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ORIGINAL ARTICLE

APHIDIINAE (HYMENOPTERA, BRACONIDAE) DIVERSITY IN CITRUS AGROSYSTEMS OF GUELMA REGION, EASTERN ALGERIA

Omar Khaladi*♦, Amira Bouderbala**, M'hamed Benada***
and
Boualem Boumaaza****

*Département d'Écologie et Génie de l'Environnement, Faculté des Sciences de la Nature et de la Vie et Sciences de la Terre et l'Univers, Université 8 Mai 1945 Guelma, BP 401, Guelma, 24000, Algérie.
**Laboratory of Agriculture and Ecosystem Functioning, Department of Agronomy Sciences, Faculty of Natural and Life Sciences, Chadli Bendjedid University- El Tarf, Algeria.
***Department of Agronomic Sciences, Faculty of Sciences and Technology, Ahmed Zabana University, Relizane, Algeria.
****Department of Agronomic Sciences, Faculty of Natural and Life Sciences, Ibn Khaldoun University, Tiaret, Algeria.
♦Corresponding author: khaladi.omar@univ-guelma.dz; khaladiomar5@gmail.com

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ABSTRACT

The importance of understanding auxiliary insects in agroecosystems has become increasingly necessary due to the overuse of chemical products that disturb their presence and sometimes lead to their extermination without our awareness of their existence. The objective of this research project is to conduct the first inventory of aphid parasitoids in the Aphidiinae subfamily in orange orchards in the Guelma Region (Eastern Algeria) and to estimate the number of species present in this region using four non-parametric estimators. The results revealed the presence of ten species, namely *Aphidius matricariae* (Haliday, 1834), *Aphidius* sp., *A. ervi* (Haliday, 1834), *Binodoxys angelicae* (Haliday, 1833), *B. acalephae* (Marshall, 1896), *Diaeretiella rapae* (McIntosh, 1855), *Ephedrus plagiator* (Nees, 1811), *Praon volucre* (Haliday, 1833), *Lipolexis gracilis* (Foerster, 1862), and *Lysiphlebus testaceipes* (Cresson, 1880). Among these species, *L. testaceipes*, *B. angelicae*, and *A. matricariae* were the most dominant during the study period. *Binodoxys acalephae*, despite being present in small numbers, had a ubiquitous ecological status, while *A. matricariae*, *A. ervi*, *B. angelicae*, *D. rapae*, and *L. testaceipes* maintained a constant status. According to the non-parametric estimators, the number of species varied, but the average was 11.39 ± 0.54 species, indicating that the richness could increase to 12 with a sampling accuracy of 87.74%. Despite their low abundance, the richness of Aphidiinae species in the orange orchards could be used in a biological control context.

Keywords: Algeria, Aphids parasitoids, Inventory, Non-parametric estimators, Orange.

INTRODUCTION

Aphids are a group of pests that cause the majority of problems in farms. Many species are considered to be extremely dangerous pests of crops, forests, and ornamental plants (Sullivan, 2008). According to Bhatia *et al*. (2011), there are about 4,000 worldwide species, of which about 250 are dangerous. When they take the sap of a plant for food, aphids inject salivary toxins and phytopathogenic viruses (Chrzanowski *et al.*, 2009).

The widespread use of insecticides to control these pests has led to the development of resistance in some species (Harmel *et al.*, 2008). Therefore, the use of biological control, which is one of the crop protection methods against phytophagy, becomes a necessity to solve the problems of these chemicals. According to Boivin (2001), the mortality of phytophagous parasitoids is higher than that attributed to predators and microorganisms combined.

Aphid parasitoids are small Hymenoptera, belonging to the families Aphelinidae (genus *Aphelinus*) and Braconidae (Aphidiinae) (Sullivan, 2004). With more than 600 species, the Aphidiinae subfamily includes the largest number of aphid parasitoid species (Mackauer and Starý, 1967).The species in this subfamily are strictly associated with their host aphids, in particular, their geographical distribution and the preferred host plants on which they feed (Starý, 1981; Žikić *et al.*, 2016).

This study is part of a research project that has been launched on aphid parasitoids in the Guelma Region (Eastern Algeria). We are focusing specifically on the Aphidiinae subfamily that preys on citrus aphids, using a simple technique to attract these beneficial insects. Furthermore, the goal is to use non-parametric estimators to estimate the potential number of species in the study region.

