

## Full Length Research Paper

## Development and longevity of Citrus mealybug *Planococcus citri* (Risso, 1813) (Insecta: Homoptera: Pseudococcidae) associated with grapevine

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The Citrus mealybug *Planococcus citri* has a wide geographical distribution and has been described as a pest of economic importance in several crops. The present work determined the developmental and biological aspects of the Citrus mealybug in order to obtain information that may support the integrated pest management (IPM) of grapevine (*Vitis vinifera* L.) cv. Syrah in the Lower Basin of the São Francisco Valley region. The research was conducted at the Laboratory of Entomology of Embrapa Semiárido, Petrolina-PE, on leaves of grapevine kept in a controlled environment ( $25 \pm 1^\circ\text{C}$ ,  $60 \pm 10\%$  R. H. and a photoperiod of 12L:12D). The first two instars had higher mortality, indicating high susceptibility in these nymphal periods. The overall nymphal period of females and males is similar at  $22.52 \pm 0.46$  and  $23.5 \pm 0.29$  days, respectively, with viability of 39%. The adult longevity of females is nearly 30 times greater than that of males, indicating that females of *P. citri* are mainly responsible for damage and injury to grapevine. The sex ratio was 0.64, indicating that females make up the majority of the adult population of *P. citri*. We conclude that the species in question completes its lifecycle on leaves of grapevine and reaches the adult phase in a short time interval.

**Key words:** Mealybugs, life cycle, grape.

### INTRODUCTION

Viticulture of the semiarid region in the Lower Basin of the São Francisco Valley (LBSFV) is characterized by

high productivity and quality of grapes and wines, and especially by the environmental conditions and its

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integrated grape production system. It is one of the major centers of fruit production in Brazil, being a pioneer in the production of grapes, juice and wine in tropical conditions (Silva et al., 2009; Camargo et al., 2011).

However, due to the expansion in cultivated area and constant changes in the agroecosystem of the grapevine some problems of a phytosanitary nature, such as the occurrence of pests, especially mealybugs, are being reported in the region (Oliveira et al., 2008). According to the literature, among the pests associated with grapevine, mealybugs stand out for causing frequent crop damage, since they limit the quality and yield of fruits (Daane et al., 2008; Bertin et al., 2010; Formolo et al., 2011; Ghini et al., 2011; Bordeu et al., 2012).

Mealybugs of the Pseudococcidae family are characterized by presenting a body covered by a thin layer of white waxy secretion. They are small sucking insects that feed on sap from the phloem of plants, live in colonies and can be found on different parts of the host plant, thus constituting an important group of insect pests associated with different cropping systems and agronomic crops (Santa-Cecilia et al., 2007; Daane et al., 2008; Cid et al., 2010; Daane et al., 2012).

On ingesting their food mealybugs excrete a sugar-rich substance known as honeydew, often associated with the development of fungi and the presence of ants. The accumulation of honeydew on leaves and fruits results in direct damage to the grapevine, and may even, in some cases, cause death of the plant. Fungi commercially depreciate the clusters, resulting in the disposal of the product (Culik and Gullan, 2005; Daane et al., 2008; Cid et al., 2010; Ahmed and Abd-Rabou, 2010).

Many species of mealybugs coexist and infest vineyards of important wine centers in various countries, hindering sustainable pest control. In this sense, each species has its own biological features, it being necessary initially to know them in order to deploy control and integrated pest management programs (Daane et al., 2008; Mahfoudhi and Dhouibi, 2009; Cid et al., 2010; Bordeu et al., 2012). Among the species that stand out in Brazil is *Planococcus citri* (Risso, 1813), which is most abundant in the vineyards of the region Serra Gaúcha, Rio Grande do Sul (Morandi Filho et al., 2008).

Given the above, the objective of this research was to determine the development and longevity of *P. citri* associated with grapevine, aiming to provide information to support the Integrated Pest Management of the grapevine (IPM-grape).

## MATERIALS AND METHODS

### Identification of *Planococcus citri*

The identification of *P. citri* was done by researchers Dr. Cherre Sade Bezerra da Silva and Dr. Caroline Viana Morgante using molecular markers developed for identifying pseudococcids (Rung et al., 2008).

