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Biodiversity of Natural Enemies of Pseudococcidae in the Semiarid Region of Brazil

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Abstract

Pseudococcidae species, also known as mealybugs, comprises a complex of pests in various cultivated and non-cultivated plant species, among which fruit plants are most affected by both direct and indirect damage. The incidence of these pest species has been constant in productive environments, causing significant losses due to sap suction, virus transmission, and damage to fruit quality. Thus, this study aimed to know and investigate the population dynamics of natural enemies of Pseudococcidae in different fruit plants cultivated in the semiarid region of Brazil. For this, mealybugs associated with roots, stems, leaves, and fruits of vine, pear, apple, persimmon, guava, and acerola trees were collected biweekly in 14 properties in the São Francisco Valley region, from July 2016 to June 2017. These mealybugs were properly separated for the removal of their predators and the emergence of parasitoids associated with them (Pseudococcidae). *Coccophagus* sp., *Aenasius* sp., *Anagyrus kamali*, *Anagyrus* sp. 1, *Anagyrus* sp. 2, *Anagyrus* sp. 3, *Anagyrus* sp. 4, *Coccidoxenoides perminutus*, *Gyranusoidea indica*, *Leptomastix dactylopii*, *Prochiloneurus* sp., *Aprostocetus* sp., and *Signiphora* sp. were identified as parasitoids and *Diadiplosis multifila*, *Cryptolaemus montrouzieri*, *Tenuisvalvae notata*, *Cycloneda sanguinea*, and *Hippodamia convergens* were identified as predators, in addition to unidentified species of Coccinellidae and Chrysopidae.

Keywords: biological control, fruit farming, parasitoids, plant protection, predators

1. Introduction

In Brazil, tropical fruit production is concentrated in the northeast region, which has a predominantly semiarid climate (Araújo, Fernandes, Silva, Ferreira, & Costa, 2015). This region exports a large part of its production, which leads to phytosanitary management necessary to ensure the quality of the products. Mealybugs (Hemiptera: Pseudococcidae) are agricultural pests responsible for a significant reduction in plant productivity and are easily spread through the international fruit trade due to their small size and rapid fixation on plant surfaces (Beltrà et al., 2015). They can feed on all parts of the plant, but the aerial part is most affected by them. When feeding, mealybugs produce honeydew, which is a carbohydrate substance that favors the fumagina proliferation and, consequently, depreciates the qualitative and quantitative value of fruits, directly affecting their commercialization (Bostanian, Vincent, & Isaacs, 2012; Bertin, Bortoli, Botton, & Parra, 2013).

Encyrtidae comprises one of the most efficient families of parasitoids for controlling mealybug species. Biological control of Pseudococcidae using these natural enemies has been applied in several regions around the world, with successful results (Tena, Nieves, Herrero, & Urbaneja, 2017), such as the use of *Acerophagus* sp. and *Leptomastix* sp. to control *Phenacoccus peruvianus* Granara de Willink, *Phenacoccus herreni* Cox & Williams, and *Paracoccus marginatus* Williams and Granara de Willink (1992). *Anagyrus* is a genus of parasitoids of *Planococcus citri* Risso (1813) and *Planococcus ficus* Signoret (1875) (Beltrà et al., 2015) and has been one of the most studied for the control of Pseudococcidae, especially in South America (Bugila, Franco, Silva, & Branco, 2014). *Coccidoxenoides perminutus* Girault (1915) (Hymenoptera: Encyrtidae) is an example of a parasitoid recently found in the São Francisco Valley, parasitizing all instars of *P. citri* (Fernades, Oliveira, Costa, & Menezes, 2016), with potential for biological control of this pest (Menezes et al., 2017).

The use of natural enemies is one of the best ways to control Pseudococcidae. However, it is necessary to know all the organisms involved so that they act satisfactorily in the management (Lima, Melo, & Barros, 2016). Thus, this study aimed to know and investigate the biodiversity of natural enemies of Pseudococcidae in fruit plants in the São Francisco Valley, semiarid region of Brazil.

