural enemies, predators and parasitoids, play a fundamental role in the control of insect herbivores in urban habitats (Losey and Vaughan, 2006; Kotze *et al.,* 2011). However, little is known about the effects of urbanization on parasitoids (reviewed in Fenoglio and Salvo, 2010) as well as on predatory species belonging to families other than Carabidae (Denys and Schmidt, 1998; Zanette *et al.,* 2005; Ferrante *et al.,* 2014) and, to date, there is no evidence of the impact of urbanization on secondary parasitoids (hyperparasitoids). Natural enemies, belonging to higher levels of the food chains, are particularly sensitive to habitat fragmentation (Hunter, 2002), even to that resulting from urbanization. Primary parasitoids may be negatively affected by urbanization or not affected at all (reviewed in Fenoglio and Salvo, 2010), whereas predatory carabid beetles might exhibit declines in species richness with increasing urbanization (Martinson and Raupp, 2013).

In the present research we assessed the effects of urbanization on the complex of natural enemies (predators, parasitoids and hyperparasitoids) of the aphid *Aphis gossypii* Glover, 1877 (Hemiptera Aphididae) present on *Jacaranda mimosifolia* D. Don, 1822 (Bignoniaceae) trees in Córdoba city, Argentina. Many aphid species are considered pests of crops and ornamental plants and their abundance, as in other herbivores, often increases with increasing levels of urbanization (Raupp *et al.,* 2010). Among their natural enemies, the alien species ladybird *Harmonia axyridis* (Pallas) has shown great potential as a biological control agent of *A. gossypii* (Flores Macías *et al.,* 2010). However, *H. axyridis* has been categorized as an invasive alien species in different parts of the world because it displaces native species (Martins *et al.,* 2009; Roy *et al.,* 2012), which may be happening also in Argentina (Saini, 2004). Therefore, studying the community structure of natural enemies of aphids in cities is important in order to develop managing strategies of pest herbivores but also for conservation of beneficial native insects.

Here we evaluated α-diversity of natural enemies and the proportion of native and alien species, and examined possible differences in species composition (β-diversity) in relation to the urbanization level in a city. To discard possible indirect effects of urbanization (mediated through changes in herbivore abundance) on natural enemy communities (Koricheva, 1994), we also estimated the effect of urbanization level on aphid abundance and, therefore, its impact on the third trophic level. We expect a higher α-diversity of natural enemies in sites with lower urbanization due to a higher dominance of native species. In addition, we expect a decrease in β-diversity at higher levels of urbanization mainly due to a higher presence of few alien species resistant to environmental disturbances.

MATERIALS AND METHODS

Study area

The present study was conducted in the city of Córdoba, located in central Argentina (31° 20’ S 64° 10’ W, 440 masl). The city covers an area of 562 km² (Dirección de

Catastro Córdoba, 2007) and has a population of 1,330,023 inhabitants (INDEC, 2010). Seven sites with different urbanization indices, located along a 10-km northwest to southeast transect, were selected from 18 sites previously used by Fenoglio *et al.* (2009). The selection of sites was based on two criteria: 1) that represented an effective gradient of urbanization based on the index (Fig. 1), 2) that jacaranda trees were infected with aphids at the period of the sampling. The urbanization index corresponds to the inverse of the first ordination axis of a Principal Component Analysis (PCA) based on several urbanization variables (distance from the city core, vehicular traffic, garden surface, vegetation coverage, ground temperature), which accumulated 74% of the variance. The index corresponding to the sites here used varied from -1.84 (least urbanized) to 4.16 (most urbanized).