### Rearing and maintenance of *Planococcus citri*

Rearing of *P. citri* was done from insects collected from grapevine clusters on a commercial farm in the municipality of Petrolina-PE. As a cleaning process, pumpkins were washed in running water, and then a neutral detergent was applied with sponges moistened with water, followed by rinsing. The pumpkins were again washed in running water and dried at room temperature. The insects were then transferred with the aid of fine tip brushes to the pumpkins (*Cucurbita moschata* Duschesne) of the cultivar Jacarézinho.

The pumpkins were kept in small plastic pots (10 × 15 cm) and placed in wooden cages (53.5 × 43 × 47.5 cm), with a glass upper surface, sides of nylon mesh screen and a front surface covered by "voile"-like material, at 25 ± 1°C, 60 ± 10% RH and a photoperiod of 12 h.

### Host plant

Grapevine leaves (*V. vinifera*) of the cultivar Syrah were collected in a vineyard at the Bebedouro Experimental Station (09° 09'S, 40° 22' W) of Embrapa Semiárid in Petrolina, PE. In the collection area and proximity there was no application of insecticides.

The leaves were washed in running water, then dried with a paper towel and observed under binocular loupes (with 22-fold increase) to check for the presence of opportunistic arthropods.

### Determination of development and longevity

The work was conducted at the Laboratory of entomology of Embrapa Semiárid, Petrolina-PE, in climatic chambers of the B.O.D. type (25 ± 1°C, 60 ± 10% R. H. and photoperiod of 12L:12D). The determination of the biology of *P. citri* was made from newly hatched nymphs, maintained on leaves of grapevine. To obtain nymphs, adult females in the reproductive phase were collected and placed in Petri dishes containing water-agar (2%) and grapevine leaf discs (7 cm diameter). After hatching, the first instars were placed on leaf discs (3 cm diameter), which were placed in Petri dishes (9 cm diameter) with the abaxial side up. To maintain turgor, the leaves were placed under a layer of water-agar (2%). Soon after, the plates were sealed with PVC film and taken to the B.O.D.

To reduce the risk of contamination by fungi and other pathogens, caused by exudates from the leaf discs, and to maintain and provide food to the insects, the Petri dishes were replaced and the water-agar solution and leaf discs were renewed at five day intervals. In addition, to prevent damage to the mouthparts and allow the natural movement of the mealybug a cut was made, with a scalpel, of a small leaf area around the insect and with the aid of forceps it was moved to the new leaf disc (Santa-Cecilia et al., 2008; Correa et al., 2011).

The leaf discs containing second and third instar male nymphs were transferred to plates without water agar because at this stage the insect's mouthparts atrophy and it does not drink (Correa et al., 2005; Santa-Cecilia et al., 2009; Ross et al., 2012).

### Parameters evaluated

The parameters evaluated were duration and viability of the nymphal period, longevity and sex ratio.

The assessment of the change of instar of males and females was based on the release of exuviae, being done daily with the aid of magnifying glasses. After registration, the exuviae were removed from the plates with the aid of brushes.

During the first and second instars the replicates were constituted of individuals of unknown sex because there is no overt sexual

**Table 1.** Duration in days (mean  $\pm$  SE) of the development of *Planococcus citri* on leaves of grapevine (*Vitis vinifera* L.) cultivar Syrah. Laboratory conditions of  $25 \pm 1^\circ$  C, 60  $\pm$  10% R. H. and photoperiod of 12L:12D.

Sex	1st instar	2nd instar	3rd instar	4th instar	Nymphal period	Adult longevity
Female	8.20 $\pm$ 0.24 <sup>a</sup>	6.60 $\pm$ 0.44 <sup>a</sup>	7.72 $\pm$ 0.25 <sup>a</sup>	*	22.52 $\pm$ 0.46 <sup>a</sup>	63.68 $\pm$ 5.45 <sup>a</sup>
Male	7.93 $\pm$ 0.22 <sup>a</sup>	7.50 $\pm$ 0.23 <sup>a</sup>	2.86 $\pm$ 0.29 <sup>b</sup>	5.21 $\pm$ 0.42	23.50 $\pm$ 0.29 <sup>a</sup>	2.07 $\pm$ 0.25 <sup>b</sup>

\* No 4th instar occurs; Means followed ( $\pm$  SE) by the same letter in the column do not differ by Tukey test ( $p < 0.01$ ).

differentiation. Sexual dimorphism becomes more evident at the end of the second instar, when the males form cocoons to complete their development (Silva et al., 2010; Correa et al., 2011).