MATERIALS AND METHODS

Description of sampling sites: The investigation of the parasitoid species was carried out during the spring sap flow of orange orchards *Citrus sinensis* (L.) Osbeck, 1765, in Guelma Province (Algeria), during 2016-2019. The coordinates of these orchards are as follows: 2016 and 2017 (36°30'13.8"N 7°24'22.4" E), 2018 (36°28'38.5"N 7°24'40.0"E and 36°28'35.5"N 7°24'35.2"E), and 2019 (36°29'18.4"N 7°26'49.7"E and 36°30'28.5"N 7°24'35.6"E). The territory of the region is characterized by a subhumid climate in the center and in the north of the area, whereas it is semi-arid in the south. Precipitation during the study months of 2016, 2017, and 2018 did not exceed 250 mm, while in 2019; we recorded 449.6 mm of precipitation. During our study period, the coldest month was January with 5.8°C and 4.3°C in 2016 and 2017, respectively, as well as February with 4.1°C and 3.6°C in 2018 and 2019, respectively. However, the warmest month was May, with 27.8°C, 30.4°C, 25.4°C, and 23.9°C in 2016, 2017, 2018, and 2019, respectively.

Sampling methods and identification: Two yellow traps are installed per hectare in the studied orchards. The traps are collected and renewed every fortnight after they have been deposited, and sometimes it’s monthly from January to May. Traps are placed in the east and southeast orientation of the tree canopy (Roth, 1971).

The identification of parasitoids was carried out using several keys, namely: Starý *et al.* (2010), Rakhshani *et al*. (2012), Kavallieratos *et al*. (2013), and Rakhshani *et al*. (2015). Moreover, the turpentine essential oil was used to detach specimens from the glue.

Statistical analysis

Diversity indexes and richness estimation: The Shannon–Wiener index (H′), and the equitability index were calculated by using PAST version 1.91 (Hammer *et al*., 2001). The diversity t-test was used to compare the Shannon–Wiener index during the four sap flows. In order to estimate the species diversity, we employed four non-parametric estimators using EstimateS software (version 9.1.0) (Colwell, 2013). These estimators, namely the average-based coverage estimator (ACE), incidence-based coverage estimator (ICE), Chao2, and Jackknife 1, were used to predict the expected number of species in our region. The average of these estimators was used to calculate the ratio of recorded species in the samples (i.e., observed richness / the average of the estimators* 100) as a measure of sampling completeness. This information was crucial in understanding the accuracy of our species data and the overall diversity in the study area. We computed a specimen rarefaction curve with the same software using 95% confidence intervals of the moment-based estimator (Sobs Mao Tau). This curve is a tool used to determine if the number of specimens taken is sufficient to capture all the biological diversity present in the given environment. The data used to generate this curve are the results of the abundances of all the parasitoid species found throughout the study period (i.e. 4 years).

Constancy: We calculated the constancy of species as well, which is the ratio, expressed as a percentage, of the number of surveys containing the species under study relative to the total number of surveys. According to Muller (1985), a species is qualified as follows:

Accidental: if C% <25%

Accessory: if 25% ≤ C% < 50%

Regular: if 50% ≤ C% < 75%.

Constant: if 75% ≤ C% < 100%.

Omnipresent: if C% = 100%.

The constant and omnipresent species are the most dominant because they have more food.

RESULTS

Taxonomic composition and relative abundance of species: A total of 173 individuals belonging to 10 species of Aphidiinae parasitoids were captured during the four sap flows. The parasitoids that were found are: *Aphidius matricariae* (Haliday, 1834), *A. ervi* (Haliday, 1834), *Aphidius* sp., *Binodoxys angelicae* (Haliday, 1833), *Binodoxys acalephae* (Marshall, 1896), *Diaeretiella rapae* (McIntosh, 1855), *Ephedrus plagiator* (Nees, 1811), *Praon volucre* (Haliday, 1833), *Lipolexis gracilis* (Förster, 1862), and *Lysiphlebus testaceipes* (Cresson, 1880). In 2016, the species *A. matricariae* was the most dominant, with almost 35.5% of the total species captured, followed by *B. angelicae* with more than 32%, while in 2017, the number of individuals caught was very low. The spring flow of the year 2018 was dominated by the parasitoid *L. Testaceipes*, with more than 46.5% of the total species captured, followed by *B. angelicae* and *B. Acalephae*, with 22.09% and 18.6%, respectively. In 2019, the dominant species remains *Lysiphlebus testaceipes* with almost 43%, followed by *B. angelicae* and *A. matricariae* with 28.57% and 21.43%, respectively (Tab. 1).

