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Nestedness between aphids and parasitoids populations in plants associated with an organic citrus grove

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Abstract: In the present study the nestedness between bipartite networks of plants, aphids and parasitoids species of an organic citrus grove located at La Selva del Camp (Tarragona, NE Spain) was determined to analyze the dynamics of the plant-aphid-parasitoid system and establish the possible reservoir of citrus aphids and their rates of parasitism. The results showed a low nested in plant-aphid assemblages, because the aphids are specialized forms to exploit certain types of plants, while the aphid-parasitoid assemblages were clearly nested because there are species of parasitoids generalists; such is the case of the parasitoid *Lysiphlebus testaceipes*, one of the most common and abundant on this type of crop, which interacts with various species of aphids. This parasitoid produced a high rate of parasitism in two species of aphids on two species of plants associated with citrus, *Dysaphis pyri* on *Pyrus communis* and *Sipha maydis* in *Avena barbata*. This positive nesting is also due to the heterogeneity of aphids attacked, because if there are few species of aphids, would lead to a competitive exclusion and therefore fewer species of parasitoids.

Keywords: bipartite networks; biological control; Lysiphlebus testaceipes

1. Introduction

The use of vegetation cover creates numerous benefits for agro-ecosystems such as an increased abundance of natural enemies, which limits the effect of some pest insects (Domínguez Gento et al., 2002). This is the case for aphids which can become very numerous on plantations and can serve as an alternative food for several beneficiary insects (Bugg et al., 1990). These species are controlled by numerous parasites (Belliure et al., 2008), which greatly reduce populations in different crops, mainly citrus (Kavallieratos et al., 2004). On the other hand, the study of networks between different populations allows one to determine symmetrical or asymmetric relationships between them. One example is the bipartite networks that illustrate the connections between species from two distinct groups (Borgatti and Everett, 1997), which is very useful in the study of parasite-host interactions. A common pattern in these interaction networks is the nestedness that occurs when the specialist species interact only with the generalists, but the generalists interact also amongst themselves, giving rise to a marked asymmetry of the specificity of the interactions (Bascompte et al., 2003). Consequently, the aim of this study was to examine the plants associated with citrus cultivation in order to analyse the dynamics of the plant-aphid-parasite system through the use of bipartite nets and the degree of nestedness among the species involved, in order to determine possible aphid reservoirs characteristic of citrus fruits and their rates of parasitism.

2. Materials and Methods

Study Area

Sampling was carried out in the associated vegetation inside and outside an ecological citrus plantation in "La Selva del Camp" (Tarragona, Northeast Spain, 41° 13 '07' 'N, 1° 08' 35"E). The citrus plantation consists of about 300 clementine trees (*Citrus clementina* var. *clemenules*) grafted on Hybrid Citrange Carrizo [*Poncirus trifoliata* (L.) Raf. × *Citrus sinensis* (L.) Osb.]. The crop has met all organic agriculture standards since 2004.

Sampling of aphids and parasites and determination

Amongst the most abundant herbaceous species were individuals with colonies of aphids, both in the perimeter area and in the intertidal of the mandarin tree plantation. Colonies of aphids were also looked for in the existing fruit trees within the crop site. Finally, one selected vegetation from the periphery of the plantation that had aphids (up to 10 m). From each selected plant, the associated species and aphids were determined and their abundance was estimated using a semi-quantitative scale of 1-5, 6-25, 26-100, and >100 individuals per shoot, branch or leaf. In order to calculate the abundance, each of the previous density scales was considered taking 3, 15, 60, and 250 individuals respectively (Piñol et al., 2009). The parasite aphids (mummies) and empty mummies were then quantified to determine the rate of parasitism in each plant and in each tree (1 to 3 branches). This procedure was carried out in mid-June 2013for a duration of one week. Later parasite aphids were collected and stored in a nursery until their emergence. The aphids were identified in the field by taxonomic codes (Nieto-Nafría et al., 2003). The parasites were separated by morphotypes and sent to specialists for their determination.

Data analysis

First, the most abundant species of each community (plants, aphids and parasites) were quantified. The rate of parasitism associated with each plant species was estimated as the ratio between the number of mummified aphids and the total number of aphids (aphids plus mummies). Subsequently the degree of nestedness in these species was analysed, for which two qualitative bipartite networks (of presence-absence) were established between plants and aphids and between aphids and parasites. To calculate the degree of nestedness, a non-parametric, permutational statistical test was carried out with the BINMATNEST (binary matrix nestedness temperature calculator) programme (Rodríguez-Gironés and Santamaría, 2006).

