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***Planococcus citri* (Risso, 1813) on Grapevine: Do Presence of the Male Influences Reproduction?**

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ABSTRACT

The reproductive biology of mealybugs is a topic of great discussion and contradiction with regard to the study of the bio-ecology of the pests. The main reason for controversy is the possibility that facultative parthenogenesis occurs, because it is reported as occurring and questioned by several authors. Thus, the objective of this study was to evaluate the reproductive biology of females of *Planococcus citri*, coming from vineyards in the Lower Basin of the São Francisco Valley and the importance of male for progeny in this species. The experiment was conducted at the Laboratory of Entomology of Embrapa Semiárido (Petrópolis-PE) in a controlled environment under the conditions of 25±1°C, 60±10% RH and a photoperiod of 12L:12D. Second instar nymphs were isolated individually in order to obtain virgin adult females. Individuals were kept in Petri dishes containing water-agar solution (2%) and a leaf disc of the grapevine (*Vitis vinifera* L.) cultivar Syrah, in the absence and presence of adult males. The studied population of *P. citri* reproduces only sexually, therefore, the presence of the male plays a key role in the reproduction of this species. In counterpart, non-copulated females preserve their morphological characteristics, such as waxy covering of the body, greater longevity and building of ovisacs.

Key words: Mealybug, fecundity, fertility, *Vitis vinifera*

INTRODUCTION

Mealybugs (Hemiptera: Pseudococcidae) comprise an important group of insect pests with a wide geographic distribution. Plants of economic importance such as cotton, coffee, citrus, mango, soy, grapevine and ornamentals are attacked by these pseudococcids (Santa-Cecilia *et al.*, 2007; Morandi Filho, 2008). Specifically in relation to grapevine, the damages caused by direct and indirect action have increased costs aimed at their control, so, the mealybugs have become an increasingly important group of pests for the crop (Daane *et al.*, 2008).

These pseudococcids are small insects, with a soft body and they feed on the sap produced in the phloem tissue of plants. Mealybugs are described as floury because their body is covered by a white waxy material (Culik and Gullan, 2005; Santa-Cecilia *et al.*, 2007).

The mealybug *Planococcus citri* (Risso, 1813) is a polyphagous and cosmopolitan species of great agronomic importance. Due to its occurrence and damages, the scientific community has conducted diverse studies aimed primarily at different temperatures and host plants, to elucidate its bioecology (Correa *et al.*, 2008; Morandi Filho, 2008; Ahmed and Abd-Rabou, 2010; Cid *et al.*, 2010). However, despite the work and ongoing discussions on this topic, there are several key points that are controversial, such as the form and the mechanisms of reproduction that occur in the species, since, there is great divergence among the information (Chong *et al.*, 2008; Zaviezo *et al.*, 2010; Correa *et al.*, 2011). The possibility that facultative parthenogenesis occurs is the main source of controversy in this area (Da Silva *et al.*, 2010; Waterworth *et al.*, 2011).

In the group of the Coccoidea, for example while some species such as *Phenacoccus solenopsis* (Tinsley, 1898) and *Dysmycoccus brevipes* (Cockerell, 1893) reproduce in the absence of the male (Vennila *et al.*, 2010; Bertin, 2011) others such as *Planococcus ficus* (Signoret, 1875) and *Pseudococcus viburni* (Signoret, 1875) require mating to produce viable offspring (Waterworth *et al.*, 2011).

In case parthenogenesis is optional in *P. citri*, as in other species of the genera *Planococcus* and *Pseudococcus*, the use of techniques and the viability of pheromones in Integrated Pest Management (IPM) programs may be adversely affected. Therefore, it is necessary to know the reproductive biology of these insects to integrate the use of pheromones into IPM. The reproduction (sexual or asexual), the fecundity of the female, the sex ratio of offspring, the mating ability and longevity of both sexes are among the main factors for the use of pheromones. Moreover, fecundity and female form of reproduction also have a close relationship with the injuries and damage levels caused by mealybugs, because these characteristics reflect the increase or decrease in the population of the insects (Walton *et al.*, 2006; Waterworth *et al.*, 2011; Waterworth and Millar, 2012).

Given the above, what are the main limitations and/or implications that may affect the reproduction of these insects? What is the importance of the presence of the male and of copulation in *P. citri*?