Table (1): Abundance and relative abundance (RA) of species found during the study campaign.

	Feb–May 2016		Feb–May 2017		Jan– May 2018		Jan– May 2019	
Parasitoids	Abundance	RA[%]	Abundance	RA[%]	Abundance	RA[%]	Abundance	RA[%]
Aphidius matricariae	22	35.48	0	0.00	3	3.49	3	21.43
Aphidius ervi	2	3.23	3	27.27	1	1.16	0	0.00
Aphidius sp.	1	1.61	0	0.00	0	0.00	0	0.00
Binodoxys angelicae	20	32.26	0	0.00	19	22.09	4	28.57
Binodoxys acalephae	2	3.23	3	27.27	16	18.60	1	7.14
Diaeretiella rapae	3	4.84	1	9.09	5	5.81	0	0.00
Ephedrus plagiator	11	17.74	0	0.00	0	0.00	0	0.00
Praon volucre	1	1.61	0	0.00	0	0.00	0	0.00
Lipolexis gracilis	0	0	1	9.09	2	2.33	0	0.00
Lysiphlebus testaceipes	0	0	3	27.27	40	46.51	6	42.86

During the four sap flows, the most abundant species was *L. testaceipes* with more than 28% of the totals followed by *B. angelicae* and *A. matricariae* with respectively 24.85% and 16.18% as shown in Diagram (1).

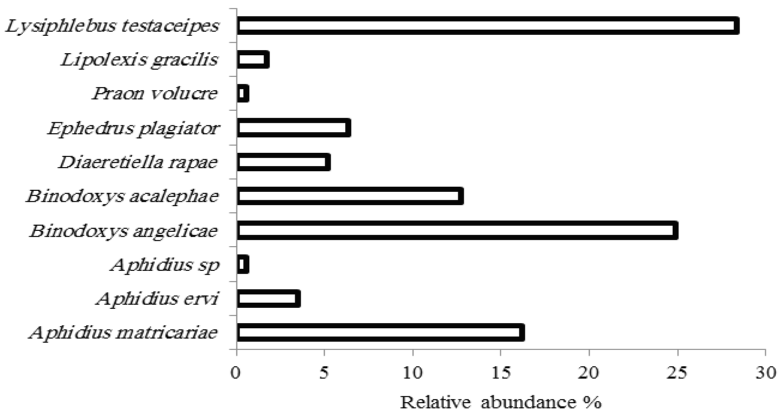

Diagram (1): Global relative abundance of the species observed during the four sap flows.

Richness estimation and diversity index: The specific richness, the Shannon index, and the equitability index were calculated from year to year during our study. The number of species found during the 2016 sap flow was the highest compared to other years with 8 species, followed by the 2018 spring flow with 7 species, while in 2017 and 2019, there were respectively 5 and 4 species (Tab. 2).

Table (2): Richness, Shannon index and equitability of the species of parasitoids found during the study. (Bootstrap Confidence interval N= 9999)

	Feb–May 2016	Confidence interval		Feb–May 2017	Confidence interval		Jan–May 2018	Confidence interval		Jan–May 2019	Confidence interval	
		Lower	Upper		Lower	Upper		Lower	Upper		Lower	Upper
Richness (S)	8	8	8	5	4	5	7	7	7	4	4	4
Shannon index (H')	1.541	1.379	1.721	1.499	1.034	1.547	1.424	1.269	1.582	1.240	0.991	1.358
Equitability (J)	0.741	0.663	0.828	0.931	0.804	0.971	0.732	0.652	0.813	0.894	0.715	0.979

The analysis of the Shannon Index showed a decrease in diversity from year to year with 1.541 bits, 1.499 bits, 1.424 bits, and 1.24 bits, respectively, in 2016, 2017, 2018, and 2019. These values are not far from their maximum values (Hmax = Ln S) which are 2.08 bits, 1.61 bits, 1.95 bits, and 1.39 bits, respectively. The diversity t-test showed a non-significant value between the four Shannon index values with $p > 0.05$.

The equitability index is a tool used to assess the distribution of species in a given study. A value of 0 to 1 is assigned to indicate how evenly or unevenly the species are distributed. During the spring flow of 2017 and 2019, the species in the study were found to be almost evenly distributed, with equitability index values of 0.931 and 0.894, respectively. However, in 2016 and 2018, the equitability index values were lower at 0.741 and 0.732, respectively; this suggests that some species were more numerous than others during those years, resulting in a less uniform distribution of species.