3. Results and Discussion

Community of plants, aphids and parasites

A total of 22 plant species associated with mandarin cultivation were sampled. The most abundant herbaceous plants containing colonies of aphids were *Sonchus oleraceus* L., 1753 (44% of the branches selected from each plant had one or more species of aphids) and *Avena barbata*

Pott. Ex Link (19%). Regarding trees, the only ones that harboured aphid colonies were pomegranate trees (*Punica granatum* L., 1753) and this was the species containing the highest number of aphids (1,350 individuals (adults + nymphs)). There were 8,469 aphids in the total of selected plants, corresponding to 27 species. The most abundant species were *Aphis punicae* Passerini, 1863 (16% of individuals counted in all plants) and *Thelaxes suberi* Del Guercio, 1911 (14%) associated to the *Punica granatum* and *Quercus ilex* L. plants respectively. Depending on the time of year and their reproductive cycle, aphids select different species of plants to start or end their biological activities. The most common aphid species in the *Aphis spiraecola* and *A. gossypii* citrus plants (Belliure et al., 2008) were not very abundant in the accompanying plants (arable and arboreal) in the citrus plantation. It has been stated that these species prefer leguminous, cucurbitaceous, composite and other herbaceous plants as secondary hosts (Blackman and Eastop, 2006). In fact, four of these species were found in our samples but with very few individuals, perhaps because the vegetation existing during the sampling period was not attractive or appropriate to complete their cycles or to become secondary hosts, mainly for the *Aphis* Species. Of the 1,852 mummies controlled in the breeding chamber, a total of 468 parasites emerged, corresponding to 25 species and associated with 13 aphid species. The most abundant were *Lysiphlebus testaceipes* Cresson, 1880 (43% of the emerged parasites), a species with a very wide range of hosts in different cultures (Michelena et al., 1994). This was followed by *Adialytus ambiguus* Haliday, 1834 (18%). In fact, our results showed a not very high species richness in the three analysed communities, especially in terms of aphids and parasites.

Rates of parasitism

A total of 1,155 mummies distributed in 14 plant species were recorded. *Dysaphis pyri* Boyer de Fonscolombe, 1841, was the aphid species with the highest parasitism rate on *Pyrus communis* L. (100%), followed by *Sipha maydis* in *Avena barbata* (50.2%) (Table 1). The first species of aphid was parasitised by *Lysiphlebus testaceipes* and *Lysiphlebus* sp. whilst the second was parasitised by *L. testaceipes* and *Adialytus ambiguus*. Given that *L. testaceipes* is one of the most important primary citrus parasites, it can be concluded that this species easily attacks the aphids in any other host plant because the aphid - parasite relationship is almost independent of the plants on which colonies of aphids are found (Michelena et al., 1994). It is also known that *L. testaceipes* can withstand higher temperatures than other parasites (Belliure et al., 2008), a fact that would justify its abundance on the dates when the sampling was done.

Table 1. The highest parasitism rates (%) of aphids, whose host plants contained mummies in the field.

Plants	**Aphid**	**% of parasitism**
Pyrus communis	*Dysaphis pyri*	100
Avena barbata	*Sipha maydis*	50,2
Hedera hélix	*Aphis gossypii*	25,0
Carduus pycnocephalus	*Brachycaudus cardui*	21,1
Malus domestica	*Dysaphis plantaginea*	20,6

Nestedness of bipartite networks

The plant-aphid network was clearly non-nested (P> 0.05 for all null models) (Table 2). Consequently, one did not find any plants that had been attacked by many species of aphids or many generalist aphids, which attacked many plant species. Only the taxon *Spartium* sp. was affected by 4 species of aphids. Therefore, a dispersed interaction between the two communities is clearly present. This behaviour could be due in part to the non-random selection procedure of the host plants (Wright et al., 1998) and sampling time: in the month of June, aphidological populations begin to diminish or disappear.

On the other hand, few generalist species of aphids were detected, perhaps because most are associated with a certain type of plant; this factor is called "reciprocal specialisation" and is the possible cause of non-nested networks (Joppa et al., 2010) and statistically non-significant results (Bascompte et al., 2006).

The aphid-parasites network presented nestedness (P <0.001 in all cases and null models; Table 2). The most significant interaction was that of the *Hyperomyzus lactucae* L., 1758 aphid with 10 species of parasites, followed by *Brachycaudus cardui* with 8 species. Regarding the species of parasites, the most generalist was *Lysiphlebus testaceipes* that parasitised with 11 species of aphids and *Pachyneuron aphidis* with 4 species. This is perhaps due to the presence of generalist parasites that attack similarly to diverse aphids, which are affected by many parasites. This is what Lewinsohn et al. (2006) found for a set of generalist species that interact with other communities of generalists forming a dense network of interactions. In our case we believe that the heterogeneity of attacked aphids is the most important factor, because if there were few species of aphids, competitive exclusion would be produced and thus there should be fewer species of parasites.

Table 2. Nestedness temperature (T) of each studied matrix and its statistical significance (*P*)

MATRIX	*P*	Average T	Variance
MATRIX 1			
Null model 1	0.05	14.92	9.18
Null model 2	0.08	14.72	10.61
Null model 3	**0.07**	**14.69**	**9.29**
MATRIX 2			
Null model 1	< 0.001	14.56	8.19
Null model 2	0.003	9.95	8.35
Null model 3	**0.001**	**10.44**	**10.23**

4. Conclusions

The common aphids in *Aphis spiraecola* and *A. gossypii* citrus had a very low presence in the plants associated with the plantation, possibly because at the time of sampling the existing vegetation was not appropriate for them to complete their biological cycles.

The *Lysiphlebus testaceipes* parasite proved to be the most significant species for this type of crop because of the large number of its individuals and its high rate of parasitism.

The degree of nestedness of the aphid species and their parasites was positive, as opposed to the plant-aphid network, due to the presence of generalist species. This added to the heterogeneity of aphids attacked by the parasites.

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Conflicts of Interest

The authors declare no conflict of interest

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