#### Analysis of experimental data

The experimental design was fully randomized with 100 replications. Initially, each replicate contained two nymphs of the same age. After the setting of these nymphs one was removed. The data for the parameters evaluated were submitted to analysis of variance and the means were compared by Tukey test ( $p < 0.01$ ) using the program BioEstat 5.0 (Ayres et al., 2007).

## RESULTS AND DISCUSSION

When kept on grapevine, males have four instars, whereas females have three instars (Table 1), corroborating other studies of the biology of mealybugs in which males and females have four and three instars, respectively (Chong et al., 2008; Morandi Filho et al., 2008; Santa-Cecilia et al., 2009; Vennila et al., 2010; Bertin et al., 2013; Fand et al., 2014).

There was not a significant difference, in days, in the duration of the first two instars for males and females. This period corresponded to approximately 14 and 15 days for females and males, respectively (Table 1).

The nymphs, principally of the first instar, were characterized by yellow coloration, little white wax, substantially reduced size and great mobility compared to later instars, corroborating the characteristics presented by Correa et al. (2005) and Santa-Cecilia et al. (2007). The small size and great mobility of the nymphs is a notable characteristic of the first two instars, favoring their spreading to other structures of the host plants, thereby hindering their location (Daane et al., 2008; Cid et al., 2010; Ross et al., 2010, 2012).

The duration of the first instar in males varied from 7 to 9 days and in females from 7 to 11 days. In the second instar, the duration in males varied from 6 to 9 days, whereas in females this variation was from 4 to 14 days. In studies of the biology and development of *P. citri*, Correa et al. (2005), Morandi Filho et al. (2008) and Santa-Cecilia et al. (2009) observed that the first instar is the longest and can last up to four days longer than the second instar. However, the duration of the first instar found by these authors was longer than that observed in this study. In the first instar, Correa et al. (2005) found

10.4  $\pm$  3.1 days for males and 11.6  $\pm$  2.6 days for females on sweet orange (*Citrus sinensis* L.) cv. Bahia. In grapevine Morandi Filho et al. (2008) found 11.17  $\pm$  0.18, 11.20  $\pm$  0.16 and 11.06  $\pm$  0.20 days in the cultivars Cabernet Sauvignon, Itália (*Vitis vinifera* L.) and Isabel (*Vitis labrusca* L.), respectively.

The inverse situation, in which the duration of the second instar is longer than the first or similar, can also be observed in *P. citri* or other pseudococcids, as reported by Ahmed and Abd-Rabou (2010) and Francis et al. (2012) in *Planococcus minor*, Bertin et al. (2013) in *Dysmicoccus brevipes*, and Fand et al. (2014) in *Phenacoccus solenopsis*. This difference is probably due to, among other factors, the use of different substrates and the nutritional quality that this furnishes to the insect, since the experimental conditions were similar.

The duration of third and fourth instars in males were of 2 to 5 days and 2 to 7 days, respectively. It was observed that the period in which the males remained in cocoons was similar to the duration of the third instar in females, which showed duration of 4 to 10 days (Table 1). Both the results are different to those found by Correa et al. (2005), who observed a mean duration of 6.3 $\pm$ 1.9 days for third instar females and 10.7 $\pm$ 2.7 days for males during the period that they remained in cocoons. Morandi Filho et al. (2008) observed that the development of males, especially during the third and fourth instars, can be inferior to that of females when fed on grapevine; moreover, when the development occurred on grapevine roots, the duration was similar or superior in males.

The viability presented by females and males during the first two instars (Table 2) showed that in this period the insects are more fragile, corroborating other studies, where high mortality of pseudococcids is observed during the first and second instars (Chong et al., 2008; Morandi Filho et al., 2008; Vennila et al., 2010; Fand et al., 2014).