2. Material and Methods

For the identification of natural enemy species of Pseudococcidae, mealybugs associated with vine, pear, apple, persimmon, guava and acerola crops were collected in different farms in the São Francisco Valley, semiarid region of Pernambuco, Brazil. Ten plants were analyzed per hectare for each crop. The plants were selected at random in order not to induce the choice of plants affected or not by scale insects. The study was conducted between July 2016 and June 2017. The observations and collections were performed from roots, stems, leaves, and fruits. Mealybugs and plant material were collected biweekly using pruning shears and fine bristle brush. Afterward, they were immediately sent to Embrapa Semiárido Entomology Laboratory, Petrolina, Pernambuco, Brazil (with geographical coordinates of 09º04′16.4″ S, 40º19′5.37″ W and altitude of 379 m).

The mealybugs mummified, together with their origin food, were kept in polypropylene jars capped with voile fabric for a week until the emergence of the parasitoids. Those emerged were placed in labeled Eppendorf tubes containing 70% alcohol and then sent for identification in the Biological Institute, in São Paulo. Predator insects were also found in the mealybug colonies, which were collected manually with the support of a fine bristle paint-brush number 0, avoiding damage to insects, identified and individualized in Eppendorf tubes also containing 70% alcohol. These parasitoids and predators were kept under refrigeration. Population fluctuation was determined according to the number of parasitoids and predators found in the collected material.

Faunistic analysis was performed using frequency indices (LF = less frequent, F = frequent, VF = very frequent and SF = super frequent), constancy (Z = accidental, Y = accessory and W = constant), dominance (ND = non-dominant, D = dominant and SD = superdominant), and abundance (R = rare, D = dispersed, C = common, A = abundant, VA = very abundant and SA = superabundant), proposed by Silveira Neto, Nakano, Barbin, and Villa Nova (1976) and made using the software for faunal analysis—AnaFau. The parasitoids were identified according to Woolley (1997) for Aphelinidae, Noyes (1980), and Noyes and Hayat (1994) for Encyrtidae, Schauff (1997) for Eulophidae, Darling (1997) for Perilampidae, Bouček and Heydon (1997), and Rueda and Axtell (1985) for Pteromalidae, and Woolley (1997) for Signiphoridae.

Entomological identification keys were used to identify insects. These keys are specific and were described by the Biological Institute in the analyzes carried out. The specimens were deposited in the "Oscar Monte" Entomophagous Insect Collection (IB-CBE) of the Biological Institute (Campinas, São Paulo, Brazil), under reference number IB-CBE-726. The applied indices (Dominance, abundance, frequency and constancy) are exposed and detailed in the results tables.

3. Results

Thirteen parasitoid species associated with Pseudococcidae were found in different cultures and locations in the São Francisco Valley. *Coccophagus* sp., a genus of parasitic species of Coccidae and Pseudococcidae (Woolley, 1997), *Aenasius* sp., *Anagyrus kamali* Moursi (1948), four other species of the genus *Anagyrus*, *Coccidoxenoides perminutus* Girault (1915), *Gyranusoidea indica* Shafee, Alam & Agarwal 1975, Howard, *Prochiloneurus* sp., *Aprostocetus* sp., and *Signiphora* sp. Noyes (2017) show details on the distribution and hosts of parasitoids identified at species level, and Noyes (1980) show details on those identified at genus level, indicating that some of these are related to Pseudococcidae, such as *Aenasius*, *Anagyrus,* and *Prochiloneurus*. *A. kamali* was classified as a superdominant, superabundant and super frequent parasitoid and as one of the most constant. *Anagyrus* sp. 1, *C. perminutus*, *Leptomastix dactylopii*, and *Aprostocetus* sp. were the most expressive species in this study, after *A. kamali* (Table 1). Predators were numerically less expressive than parasitoids regarding the total number of natural enemies of mealybugs. The predatory fly, *Diadiplosis multifila* Felt (1907) (Diptera: Cecidomyiidae), was the most expressive natural enemy found in the sampled material, followed by Chrysopidae species and the ladybug, *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) (Table 1).