Based on the rarefied data, we observed 10 species in the study region during the monitoring period with a confidence interval of 2.47, and the non-parametric estimators indicate that the expected richness is 11.39 ± 0.54 species; for instance, the number of species could theoretically reach 12. We assumed that our sampling effort was sufficient because the mean ± SD specimen coverage per site was 87.74% (Diag. 2, Tab. 3).

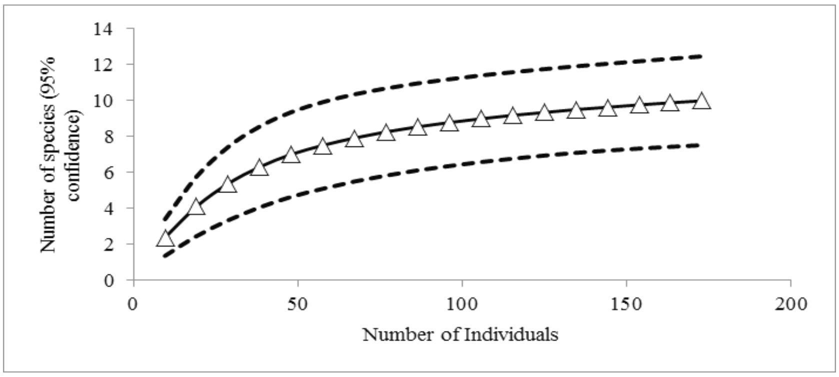

Diagram (2): Specimens rarefaction curve of the number of parasitoids species found during our study "Dashed lines indicate 95% confidence intervals".

Table (3): Observed and expected number of species in the study region.

Observed richness	Nonparametric richness estimator						Observed richness/Means estimated
	ACE	ICE	Chao 2	Jack 1	Mean	SD	
10	11.84	10.97	10.89	11.89	11.39	0.54	87.74 %

The global Shannon index was around 1.83 bits and its maximum value was 2.30 bits, which also indicates that the diversity is not far from its maximum value.

Availability of parasitoids: After conducting surveys to detect the presence of parasitoids, we evaluated the ecological status or constancy of the Aphidiinae species. The global status was determined by considering the four sap flows, and is presented in Table (4). This ecological status varies across species. We observed that 50% of the species found in our study area are constant, including *A. matricariae*, *A. ervi*, *B. angelicae*, *D. rapae*, and *L. testaceipes*. One species, *B. acalephae*, was omnipresent during the four-year study period, suggesting that these species have found sufficient food in the area. However, the remaining species had a low occurrence rate.

Table (4): Ecological status of parasitoids in the study region.

Parasitoids	Feb–May 2016	Feb–May 2017	Jan–May 2018	Jan–May 2019	Constancy [%]	Ecological status
Aphidius matricariae	1	0	1	1	75	Constant
Aphidius ervi	1	1	1	0	75	Constant
Aphidius sp.	1	0	0	0	25	Accessory
Binodoxys acalephae	1	1	1	1	100	Omnipresent
Binodoxys angelicae	1	0	1	1	75	Constant
Diaeretiella rapae	1	1	1	0	75	Constant

Ephedrus plagiator	1	0	0	0	25	Accessory
Lipolexis gracilis	0	1	1	0	50	Regular
Lysiphlebus testaceipes	0	1	1	1	75	Constant
Praon volucre	1	0	0	0	25	Accessory

[0: absence; 1: presence]

DISCUSSION

There are various techniques for insect inventory, mainly grouped into two categories: interception traps and colored traps (Nageleisen and Bouget, 2009). In our study, we chose to use colored traps because they actively attract flying hymenopterans, especially parasitoids. Other researchers prefer to use interception traps like Malaise traps, which, despite their passive nature in attracting insects, have shown the ability to provide interesting results (Fraser *et al*., 2008; Lotfalizadeh *et al.*, 2016; Piruznia *et al.*, 2022).

In this study, we did not look for the aphid-parasitoid association, but rather conducted a preliminary inventory of the existing Aphidiinae species in the selected orange orchards. To avoid disrupting the auxiliary fauna in the orchards, we limited the number of traps used to two per hectare because our objective was to confirm the presence of parasitoids in the area.