Thus, this study aimed to evaluate the reproductive biology of females of *P. citri* on grapevine and the importance of the male for the progeny of this species, occurring in the Lower Basin of the São Francisco Valley.

MATERIALS AND METHODS

The study was conducted at the Laboratory of Entomology of Embrapa Semiárida, Petrolina, PE, in the B.O.D. climatic chambers (25±1°C, 60±10% RH and photoperiod 12L:12D).

Second instar females were collected with the aid of fine tip brushes from pumpkins (*Cucurbita moschata* Duschene) of the cultivar Jacarézinho and reared and maintained individually in Petri dishes (9 cm diameter) on leaf discs (3 cm diameter) of grapevine (*Vitis vinifera* L.) cultivar Syrah with the abaxial surface facing upward. To maintain the turgor of the leaves a water-agar solution (2%) was used. Afterwards, the plates were sealed with PVC film, labeled and taken to the B.O.D. Females were kept under these conditions to ensure that copulation was performed with virgin adult females, remaining under the same experimental conditions for the complete life cycle (Da Silva *et al.*, 2010).

Adult males were collected with the aid of fine tip brushes and placed inside Petri dishes which contained a virgin adult female.

The leaf discs and agar-water mixture were renewed every seven days by replacing the Petri dishes and the PVC film. To avoid damage to the mouthparts and allow the natural

movement of insects, a cut was made with a scalpel of the area around the insect and with the aid of tweezers it was moved to the new leaf disc (Santa-Cecilia *et al.*, 2008; Correa *et al.*, 2011).

The exchange of exuviae of females, the pre-oviposition period (duration between changes of the adult female and the first egg production), oviposition (duration between the start and end of egg production) and post-oviposition (duration of last production of eggs to death female), number of ovisacs per female, number of eggs per mass, viability and duration of incubation of eggs and longevity of females were assessed daily with the aid of stereoscopic microscopes.

The ovisac and eggs were removed with the aid of fine tip brushes moistened with distilled water, placed in Petri dishes on moistened filter paper and counted. Around the eggs a circle was made with a black pencil (No. 2) to facilitate locating them. Then, the plates were covered with PVC plastic wrap, labeled and placed in the B.O.D. under the same experimental conditions.

The experimental design was fully randomized with two treatments and twenty replicates. The first treatment consisted of virgin adult females in the absence of the male and the second, in the presence of an adult male, on average one day old. In the second treatment, each female received only one male placed always in the morning between 7:45 and 08:00, since, it was observed that at that time there were a large number of males in the maintenance breeding area. Adult females were on average 8 days of age. The male remained with the female for 1 day and was replaced the following day. During the experiment it was not observed if there is rejection of mating by either females or males.

The data of the evaluated parameters were subjected to analysis of survival and means were compared by log-rank test at 1% significance using BioEstat 5.0 (Ayres *et al.*, 2007) statistical software.

RESULTS AND DISCUSSION

Description of the moment of copulation: The copulation of insects was visualized and observed only in the first 4 h on the first day of contact between females and males, hence, the description that follows refers to the first instances of copulation of the insects (Fig. 1).



Fig. 1: Copulation of *Planococcus citri*, Male is holding the female for copulation

As males were placed in the Petri dishes, it was noted that females seemed to notice the presence of the male, since, they lifted the edge of their abdomens in order to attract and/or allow the introduction of the male copulatory organ. However, females did not move during this time, probably because they are fixed to the leaf disc.

On perceiving this signal, immediately, the males went to the females. First, they roamed around the body of the female then positioned themselves at the back to perform copulation.

During the evaluations, it was also observed that females attract males even with the plate sealed or within the B.O.D., because when being evaluated, males were seen standing on the PVC film and seeming to search for a way to enter the plate to copulate with the female. In the morning a large number of males were noticed on the floor around the B.O.D. This behavior can be explained by the relationship between the production and perception of pheromones, in which the males, after perceiving the emission, fly towards the females to mate. According to Waterworth and Millar (2012) this behavior is common among these insects, because adult females, mated or not, produce pheromones constantly. The emission of pheromones can overcome certain barriers, because even confined according to the methodology. The pheromone was perceived by the males which in turn responded positively, flying toward the female.