Table 1. Faunistic analysis of parasitoids and predators of mealybug in the Submedio of the São Francisco Valley, Pernambuco, Brazil

Natural Enemies	No. ind.	Dominance	Abundance	Frequency	Constancy
Parasitoids					
Coccophagus sp.	2	ND	D[1]	LF	Z
Aenasius sp.	2	ND	D[1]	LF	Y
Anagyrus kamali	86	SD	SA	SF	W
Anagyrus sp. 1	40	D	VA	VF	Z
Anagyrus sp. 2	1	ND	D[1]	LF	Z
Anagyrus sp. 3	4	ND	C	F	Z
Anagyrus sp. 4	1	ND	D[1]	LF	Z
Coccidoxenoides perminutus	24	D	VA	VF	W
Gyranusoidea indica	4	ND	C	F	Y
Leptomastix dactylopii	12	D	C	F	Y
Prochiloneurus sp.	1	ND	D[1]	LF	Z
Aprostocetus sp.	19	D	VA	VF	W
Signiphora sp.	5	ND	C	F	W
Predators					
Diadiplosis multifila	79	SD	SA	SF	W
Cryptolaemus montrouzieri	18	D	VA	VF	Y
Tenuisvalvae notata	1	ND	C	F	Y
Cycloneda sanguinea	2	ND	C	F	Y
Hippodamia convergens	3	ND	C	F	Y

Note. No. ind.: number of individuals. ND: non-dominant, D: dominant, SD: superdominant; D[1]: dispersed, SA: superabundant, VA: very abundant, C: common; SF: super frequent, VF: very frequent, F: frequent, LF: less frequent; Z: accidental, Y: accessory, W: constant.

Regarding the crops where these natural enemies were found associated with mealybugs, *Anagyrus* sp. 1 had the highest dominance, abundance, frequency and constancy, followed by *L. dactylopii* in vine. In this culture, the predatory fly (*D. multifila*) was the most frequent and constant natural enemy found in the sampled material, followed by Chrysopidae species and the ladybug (*C. montrouzieri*). The parasitoid *A. kamali* and chrysopid predators were the most prominent in pear trees. The parasitoids were found in a smaller number in persimmon trees, with higher prominence for the predatory fly (*D. multifila*). *C. perminutus* was dominant, very abundant, very frequent and constant in apple trees; no predators associated with this culture were found and no predators and parasitoids were found in guava and acerola trees (Table 2).

Table 2. Faunistic analysis of parasitoids and predators of mealybug in different crops in the São Francisco Valley, Pernambuco, Brazil

Culture	Natural Enemies	No. ind.	Do.	Ab.	Fr.	Con.
Parasitoids						
Vine	*Anagyrus kamali*	6	D	C	F	Y
	Anagyrus sp. 1	40	SD	SA	SF	W
	Leptomastix dactylopii	11	D	VA	VF	W
	Aprostocetus sp.	7	D	C	F	Y
	Pachycrepoideus vindemmiae	6	D	C	F	Y
	Signiphora sp.	1	ND	R	LF	Z
	Aphelinus sp.	1	ND	R	LF	Z
Pear	Bethylidae	1	ND	C	F	Z
	Aprostocetus sp.	6	D	VA	VF	Y
	Anagyrus kamali	29	SD	SA	SF	W
	Signiphora sp.	2	ND	C	F	Z
	Gyranusoidea indica	2	ND	C	F	Z
	Encyrtidae	1	ND	C	F	Z
	Anagyrus sp. 2	1	ND	C	F	Z
	Prochiloneurus sp.	1	ND	C	F	Z
	Coccidoxenoides perminutus	2	ND	C	F	Z
Apple	*Aprostocetus* sp.	3	ND	VA	F	Y
	Coccidoxenoides perminutus	16	D	VA	VF	W
	Gyranusoidea indica	1	ND	VA	F	Z
Persimmon	*Aenasius* sp.	1	ND	D	LF	Z
	Coccidoxenoides perminutus	3	ND	A	VF	W
	Aprostocetus sp.	1	ND	D	LF	Z
	Coccophagus sp.	2	ND	C	F	Y
	Perilampus sp.	1	ND	D	LF	Z
	Signiphora sp.	1	ND	D	LF	Z
	Anagyrus kamali	4	ND	VA	VF	W
	Gyranusoidea indica	1	ND	D	LF	Z
	Anagyrus sp. 3	4	ND	VA	VF	W
Predators						
Vine	*Diadiplosis multifila*	61	D	VA	VF	W
	Cryptolaemus montrouzieri	15	D	VA	F	Y
	Tenuisvalvae notata	1	ND	VA	F	Z
	Cycloneda sanguinea	2	ND	VA	F	Z
Pear	*Diadiplosis multifila*	1	ND	R	LF	Y
	Cryptolaemus montrouzieri	3	ND	C	F	Y
	Hippodamia convergens	3	ND	C	F	Y
Persimmon	*Diadiplosis multifila*	17	D	VA	F	W

