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Mealybugs on fruit crops in the Sao Francisco Valley, Brazil

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Mealybugs are agricultural pests occurring all over the world. The Sao Francisco Valley is one of the most productive regions regarding the cultivation of fruit crops in Brazil, demanding a proper phytosanitary management that can be obtained through the biological control of pests. Fortnight samples of mealybugs associated with roots, trunk, leaves and fruits of grapevines, pear, apple, persimmon, guava and Barbados cherry were performed in commercial farms in the sub-medium Sao Francisco Valley, Brazil, during twelve months. In order to identify mealybugs based on morphological characteristics from adult female specimens, samples were stored in Eppendorf tubes containing ethanol 70%, labeled and then identified. A total of 10,189 mealybugs, including nymphs and adult females were collected, with the highest number of specimens found in grapevines. The second most affected crop was persimmon, followed by pear. The lowest infestation was observed in guava, apple and Barbados cherry. Pseudococcidae evidenced preference for fruits. The following mealybugs were observed in the sub-medium region of the Sao Francisco Valley: *Planococcus* sp., *Planococcus citri*, *Phenacoccus solenopsis*, *Dysmicoccus brevipes*, *Maconellicoccus hirsutus*, *Ferrisia virgata* and *Pseudococcus elisae*.

Key words: Fluctuation, identification, morphology, pests.

INTRODUCTION

Brazil is one of the world leading fruit producers, with an area of 2.5 million hectares and production of around 40 million tons per year. The municipalities of Petrolina-PE and Juazeiro-BA are the main exporters of table grapes in Brazil, with future perspectives to increase production (Silva et al., 2014).

Besides table grapes, guava and Barbados cherries are also consolidated crops in the Sao Francisco Valley region, while some other fruit crops are being implemented, such as apple, pear and persimmon (Lopes et al., 2013; Oliveira et al., 2015). In apple, mealybugs directly affect the fruit calyx and tree trunk, negatively affecting apple exportation due to quarantine restrictions for this pest (Lo et al., 2012). Also, persimmon is being adapted to the São Francisco Valley region. In Brazil, persimmon has a planted area equivalent to 9,000 ha, producing more than 170,000 tons per year, with emphasis in the State of Rio Grande do Sul, where mealybugs were found in at least 50% of the planted areas. On a global scale, ten species were identified in

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association with persimmon, namely: *Dysmicoccus brevipes* Cockerell, 1893, *Maconellicoccus hirsutus* and *Planococcus citri* (Hemiptera: Pseudococcidae) (García et al., 2016; Silva et al., 2016). Agricultural pests are one of the most limiting factors for agriculture in the country; losses of around 1.6 billion dollars per year are estimated in Brazil due to incidence of insect pests. Sometimes chemical control used against insect pests has proven inefficient and may contribute in an antagonistic manner to its original purpose (Silva et al., 2017).

Mealybugs are sucking insects with oval soft bodies and rose to whitish color, with their bodies covered by a white waxy substance similar to powdery or floury cotton; they have a pair of waxy filaments around the top of their bodies (Silva et al., 2016). The incidence of mealybugs results in direct and indirect injuries, causing photosynthetic reduction to the plant, reducing plant vigor and fruit quality (Morandi Filho et al., 2015). By releasing honeydew, mealybugs cover the leaf surface with a sugary substance that is rich in carbohydrates, leading to the development of sooty mold (Daane et al., 2012) and, consequently, depreciating the product (Bertin et al., 2013).

These insects belong to the family Pseudococcidae, the second largest family in Coccomorpha, behind only Diaspididae family and representing around 2020 species within 260 genera, with 223 species, within 44 genera, of mealybugs registered in the Neotropical region (García et al., 2016). One of the main issues concerning mealybugs is the propagation of plant viruses, such as GLRaV (Closteroviridae) associated with grapevine (Naidu et al., 2014), which reduces grapes' quality, delays maturation and weakens plants (Golino and Almeida, 2008). In South America, Australia, USA and Europe, mealybugs were documented as the main vector for GRLaV-3 virus in grapevines (Charles et al., 2012).