Longevity differed between treatments ($p < 0.001$) and was influenced by the presence of males. Note that copulated females lived for a shorter period, on average, 31 days. In this experiment we registered longevity of 17-45 days and 14-83 days for copulated and not copulated females, respectively. Studies conducted by Chong *et al.* (2008), Correa *et al.* (2011) and Francis *et al.* (2012) showed that adult virgin females, kept in isolation, of the species *Maconellicoccus hirsutus* (Green, 1908), *P. citri* and *P. minor*, respectively, can live for up to 2 months.

On studying the reproductive biology of females of *Pseudococcus maritimus* (Ehrhorn, 1900), Grimes and Cone (1985) and Waterworth and Millar (2012) observed that females have shorter longevity when mated. Grimes and Cone (1985) explain that this decrease probably occurs because the metabolic expense to produce the ovisac and perform oviposition.

Another difference observed between treatments was with respect to morphology, as on reproducing and generating offspring copulated females became smaller and had less waxy material covering the body (Fig. 2a, b).



Fig. 2(a-b): Morphological differences between *Planococcus citri* females of the same age, (a) Non-copulated and (b) Copulated

Table 1: Longevity and reproductive parameters (Mean±SE) of females of *Planococcus citri* (Hemiptera: Pseudococcidae) in the absence (T1) and the presence of the adult male (T2)

Treatments	Longevity	Pre-ovi.	Oviposition	Post-ovi.	Daily Fec.	Total Fec.
T1	52.70±6.28 ^a	*	*	*	*	*
T2	31.60±2.01 ^b	12.30±0.57	15.45±1.58	3.85±0.81	8.85±0.79	129.05±12.79

Means±SE followed of different letters vertically differ significantly by Log-rank test ($p < 0.001$), *Females did not oviposit, Pre-ovi: Pre-oviposition, Post-ovi: Post-oviposition, Daily Fec: Daily Fecundity, Total Fec: Total fecundity, Laboratory conditions of 25±1°C, RH 60±10% and photoperiod 12L:12D

Based on the results obtained, it was possible to confirm that virgin females kept in isolation (no contact with the adult male) did not reproduce (Table 1) but even in the absence of copulation a small percentage of these (20%) built the ovisac. It was also observed that copulated females could not form the ovisac and perform oviposition for one or more days. The presence of males and mating are essential for reproductive success and progeny in this population and it appears the formation of the ovisac is a process independent of copulation, corroborating the observations of Chong *et al.* (2008), Da Silva *et al.* (2010), Zaviezo *et al.* (2010), Correa *et al.* (2011) and Francis *et al.* (2012), who reported similar behavior in other pseudococcids.

According to Da Silva *et al.* (2010) non-copulated females of *P. viburni* and *P. calceolariae* (Maskell, 1879) can produce ovisacs, however, only a small percentage of these perform oviposition (maximum 8) and the eggs are sterile. The authors also point out that non-copulated females of *P. citri* do not build an ovisac. However, they conclude that in *P. viburni* and *P. calceolariae* the formation of the ovisac and oviposition are independent of copulation while in *P. citri* this process depends on copulation.

The oviposition period showed the greatest duration and corresponded to about half of longevity, being followed by pre-oviposition. The post-reproductive period showed the shortest duration (Table 1). Francis *et al.* (2012) reported that in *P. minor* the duration of the pre-reproductive and reproductive periods were similar. However, the proximity or distance between the duration of pre-oviposition and oviposition may be related to the mode of reproduction (Bertin, 2011) which was sexual in this study.

The fecundity and egg viability were satisfactory (Table 1), when compared with other studies on the bio-ecology of *P. citri* and other pseudococcids such as *D. brevipes* and *P. viburni*, also conducted in grapevine leaves (*Vitis vinifera*). We registered a range of 27-242 eggs per female, with viability to 86.90±1.54%. Compared to other studies involving the reproductive parameters mentioned above, the results observed in this study were average or greater in quantity than observed by Morandi Filho (2008), Zaviezo *et al.* (2010) and Bertin (2011).

It was also observed that with the passage of days females reduced the number of eggs produced, as evidenced by a negative correlation between reproduction days and the number of eggs (Fig. 3). The first to the third day of the reproductive period, females oviposit in larger quantities. It was noted that from the seventh day there was a decline in oviposition.