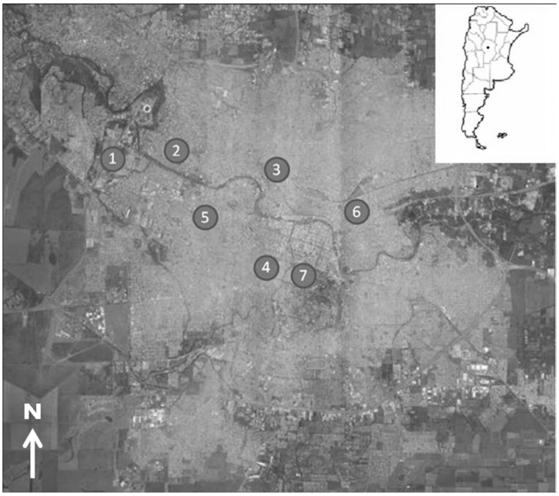

Fig. 1. Location of sampling sites arranged in increasing order of urbanization (*sites 1 to 7*) in Córdoba city (Argentina).

Study system

The host plant *J. mimosifolia*, commonly known as “jacaranda”, is a subtropical tree widely present in cities, with deciduous foliage and large blue-violet flowers, grouped in panicles, which bloom in October in the study area (Dimitri, 1980). The aphid *A. gossypii* is a polyphagous herbivore with cosmopolitan distribution (Delfino *et al.,* 2007; Flores Macías *et al.,* 2010), which attacks crops and ornamental plants (Margaritopoulos *et al.,* 2006). In Córdoba city, it is associated with jacaranda trees, among several other plant species (Delfino and Buffa, 2008). Aphids have a varied fauna of natural enemies, comprising predators (Coleoptera, Neuroptera and Diptera) and primary parasitoids and hyperparasitoids (Hymenoptera).

Sampling

During October and November 2012, three jacaranda trees attacked by *A. gossypii* were selected at each site, with the exception of two sites where only one and two jacaranda trees with aphids were found. The selected trees were similar on their age and architecture (estimated as trunk diameter at breast height, ranging from 100 to130 cm, and a height between 3-5 m), and no chemical treatment was applied to them. In cases where more than three attacked trees were observed, focal trees were selected based upon their foliage accessibility (canopy height not longer than the length of the pruning scissor which was of 7 m), and separated from each other by a distance equal to or greater than 100 m. In each tree, 20 inflorescences of about 30 cm in length were selected at random around the outline of trees, located between 1 and 7 m in height, to account for the abundance of aphids and their natural enemies. Aphid colonies are aggregated; hence, to facilitate quantification of *A. gossypii* abundance, an index proposed by Yokomi and Tang, (1996) was used. Thus, aphid samples were classified as follows: 0 = no aphids; 1 = 1-20 aphids; 2 = 21-50 aphids; 3 = 51-100 aphids; 4 = 101-200 aphids; 5 => 200 aphids. Then, the abundance of aphids was estimated in each sample using the midpoints of the above categories: 0, 10, 35, 75, 150 and 300, respectively, following Yokomi and Tang (1996).

To determine species richness and abundance of natural enemies, other five inflorescences of about 30 cm in length were randomly taken, placed in labeled plastic bags and transported to the laboratory where material was bred. The inflorescences were placed in a vase containing water to keep them hydrated, all inside a plastic container of 30 cm in height and 30 cm in diameter covered with a plastic mesh to allow proper ventilation. The immature stages of natural enemies were maintained at 18°C -22°C temperature, 55%- 68% R.H., and 12L:12D photoperiod, and were fed daily with *A. gossypii* individuals, until they reached the adult stage. Adults were placed in tubes with 70% ethanol. Species were identified using dichotomous keys (Gonzalez 2006; Zumoffen *et al.,* 2015) and the assessment of a specialist (Dr. Leticia Zumoffen, INTA Rafaela). The origin and the feeding habit of natural enemies were obtained from literature (Weems 2004; Gonzalez 2006; López *et al.,* 2012) and the assessment of a specialist (Dr. Leticia Zumoffen, INTA Rafaela). The specimens were deposited in the Collection of Entomology Department, National University of Córdoba, Argentina.

The following variables related to natural enemies were estimated for each site: α-diversity measured through species richness (total number of species found in the period sampled), abundance (total number of individuals found in the period sampled) and Shannon index; relative richness of native species; relative abundance of native species and β-diversity estimated by the quantitative Sorensen index. The δ-diversity was measured as the total number of natural enemies found throughout the study sites.