Monitoring pests in agro-ecosystems works as a basis for preventing environmental impacts and may even be used in a preventive fashion (Azevedo et al., 2015). Identification of Pseudococcidae species may be achieved through the morphological analysis of adult females (Williams and Watson, 1988). In the present work, we aim to identify mealybugs associated with grapevines, pear, apple, persimmon, guava and Barbados cherry crops cultivated in the Sao Franciso Valley-Brazil and to study the behavior of these insects by means of their population dynamics, distribution and faunistic analysis.

MATERIALS AND METHODS

Mealybugs associated with grapevines, pear, apple, persimmon, guava and Barbados cherry were sampled in different farms, analyzing 10 plants per hectare for each fruit crop. From July 2016 to July 2017, the roots, trunk, leaves and fruits from these crops were exanimated at intervals of fifteen days, collecting mealybugs and vegetal material in the field with the aid of pruning shears and thin bristles brushes, before being transported to the Laboratory of Entomology from Embrapa Semiarido. All samples were collected in the same period and under the same climatic conditions, not specific as phenological phases and the age of each plant. To identify mealybugs based on morphological characteristics of adult females, approximately 20 specimens were stored in Eppendorf tubes containing ethanol 70% and labeled, before being sent for identification at the Paulista State University Júlio de Mesquita Filho-Jaboticabal, Brazil. Melaybugs were mounted in permanent slides and identified by morphology as described by Granara de Willink (1990), consisting in accomplishing 3 or 4 perforations in the ventral region of the specimens using fine needles and clearing the specimen in KOH 10% solution, in hot bath, before washing the specimen in distilled water, performing dehydration in ethanol series at 70 and 100% (15 min each), staining the exoskeleton with acid fuchsine while the specimens are still in 70% ethanol solution; completing dehydration in ethanol 100% and finalizing clarification in eugenol for about 4 h; mounting slides in Canada balsam and drying slides in an oven until the balsam is completely dried. Insects were identified with the aid of a light microscope, using identification keys from Williams and Granara de Willink (1992), Granara de Willink (2009); Gullan et al. (2010) and Kaydan and Gullan (2012).

Population fluctuation was assessed by sampling nymph and adult of the mealybugs throughout the year, with the aid of stereomicroscope, automatic counter and fine bristles brush. Preference of Pseudococcidae for different plant organs within the same crop was analyzed under a completely randomized design with factorial scheme and Tukey test at 5% probability, using the software R version 3.2.5 and the figures were generated using the software Sigma Plot version 11.0. Fruit crops constituted the first factor and plant structure the second, with treatments being 'Grapevine', 'Pear', 'Apple', 'Persimmon', 'Guava' and 'Barbados cherry'; and 'Roots', 'Trunk', 'Leaves' and 'Fruits', respectively. The number of 24 samples performed refers to the number of replicates.

The faunistic analysis was completed using the frequency index (PF-rarely frequent, F-frequent, MF-very frequent and SF-extremely frequent), consistency (Z-accidental, Y-accessory and W-constant) and abundance (R-rare, D-disperse, C-commom, A-abundant, MA-very abundant and SA-extremely abundant) as proposed by Silveira Neto et al. (1976), using the software for faunistic analysis – AnaFau®.

The climate in the municipalities of Petrolina and Lagoa Grande-PE, Brazil, where the samples were collected, is classified as tropical semi-arid, BSh', characterized by scarce and irregular rainfall with summer rains and intense evaporation due to high temperatures. Climate data were collected from INMET – National Institute of Meteorology; where the accumulated rainfall (mm), relative humidity of air (%) (Figure 1A), temperature (°C) and wind velocity (m s^{-1}) (Figure 1B) was verified as monthly averages obtained during the experimental period.

RESULTS AND DISCUSSION

Mealybugs were identified as *Planococcus* sp., *Planococcus citri* Risso (1813), *Phenacoccus solenopsis* Tinsley (1898), *Dysmicoccus brevipes* Cockerell (1893), *Maconellicoccus hirsutus* Green (1908), *Ferrisia virgata* Cockerell (1893) and *Pseudococcus elisae* Borchsenius (1947).