Previous studies that have examined the association between parasitoids and citrus aphids have reported a low richness of primary parasitoids emerging from mummies. For instance, Sellami *et al.* (2013) found only five species belonging to three genera, which are *Lysiphlebus testaceipes*, *L. fabarum* (Marshall, 1896), *Aphidius matricarea*, *A. coleamani* (Viereck, 1912), and *Binodoxys angelicae* in Tunisia, with the last species being found only on *Aphis gossypii* (Glover, 1877). Similarly, Gómez-Marco (2016) identified only *Binodoxys angelicae* on *Aphis spiraecola* (Patch, 1914) in Spain. A subsequent study in western Algeria by Labdaoui and Guenaoui (2018), which was conducted in 2016 and 2017 on a Citrus clementina orchard, found only two species of Aphidiinae on *Aphis spiraecola*.

We were able to create a comprehensive list of Aphidiinae wasps in orange orchards for the first time in the Guelma Region through this study. The identified species come from seven distinct genera, namely *Aphidius* (Nees, 1819), *Binodoxys* Mackauer, 1960, *Ephedrus* Haliday, 1833, *Praon* Haliday, 1833, *Lipolexis* Förster, 1862, *Diaeretiella* Stary, 1960, and *Lysiphlebus* Förster, 1862.

According to Kambhampati *et al*. (2000), cited by Choi and Kim (2018), the Aphidiinae comprises about 50 to 63 genera of endoparasitic wasps that use aphids as hosts. This indicates a high dependency of these parasitoids on aphids as a food source in agricultural crops. In the present study, the orange tree was the main crop, and the possible hosts of the parasitoids included *Aphis spiraecola*, *Aphis gossypii*, *Toxoptera aurantii* (Boyer de Fonscolombe, 1841), *Toxoptera citricida* (Kirkaldy, 1907), and *Myzus persicae* (Sulzer, 1776). Khaladi and Guendouz-Benrima (2019), reported the presence of *Aphis spiraecola* and *Aphis gossypii* in the Guelma Region. Weeds such as broad bean (a previous crop in the orchard of 2016–2017), sowthistle, buttercup oxalis, curly dock, alfalfa, charlock, and others also colonized the orchards, indicating the potential presence of other aphid species besides those found on citrus.

The same genera that we found in our region were also found by Kavallieratos *et al*., (2013), in Southeastern Europe, but with 22 species of herbaceous and shrubby ornamental plants. However, they didn't find *Aphidius ervi.* They found that these wasps parasitize several aphid hosts, including *Aphis gossypii*, *A. spiraecola*, *A. fabae* (Scopoli, 1763), *Myzus persicae*, *Toxoptera aurantii*, and *Aphis craccivora* (Koch, 1854).

In the Middle East, Barahoei *et al*. (2014) identified our species in Iran as part of a group of 78 species belonging to 17 genera that have been found in association with several aphids, including *Aphis gossypii*, *A. spiraecola*, *A. fabae*, *Myzus persicae*, *Toxoptera aurantii*, and *Aphis craccivora*. Similarly, in Turkey, Satar *et al*. (2014) found most of the species we found in our study. In the United States, *Lysiphlebus testaceipes* has been identified as a parasitoid of *Toxoptera citricida* in citrus orchards (Tang *et al.*, 2002).

In Tunisia, Boukhris-Bouhachem (2011) reported four species in common with ours among a total of eight, which were *Aphidius matricariae*, *Diaeretiella rapae*, *Praon volucre*, and *Lysiphlebus testaceipes.*

Hemidi *et al*. (2013) conducted an investigation in a natural environment in the Biskra Province (Algeria) and reported 11 species from six different genera. A subsequent study by Hemidi and Laamari (2020) proved that *Aphidius matricariae* and *Lysiphlebus testaceipes* were the most abundant. These species belong to the same six genera as those found in our study, except for *Lipolexis*, which was unique to our investigation. Laamari *et al*. (2011) found a similar number of genera (6) in their surveys of natural environments and cultivated areas in eastern Algeria, but identified 28 species.

Another study by Chaouche and Laamari (2015) in the Biskra Region focused on herbaceous plants and shrubs, and identified 14 species from six genera, including *Aphidius*, *Binodoxys*, *Diaeretiella*, *Lysiphlebus*, *Praon*, and *Trioxys*, found on a variety of herbaceous plants such as alfalfa, sowthistle, mallow, and charlock.,…etc

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In Blida Province, Algeria, Aroun (2015) identified 11 aphid-associated species in various settings, including cultures, spontaneous and ornamental plants, and coniferous forests. These species include *Aphidius matricariae*, *Aphidius ervi*, *Diaeretiella rapae*, *Ephedrus persicae* (Froggatt, 1904), *E. plagiator*, *Lipolexis gracilis*, *Lysiphlebus fabarum*, *Praon volucre*, *Trioxys angelicae* (Haliday, 1833), *Pauesia cedrobii* (Stary and Leclant, 1977), and *P. Silana* (Tremblay, 1969). According to the same author, *A. matricariae* is a polyphagous species that can be found in 36 different aphid species on 34 different plant species. This may explain why this species is considered "Contant" in our region during the four spring flows.