Statistical analyses

The effect of urbanization on the natural enemies of the aphid *A. gossypii* was evaluated using simple linear regression analyses, considering the index of urbanization as the independent variable and α-diversity, relative richness and abundance of native species as dependent variables. Richness was estimated using the rarefaction method with the package vegan in R 2.9.2 statistical software (R Core Team, 2010), which enables the comparison of samples with different numbers of individuals (Magurran, 2004). Moreover, to discard possible indirect effects of urbanization on natural enemies due to changes in resource availability, we first examined whether aphid abundance varied according to the urban gradient and then if/how resource abundance influenced indicators of diversity. It was not possible to perform multiple linear regression analysis due to the limited number of degrees of freedom available and multicollinearity problems (Graham, 2003). In all cases, α was of 0.05, although probability values ranging between 0.05 and 0.1 were considered marginally significant.

The effect of urbanization on the composition of natural enemies in different sites was evaluated using a partial Mantel test (Legendre and Legendre, 1998) between the distance matrix of Sorensen, expressed as the additive inverse (1 - Sorensen index) and the Euclidean matrix of urbanization distances (expressed as the differences between sites in terms of their urbanization index), controlling for geographical distances between sites (expressed in km). This analysis was performed using the vegan package in R software.

RESULTS

The 90 jacaranda inflorescences sampled held 230 individuals corresponding to 14 species of natural enemies (δ-diversity) associated with the aphid *A. gossypii* (Table 1). Predators made the greatest contribution (72%) to the species richness of natural enemies. In terms of abundance, primary parasitoids and hyperparasitoids together were the group most abundant with 66% of all natural enemies (Table 1). Among the predators, *H. axyridis* was the dominant species. The species richness of natural enemies associated with *A. gossypii* showed a slight increasing trend at sites with high urbanization level (Table 2 and Fig. 2a). The same trend was observed for the richness corrected by rarefaction (Table 2). On the other hand, neither abundance nor Shannon index of natural enemy community changed with the urban gradient (Table 2). Although abundance of aphids significantly increased as the level of urbanization increased (Table 2 and Fig. 2b), none of the variables indicative of α-diversity were related to aphid abundance (Table 2).

In terms of richness, a similar proportion of native and alien species associated with *A. gossypii* was recorded (Table 1 and Fig. 3); however, when considering the abundance, more than 80% of individuals belong to alien species (Fig. 3). Moreover, most of these alien species were present in a high proportion of the studied sites (Table 1). Both relative richness and abundance of native natural enemies per site were less than 41% and showed no significant changes in relation to the urbanization index or the abundance of aphids (Table 2).

The Partial Mantel test showed that species composition of natural enemies evaluated through β-diversity (Sorensen distances) was independent of the level of urbanization ($r_M = 0.22$, $p = 0.19$).

Table 1. Taxonomic classification, origin, and abundance of natural enemies associated with the aphid *A. gossypii* in the sampling sites of Córdoba city, and proportion of sites where the species was recorded.

Natural enemies	**Order**	**Family**	**Species**	**Feeding habit**	**Origin**	**Abundance**	**Proportion of sites with the species (n=7)**
Predators	Coleoptera	Coccinellidae	*Harmonia axyridis* (Pallas)	Generalist/A	Alien	28	1.00
			Adalia bipunctata (Mulsant)	Generalist	Alien	11	0.71
			Hippodamia convergens (Guerin-Meneville)	Generalist	Alien	7	0.57
			Olla v-nigrum (Mulsant)	Generalist	Alien	7	0.71
			Cycloneda ancoralis (Germar)	Generalist	Native	1	0.14
			Scymnus rubicundus (Erichson)	Generalist	Native	7	0.29
	Diptera	Syrphidae	*Pseudodoros clavatus* (Fabricius)	Generalist	Native	7	0.57
			Allograpta exotica (Wiedemann)	Generalist	Native	1	0.14
			Allograpta obliqua (Say)	Generalist	Native	9	0.57
	Neuroptera	Chrysopidae	Chrysopinae *sp*	-	Unknown	1	0.14
Parasitoids	Hymenoptera	Braconidae	*Lysiphlebus testaceipes* (Cresson)	Generalist	Alien	45	0.86
		Aphelinidae	*Aphelinus mali* (Haldeman)	Generalist	Alien	66	0.86
Hyperparasitoids		Pteromalidae	*Asaphes vulgaris* (Walker)	Generalist	Alien	39	1.00
		Megaspilidae	*Dendrocerus sp*	-	Unknown	1	0.14