In grapevines, *P. citri* and *D. brevipes* were identified in bunches of grapes and the trunk, while *P. solenopsis* and *M. hirsutus* were found only associated with fruits. However, *P. solenopsis*, *F. virgata*, *M. hirsutus* and *Planococcus* sp. Occurred in pear fruits; *D. brevipes* occurred in pear roots and fruits. In apple, *M. hirsutus*

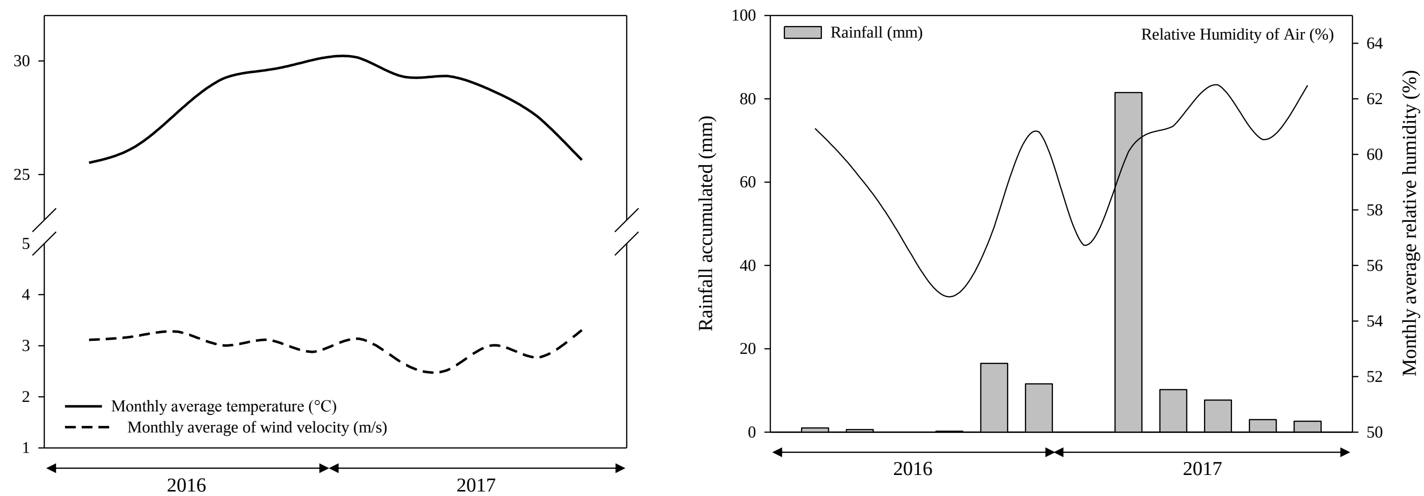

Figure 1. Climate data obtained from 07/2016 to 06/2017. (A) Accumulated rainfall (mm) and monthly average of the relative humidity of air (%); (B) Monthly average temperature (°C) and monthly average of wind velocity (m/s). Cata from INMET, average values from municipalities of Petrolina and Lagoa Grande, Pernambuco- Brazil.

Table 1. Species of mealybugs associated with different plant parts in grapevines, pear, apple, persimmon, guava and Barbados cherry crops in the Sub-medium São Francisco Valley.

Crop	Mealybug	Plant part
Grapevines	*Planococcus citri, Dysmicoccus brevipes*	Trunk
	Planococcus citri, Dysmicoccus brevipes, Planococcus sp.,*Phenacoccus solenopsis, Maconellicoccus hirsutus*	Fruit
Pear	*Dysmicoccus brevipes*	Root
	Phenacoccus solenopsis, Ferrisia virgata, Planococcus sp., *Dysmicoccus brevipes, Maconellicoccus hirsutus*	Fruit
Apple	*Pseudococcus elisae*	Leaf
	Pseudococcus elisae, Maconellicoccus hirsutus	Fruit
Persimmon	*Dysmicoccus brevipes, Planococcus* sp.,*Phenacoccus solenopsis, Maconellicoccus hirsutus, Pseudococcus elisae*	Fruit
Guava	*Pseudococcus elisae*	Fruit
Barbados cherry	*Dysmicoccus brevipes*	Fruit

occurred in fruits, while *P. elisae* is found in fruits and leaves. In persimmon, the species *D. brevipes*, *Planococcus* sp., *M. hirsutus*, *P. solenopsis* and *P. elisae* were identified in fruits. *P. elisae* was associated with guava fruits, while *D. brevipes* was associated to Barbados cherry (Table 1).