In the northwest of Algeria, Labdaoui and Guenaoui (2018) found that *Lysiphlebus testaceipes* and *Binodoxys angelicae* parasitized *A. spiraecola* on *Citrus clementina* Hort. ex.

CONCLUSIONS

The technique used during our study allowed us to detect 10 species of Aphidiinae on orange trees during the spring sap flow over four consecutive years (2016–2019). Notably, some of these species were found consistently throughout the four-year study period, which could make them valuable in the development of a potential biological control program for this region.

The non-parametric estimators used to predict the number of species in our region confirmed that the estimated number of species is approximately 12, which is two more than what we found, and our sampling effort was deemed sufficient, with an accuracy rate of 87.74%.

CONFLICT OF INTEREST STATEMENT

"The authors have no conflicts of interest to declare ".

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تنوع عويلة Aphidiinae (Hymenoptera, Braconidae) في النظم الزراعية للحمضيات في منطقة قالمة (شرق الجزائر)

عمر خلادي*، أميرة بودربالة**، محمد بن عدّة*** و بوعلام بومعزة****

*قسم البيئة وهندسة المحيط، كلية علوم الطبيعة والحياة وعلوم الأرض والكون، جامعة 8 ماي 1945 قالمة، ص.ب. 401، قالمة، 24000، الجزائر.

**مختبر أداء الزراعة والنظم البيئية، قسم العلوم الزراعية، كلية العلوم الطبيعية والحياة، جامعة الشاذلي بن جديد - الطارف، الجزائر.

***قسم العلوم الزراعية، كلية العلوم والتكنولوجيا، جامعة أحمد زبانة غليزان، الجزائر.

****قسم العلوم الزراعية، كلية علوم الطبيعة والحياة، جامعة ابن خلدون تيارت، الجزائر.

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الخلاصة

أصبحت أهمية فهم الحشرات المساعدة في النظم الزراعية ضرورية بشكل متزايد بسبب الإفراط في استخدام المنتجات الكيميائية التي تعكر وجودها وتؤدي في بعض الأحيان إلى إبادتها دون وعينا بوجودها. الهدف من هذا المشروع البحثي هو إجراء عملية جرد لطفيليات المن من فصيلة Aphidiinae في بساتين البرتقال في منطقة قالمة (شرق الجزائر) وتقدير عدد الأنواع الموجودة في هذه المنطقة باستخدام أربعة مقدرات غير معيارية. كشفت النتائج عن وجود عشرة أنواع وهي: *Aphidius matricariae* Haliday, 1834، *Aphidius* sp.، *A. ervi* Haliday, 1834، *Binodoxys angelicae* Haliday, 1833، *B. acalephae* Marshall, 1896، *Diaeretiella rapae* McIntosh, 1855، *Ephedrus plagiator* Nees, 1811، *Praon volucre* Haliday, 1833، *Lipolexis gracilis* Foerster, 1862، و *Lysiphlebus testaceipes* Cresson, 1880. من بين هذه الأنواع، كانت *L. testaceipes* و *B.*

angelicae و *A. matricariae* هي الأكثر انتشارًا خلال فترة الدراسة. فيما يتعلق بـ *B. acalephae*، على الرغم من وجودها بأعداد صغيرة، كان لها وضع بيئي في كل مكان، في حين ان الانواع *A. matricariae*، *A. ervi*، *B. angelicae*، *D. rapae*، و *L. testaceipes* حافظوا على وضع ثابت. وفقًا للمقدرات الغير معيارية، تباين عدد الأنواع، لكن المتوسط كان 11.39 ± 0.54 نوعًا، مما يشير إلى أن الثراء يمكن أن يرتفع إلى 12 مع دقة أخذ النماذج تبلغ 87.74%. على الرغم من قلة وفرتها، يمكن استخدام ثراء أنواع عويلة Aphidiinae في بساتين البرتقال في سياق المكافحة الحيوية.