Table 2. Parameters of the regression analyses performed for different variables of the natural enemy community associated with *A. gossypii* in relation to the urbanization index and aphid abundance. Significant and marginally significant P-values are in bold.

Response variable	**Urbanization index**			**Aphid abundance**		
	$F_{1,5}$	***P***	**R**	**$F_{1,5}$**	***P***	**R**
Species richness	4.32	**0.09**	0.46	2.03	0.21	0.29
Rarefied species richness	4.79	**0.08**	0.49	-	-	-
Species abundance	0.32	0.59	0.06	<0.01	0.99	<0.01
Shanon index	2.61	0.17	0.34	0.16	0.70	0.03
Relative richness of native species	3.30	0.13	0.40	0.98	0.37	0.16
Relative abundance of native species	1.05	0.35	0.17	<0.01	0.98	<0.01
Abundance of aphids	11.96	**0.02**	0.71	-	-	-

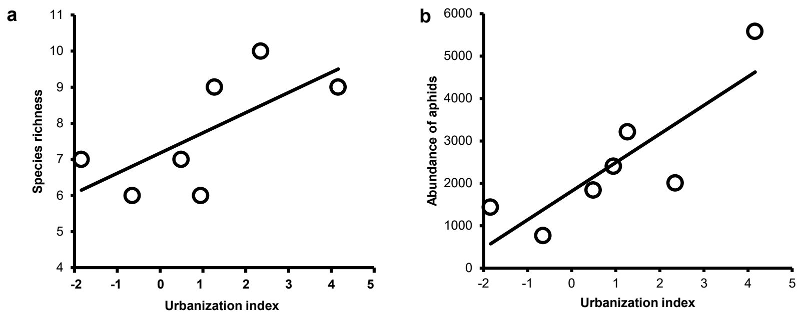

Fig. 2. Relationship between the urbanization index for Córdoba city (high values of the index mean high urbanization degree) and a) observed species richness of natural enemies of the aphid *A. gossypii* y = 0,56x + 7.18 and b) the abundance of *A. gossypii* y = 674,54x + 1817.19.

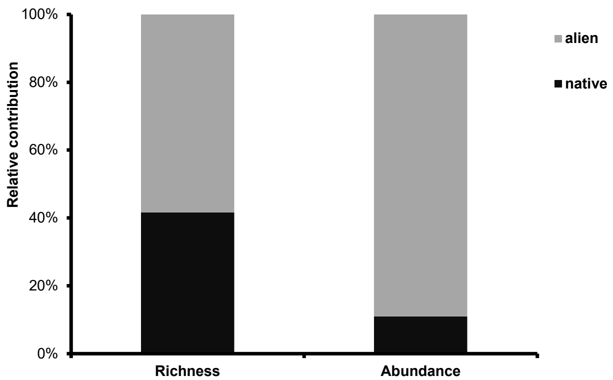

Fig. 3. Relative contribution (%) in terms of species richness and abundance of native and alien species of *A. gossypii* natural enemies considering all sampled sites in Córdoba city.