Note. No. ind.: number of individuals. Do.: dominance; Ab.: abundance; Fr.: frequency; Con.: constancy. ND: non-dominant, D: dominant, SD: superdominant; D[1]: dispersed, A: abundant, SA: superabundant, VA: very abundant, C: common; R: rare; SF: super frequent, VF: very frequent, F: frequent, LF: less frequent; Z: accidental, Y: accessory, W: constant.

With regard to parasitoids of mealybugs, persimmon trees had the highest number of these natural enemies, with peaks of 106 and 98 individuals per collection, found in most of the collections performed. The pear trees (178 parasitoids collected) had a uniform distribution over time with a low number of insects, except for the population peaks of 68 and 31 individuals per collection. The apple trees and vine had a low number of parasitoids (Figure 1). There was a higher number of parasitoids in crops subjected to less phytosanitary treatments, such as persimmon and pear trees, unlike the vine, which is subjected to constant applications of products for the control of various pests and diseases, which may affect the development of these beneficial organisms. The number of predators was less expressive than that of parasitoids throughout the experimental period and in all cultures, considering the highest population peak of 14 individuals (Figure 2). This is related to predators' independence on their prey, *i.e.*, they do not need to be directly associated with the insect, differently from parasitoids, which need to complete their cycle within the host and thus become dependent.

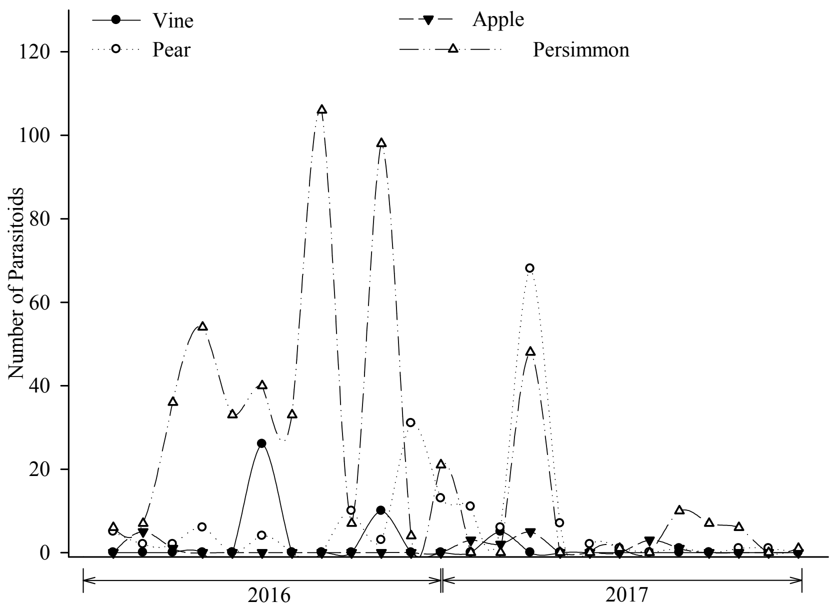

Figure 1. Population fluctuation of parasitoids in different cultures in the São Francisco Valley, Pernambuco, Brazil