All mentioned fruit crops are being increasingly cultivated; however, there are huge numbers of insects associated with these crops (Ülgentürk and Ayhan, 2014). One hundred and forty-eight insect species (pests or not) are associated to grapevines, 69 to persimmon and 26 to apple, which may feed from different parts of the fruit plants or from nearby spontaneous plants (Silva et al., 2017). *M. hirsutus* is a polyphagous pest distributed in 93 countries around the world, including Brazil and other South American countries, gradually increasing their expansion (Culik et al., 2013). In countries like Mexico, *M. hirsutus* is a quarantine pest with serious risks of entering the country and, consequently, damaging agricultural production and restricting free circulation of vegetals (García-Álvarez et al., 2014). There are approximately 124 species belonging to the genus *Dysmicoccus* around the globe, especially within the Neotropical region (Williams and Granara de Willink, 1992). Species such as *D. brevipes* affect diverse crops, especially by the transmission of plant virus (Granara de Willink, 2009). In persimmon, ten species of mealybugs were registered, among *D. brevipes*, *M. hirsutus* and various species of *Phenacoccus* (García et al., 2016) also observed in the present work. *P. citri* and *Dysmicoccus* sp. are the most frequent mealybugs found in grapevines in Rio Grande do Sul (Morandi Filho et al., 2015). It is known that *P. solenopsis* infest 202 plant species within 55 botanical families in Africa, Asia and North and South America (Williams and Hodgson, 2014).