DISCUSSION AND CONCLUSION

Overall, our results show that urbanization does not have significant effects on the natural enemy community of the aphid *A. gossypii*. Two of the indicators here used to determine α-diversity of natural enemy communities (abundance and Shannon index) showed no significant changes along the urban gradient (urbanization index), which is in disagreement with our predictions. However, species richness showed a marginally significant increase with increasingly urbanized sites of the city, regardless of aphid abundance. Generalist and/or alien natural enemy species, such as those found in our study (Table 1), tend to have higher tolerance to disturbance than specialists and/or native ones (Forbes and Kendle, 1998) and therefore are likely to prevail in areas of increased urbanization (Kotze *et al.,* 2011, Burkman and Gardiner, 2014). Moreover, studies focusing on other group of predators such as carabid communities, found an increment of species richness in urban habitats, which was attributed to the dominance of generalist and open-habitats species (Magura *et al.,* 2004, 2010; Elek and Lovei, 2007). In our study, all the found natural enemies are generalist species (i.e. they consume more than one host/prey genus), which could explain the increase in species richness at sites of increased urbanization.

Native species are usually the most negatively affected by increasing urbanization, whereas alien species may even be benefited by urban environments (McKinney, 2006). In our system, alien natural enemies were clearly dominant, mainly represented by all parasitoid and hyperparasitoid species, and most of predator species of the Coccinellinae subfamily. In a literature review, Faeth *et al.,* (2005) found that in urban environments the relative abundance of most of the alien species show a tendency to increase at the expense of the native ones. In our work, the values of relative richness and abundance of native natural enemies were low (less than 41% in both cases) and similar across sites, revealing that alien species were dominant even in those city sites with low levels of urbanization. In line with these results, the similarity of the complexes of *A. gossypii* natural enemies between sites represented by β-diversity did not depend on the level of urbanization.

In the particular case of ladybirds, the dominance of alien species was even more evident, since highly competitive alien species were present in all sites. Species like *H. axyridis* may be displacing (Alyokhin and Sewell, 2004; Saini, 2004; Roy *et al.,* 2012) native species such as *C. ancoralis* and *S. rubicundus*, which were present at very low abundances in only one and two sites, respectively. Available evidence suggests that *H. axyridis* is an intraguild predator (Roy *et al.,* 2012) that would cause detrimental effects on smaller species, like the native ones mentioned above (Mirande *et al.,* 2015). Moreover, notably *Eriopis connexa* Germar (Coleoptera: Coccinellidae) has not been found in any of the sites, although individuals of this species were frequently collected in the city of Córdoba in previous years (as observed in labels of the several specimens deposited in the Collection of the Department of Entomology, National University of Córdoba).

As expected, the abundance of *A. gossypii* increased with the level of urbanization; indeed, due to the biological characteristics of aphids (small body size, sucking mouthparts, limited mobility and multiple generations in the same host plant), and to features of the urban environment (e.g. high temperatures, stressed plants and pollution), they would benefit from disturbance (Braun and Flückiger, 1985; Raupp *et al.,* 2010; Dale and Frank, 2014). However, the increase in prey abundance was not related to natural enemy species diversity, but we cannot completely discard an effect of urbanization mediated by the availability of resources, since the whole aphid community developing in other plant species was not sampled.

In conclusion, the community of natural enemies of *A. gossypii* was in general not affected by urbanization, either directly or indirectly via increased food resources in jacaranda trees. The absence of a relationship between indicators of community structure and the urban gradient could be explained by the numerical dominance of alien species, which are resistant to disturbance in all urban sites. Homogenization of the biota could be occurring in the urban environment, where native species are replaced by alien ones (McKinney, 2006). However, historical data on biodiversity necessary to confirm this hypothesis (Alvey, 2006) are not available for this system.

Beneficial insects play an important role as suppliers of ecosystem services such as biological pest control (Burkman and Gardiner, 2014) and have potential as biological indicators of environmental disturbance (McIntyre, 2000). *Harmonia axyridis* was the main natural enemy of *A. gossypii* in Cordoba city, but considering the invasive status of this species (Martins *et al.,* 2009; Roy *et al.,* 2012) it would not be suggested as a biological control agent from a diversity conservation point of view. We recommend continuing this line of research, incorporating a higher number of sampling sites in order to corroborate the present results and to provide new tools that improve both the management and conservation of these insects in urban environments.

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