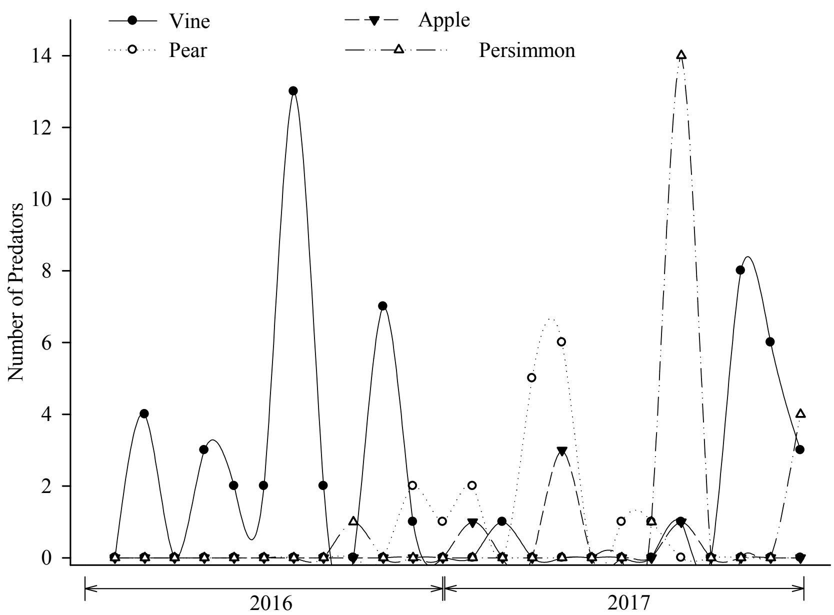

Figure 2. Population fluctuation of predators in different cultures in the São Francisco Valley, Pernambuco, Brazil

4. Discussion

For the success of agricultural pest control by biological control, the effectiveness of associated natural enemies is crucial (Menezes et al., 2017). The parasitoid *C. perminutus* is one of these enemies, which was recently recorded parasitizing Pseudococcidae naturally in the São Francisco Valley (Fernandes et al., 2016). Identify the presence of natural enemies in the region is a factor that validates the mass application of these organisms in production scale, since the adaptation of these insects is already well defined, that is, the use of natural enemies has a high success in relation to its efficiency.

In a study conducted for sixteen years, natural enemies associated with *Pseudococcus* sp. and *Paracoccus abnormalis,* in vine, were collected and reared. Accounting for seven parasitoid species, among which, *Anagyrus* sp., *Gyranusoidea* sp. and *Coccophagus* sp., and four predator species, among which, *C. montrouzieri* and *Diadiplosis* sp. (Charles, Bell, Lo, Cole, & Chhagan, 2012). Among these natural enemies with high potential for biological control, rearing in New Zealand, there are also species found in the São Francisco Valley, which may have an important parasitism and predation relationship, such as *Anagyrus* sp., *G. indica*, *C. montrouzieri*., and *D. multifila*, which may be used in biological control programs in integrated management of mealybug pests. The similarity between the climatic conditions of New Zealand and Brazil indicates the success in applying these organisms not only to fruit production in the São Francisco Valley, but in all cultures where these pests occur throughout the country.