In the third instar there was not a significant difference between the viability of females and males. Survival results similar to those found in our study were reported by Francis et al. (2012) and Fand et al. (2014). It was observed that even in the cocoon the males are susceptible, since mortality occurred in the passage from the third to the fourth instar. This result differs from those found by Correa et al. (2005), in which mortality did not occur in the change of male instars. Comparing our results for longevity of males to those reported in the scientific literature, there is evidence that the presence of

**Table 2.** Viability (mean  $\pm$  SE, %) of *Planococcus citri* on leaves of grapevine (*Vitis vinifera* L.) cultivar Syrah. Laboratory conditions of  $25 \pm 1^\circ$  C,  $60 \pm 10\%$  R. H. and photoperiod of 12L:12D.

Sex	1st instar	2nd instar	3rd instar	4th instar	Nymphal period
Female	70.00 $\pm$ 5.77**	71.48 $\pm$ 3.12**	79.17 $\pm$ 7.42 <sup>a</sup>	-*	39.00 $\pm$ 4.92**
Male			77.77 $\pm$ 9.03 <sup>a</sup>	100	

\* No 4th instar occurs; \*\*Both sexes, males and females; Means followed ( $\pm$  SE) by the same letter in the column do not differ by Tukey test (p <0.01).

cocoons does not seem to be a factor that ensures survival, whether in the third or fourth instar, since the presence of a cocoon also did not impede the occurrence of mortality in the studies conducted by Morandi Filho et al. (2008), Francis et al. (2012) and Fand et al. (2014)

In the adult phase, the longevity of females was much greater than that of males, nearly 30 times greater (Table 1). Maximum durations of 99 and 4 days of longevity were registered for females and males, respectively, in this phase. For females, the result found in this study is much longer than that reported by Morandi Filho et al. (2008), Vennila et al. (2010), Francis et al. (2012), Bertin et al. (2013) and Fand et al. (2014). Possibly this result occurred due to the absence of the reproductive period of *P. citri*.

The total cycle, here understood as the nymphal phase and the adult phase, also differed between females and males, which presented approximately 86 and 25 days respectively, such that in females the cycle was 3.4 times greater than that of males. In this case, the difference is due to the adult longevity of the females, which was much greater than that of the males (Table 1).

It was observed that in both of the sexes the period of nymphal development is similar, although the number of instars is different. In studying the biology of *P. citri* and *P. minor*, respectively, Correa et al. (2008), Francis et al. (2012) and Fand et al. (2014) report that the duration of nymphal states as well as the nymphal-adult cycle of females and males can be close or similar, even at different temperatures. However, Morandi Filho et al. (2008) and Correa et al. (2005) observed a prolongation of 7 to 8 days in the nymphal period of females.

Assessment of viability in the nymphal period shows a low rate of survival associated with high mortality during the first two nymphal instars; after this period the mortality was reduced, demonstrating that the later instars are less susceptible. Similar results with a low rate of survival were described by Chong et al. (2008), Morandi Filho et al. (2008) and Vennila et al. (2010).

The sex ratio obtained was 0.64; this shows the greater number of females in the adult phase relative to the number of males. This corroborates the reports of Chong et al. (2008) and Vennila et al. (2010), where there was a greater proportion of females relative to males. In agreement with Francis et al. (2012), females can correspond to between 60 and 73% of the population of mealybugs.

The similarities or differences between the results found in this study compared with the literature can be attributed to different factors, such as temperature and host plant. According to Chong et al. (2008), Lazzari and Zonta-de-Carvalho (2009), Vennila et al. (2010), Francis et al. (2012) and Fand et al. (2014), these are among the main factors that exert influence on the biology of insects, especially sap sucking insects, as is the case for mealybugs. Notwithstanding, understanding the biology of *P. citri* from the results obtained is the basis for the beginning of integrated pest management programs in vineyards of the LBSFV, since despite being pioneering research in the region, concrete and precise data are presented about the biology of the pest in question, generating information for future work.

## Conclusions

The mealybug *P. citri* completes its life cycle in leaves of grapevine (*Vitis vinifera* L.) cultivar Syrah showing high longevity for adult females. In grapevine, the nymphal period of females and males of *P. citri* is similar.

## Conflict of Interest

The authors have not declared any conflict of interest.

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