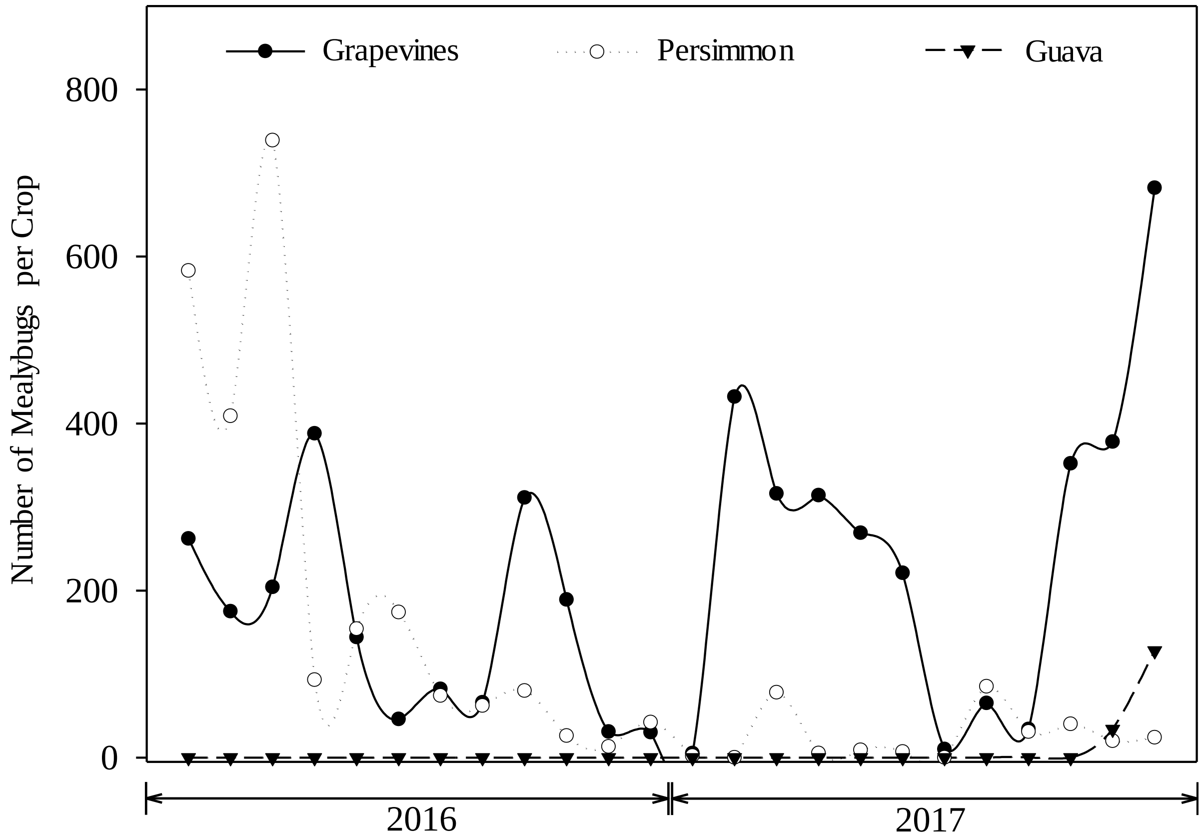

Figure 2. Population fluctuation of mealybugs in grapevines, persimmon and number crops in the São Francisco Valley.

During the evaluation period, 24 samplings were performed with a total of 10,189 mealybugs collected between nymphs and adult females. Higher infestation was observed in grapevines, with 5006 individuals. Persimmon was the second most affected crop by mealybugs, with 2750 Pseudococcidae. Pear crops showed high infestation by Pseudococcidae, with 2180 individuals. The lowest infestations were observed in guava, apple and Barbados cherry, with 162, 68 and 23 mealybugs, respectively. In grapevine, which is the most affected crop by mealybugs, the population remained with fluctuation along the sampling period, confirming a frequent presence in this crop with slightly variable population apices. Persimmon showed an elevated population peak in the first three samples, with a drastic population reduction in all other samplings. The presence of mealybugs in guava crops was observed only in the last two samplings, with 34 and 128 mealybugs (Figure 2). In pear, an elevated number of mealybugs were observed; the population was higher with few population peaks in the median third of the sampling period. Apple was slightly affected by Pseudococcidae, with few samplings recording these insects, as well as in Barbados cherry which showed a population peak with only 8 mealybugs per plant (Figure 3).

Family Pseudococcidae was present in all crops evaluated. In grapevines the results are in agreement with Bertin et al. (2013), who reported the presence of mealybugs in Brazil and Oliveira et al. (2014) reported the presence of the species *M. hirsutus* in the Sao Francisco Valley in grapevines. Lo (2012), remarks the difficulty to control mealybugs in apples in New Zealand. At a global scale, species such as *D. brevipes*, *M. hirsutus* and *P. citri* are associated with persimmon (García et al., 2016; Silva et al, 2016). In Maceió, Alagoas, in the Northeastern Brazil, Pseudococcidae species were associated with guava and Barbados cherry (Broglio et al., 2015). Seasonality of temporal and climatic factors may directly influence mortality, egg laying, feeding, growth, development and migration of insects. Temperature, rainfall, relative humidity and wind velocity are the main climatic factors affecting population dynamics in insects, including pest insects and natural enemies (Calore et al., 2013).

Inadequate control of pests with synthetic insecticides is one of the main reasons for the increase of insect resistance and other behavioral modifications (Ootani et al., 2013). The farms did not allow access to the spraying records. Use of chemical insecticides has been frantically increased, leading to diverse problems such as the