About thirty-nine parasitoid and forty-two predator species of *Maconellicoccus hirsutus* (Chong, Aristizábal, & Arthurs, 2015) are known in the world, with twenty-seven species in the Neotropical region (Culik et al., 2013; Chong et al., 2015), considering that Brazil is one of the richest countries in biodiversity, both in fauna and flora. In biological control programs in various countries, several species of natural enemies have already been used, such as the predatory ladybug, *C. montrouzieri* Mulsant (Coleoptera: Coccinellidae) and the parasitoids *A. kamali* Moursi and *G. indica* Shafee, Alam & Agarwal (Hymenoptera: Encyrtidae) (Culik et al., 2013; Chong et al., 2015), all found in the present study, evidencing the possibility of applying a clean, efficient and productive control, as occurs when using natural enemies. Once again, São Francisco Valley shows a high perspective for the use and scale production of these organisms.

In Israel, which also has a semiarid climate, fourteen species of natural enemies are associated with *P. solenopsis*; however, *Aenasius arizonensis* (Girault) (Hymenoptera: Encyrtidae) stood out. This parasitoid has controlled the population density of the mealybug in question in that country (Spodek et al., 2018), making it potentially usable at São Francisco Valley because of the high adaptability of the genus *Aenasius* sp. in the region. Some insects may become more adapted to a given region, which indicates the need to continue the current work with more research to determine which natural enemies are most efficient in applied biological control and, therefore, develop their potential. Brazil, especially the Northeast region, promote greater development of the life cycle of insects, given their favorable climatic conditions.

Biological control is the most appropriate method to control mealybugs, because these pests have a high diversity of hosts, easily disperse to various regions, have as main characteristic the presence of wax on their body, which prevents full penetration of chemical compounds, and protect themselves in plant regions that are difficult to access by chemical applications (Peronti et al., 2016).

Reports of predators and parasitoids controlling mealybug populations in Brazil are common. Aphelinidae, Encyrtidae, Eulophidae, Eupelmidae, Pteromalidae, Signiphoridae, Azotidae, Mymaridae, and Platygastridae stand out among the main parasitoid families, and Chrysopidae, Cecidomyiidae, Drosophilidae, Syrphidae, and Coccinellidae among the main predator families, in which Coccinellids of the genus *Cryptolaemus*, *Scymnus*, *Coccidophilus*, *Azya*, *Cycloneda*, *Chilocorus*, *Exoplectra*, *Harmonia*, *Tenuisvalvae*, and *Pentilia* are commonly found associated with mealybug species (Cruz, 2018).

Less frequently, stinkbugs predators of mealybugs showed good responses for predation, satisfactorily reducing the population of Pseudococcidae and selective insecticides (Kitherian et al., 2018). In addition to insects, Rhabditida nematodes are entomopathogenic and have also been studied for applications in the control of mealybugs (Platt, Stokwe, & Malan, 2018).

Parasitoids have a higher preference for mealybugs in later stages of their life cycle, showing little or no parasitic activity on younger mealybugs in their first instars, as observed by Silva, Garcia, and Botton (2017) on *Blepyrus clavicornis*, and also reported for *Anagyrus* sp., *Aenasius bambawalei* and *Aenasius vexans*. The biological control using the parasitoid *Anagyrus kamali* has reduced the mealybug populations, in combination with other control methods (Negrini, Morais, Batista, & Chagas, 2018). This is a fundamental factor for integrated pest management, using all available resources when necessary, in a sustainable manner, preserving the entire environment and natural resources.

Parasitoids are most commonly used in biological control since they are generally pest specialists and are always associated with their host, unlike predators, which are generalists. They have great potential for reproduction and rearing provided that the researcher has a thorough knowledge of the specific characteristics of the natural enemy and its pest; thus, there will be successful management (Bostanian et al., 2012).

Anyway, the use of predators and parasitoids still needs to be studied and disseminated in agriculture, mainly due to the countless benefits that integrated pest management brings, protecting nature and all its resources that are fundamental to human existence.

5. Conclusions

The genera *Anagyus* and *Aprostocetus* and the species *Coccidoxenoides perminutus* are parasitoids with high potential for biological control of mealybugs in this region.

Anagyrus kamali is a species of parasitoid that stood out in the study, being the greatest potential for sequential work, as a species of predator *Diadiplosis multifila* in mealybug statistics in the Brazilian Semiarid.

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