The period of incubation and egg viability were evaluated until the fourteenth day of oviposition, because from then on, the quantity of eggs produced was considerably reduced (Fig. 3). It was observed that the incubation time increases from the twelfth day and egg viability decreased from the ninth day (Fig. 4).

Reproductive parameters (Table 1) are essential for the maintenance of the offspring of *P. citri*, whether in controlled environments or not. Although, they were performed in the laboratory, the observations and data obtained are relevant to research on the behavior and

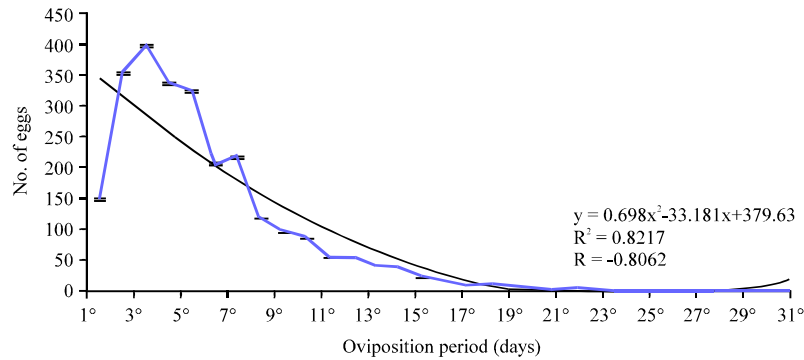


Fig. 3: Quantity of eggs produced during oviposition period by copulated females of *Planococcus citri*

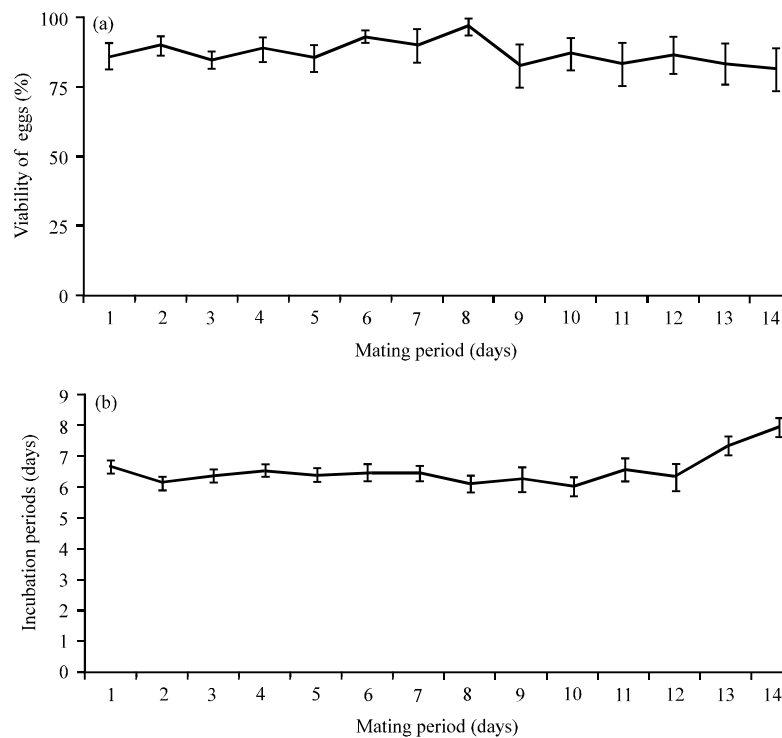


Fig. 4(a-b): Incubation period (days) and viability (%) of eggs (Mean $\pm$ SE) of female *Planococcus citri* during the first two weeks of the mating period

reproductive biology of these insects, because there is little information about the topic, especially in Brazil. Considering the socio-economic importance of the region and pest species mentioned, it noted that *P. citri* is a real threat, since it is part of a complex of insects responsible for the drop in yield and quality of grape and derivatives in many wine regions.

CONCLUSION

Reproduction in the population of *Planococcus citri* originating from vineyards of the Lower Basin of the São Francisco Valley is sexual, because non-copulated females do not produce eggs.

The construction of the ovisac is a process independent of copulation, however, in its absence egg production does not occur. The absence of descendants is due solely to the absence of the male and copulation.

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