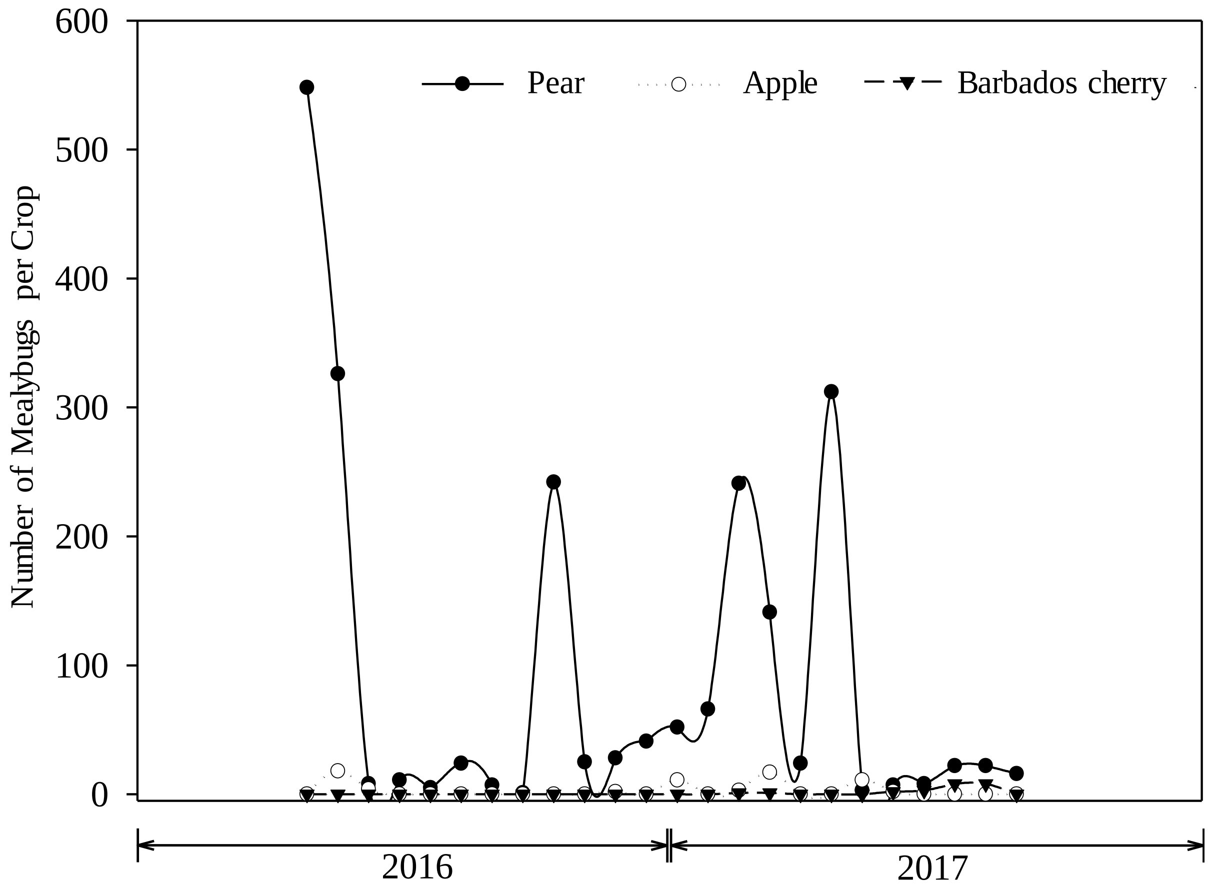

Figure 3. Population fluctuation of mealybugs in pear, apple and Barbados cherry crops in the São Francisco Valley.

reappearance of pests and the insertion of insects in the status of pests (Holtz et al., 2016); therefore, mealybugs' population fluctuation is directly related to the phytosanitary management applied in farms, that is to say, inadequate control may result in pest super populations, reducing the beneficial entomological fauna and producing environmental contamination.

When analyzing which crop was more affected within each specific part, we verified there were no significant difference in roots, trunks or leaves, while in fruits, the most affected were grapes and the less affected were apples, guava and Barbados cherry. Grapevines, pear and persimmon showed a statistically superior number of mealybugs in fruits, when compared to other plant parts, while mealybugs observed in apple, guava and persimmon were statistically equal in all plant parts, by the Tukey test at 5% probability. Pseudococcidae preference by different plant parts can be observed in Table 2.

The total soluble solids and concentration of carbohydrates and nutrients predominate in fruits when compared with other plant parts; according to the model of pressure flow, the pressure gradient between source and sink activate sap transport in the phloem through the elements of sieve tubes. This osmotic gradient results from sap loading in the source and unloading in the sink. Being considered as sinks, fruits receive more nutrients that the other plant parts and this fact is directly related with the attraction of mealybugs (Santa-Cecília et al., 2013; Taiz and Zeiger, 2017). Roots and trunk are more resistant structures than leaves and fruits. Due to the external physical structure of fruits which are less thick compared to the other plant parts, mealybugs tend to preferably feed on fruits, once their mouthparts penetrate easily in fruit tissues for feeding (Santa-Cecília et al., 2013; Snodgrass, 1935). Due to the preference of Pseudococcidae for fruits, they are more harmful for fruit crops. Mealybugs produce honeydew, a substance composed of sugars which enhances the proliferation of sooty mold, and consequently reduces the qualitative and quantitative value of fruits, directly affecting commercialization (Daane et al., 2012; Bertin et al., 2013). Even when the whole fruit is not lost, its commercial price reduces significantly, sometimes even under production prices.

The results obtained for faunistic analysis are in agreement with the above, where higher abundance and frequency of mealybugs is associated with grapevines. In

Table 2. Distribution of mealybugs associated to plant parts from different crops in the São Francisco Valley

Crop	Plant part			
	Root	Trunk	Leaf	Fruit
Grapevines	0.2^{aB}*	11.2^{aB}	5.60^{aB}	183.0^{aA}
Pear	1.3^{aB}	0.40^{aB}	13.8^{aB}	74.8^{bA}
Apple	0.0^{aA}	0.10^{aA}	1.90^{aA}	0.80^{cA}
Persimmon	0.0^{aB}	2.70^{aB}	21.3^{aB}	91.0^{bA}
Guava	0.0^{aA}	0.00^{aA}	0.00^{aA}	6.90^{cA}
Barbados cherry	0.0^{aA}	0.00^{aA}	0.00^{aA}	1.00^{cA}

*Means followed by the same non-capital letter within the column and capital letter within rows are not statistically different by the Tukey test at 5% probability.

Table 3. Faunistic analysis of mealybugs present in crops grapevines, pear, apple, persimmon, guava and Barbados cherry in the São Francisco Valley.

Crop	No. of individuals	Abundance	Frequency	Constancy (%)
Grapevines	5006	MA	MF	W
Pear	2180	C	F	W
Apple	2750	C	F	W
Persimmon	68	R	PF	Z
Guava	162	R	PF	Z
Barbados cherry	23	R	PF	Z

MA: highly abundant, C: common, R: rare; MF: very frequent, F: frequent, PF: rarely frequent; W: constant, Z: accidental.

persimmon and pear, mealybugs were considered frequent, constant and of common abundance while in apple, guava and Barbados cherry they were considered of rare abundance, rarely frequent and accidental, as shown in Table 3.

In order to achieve a successful integrated management of pests in an efficient and agile manner, it is fundamental to perform a coherent sampling of pest population. Here, faunistic analysis is widely used to assist in determining variables such as frequency and abundance, as well as fundamental for decision making in pest management (Silva et al., 2016).

The Sao Francisco Valley region is of special consideration due to its high productivity and quality of fruits produced, which occurs mainly due to the local environmental conditions and specialized management of crops (Silva et al., 2015). The environment also contributes to the development of pests such as mealybugs, which require an adequate management, and therefore, the knowledge of each species is fundamental for an efficient control (Bordeu et al., 2012).

Conclusion

Species of mealybugs observed in the sub-medium Sao Farncisco Valley were: *Planococcus* sp., *Planococcus citri* Risso (1813), *P. solenopsis* Tinsley (1898), *D. brevipes* Cockerell (1893), *M. hirsutus* Green (1908), *F. virgata* Cockerell (1893) and *P. elisae* Borchsenius (1947), distributed along the whole year. Pseudococcidae has higher preference for fruits, with the most infested crops being grapevines, persimmon and pear.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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