

# Interaction between mealybugs (Pseudococcidae) and coffee plants

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## ABSTRACT

Mealybugs, *Planococcus citri* (Risso) and *Planococcus minor* (Maskell) (Hemiptera: Pseudococcidae) are able to colonize several plants, such as coffee (*Coffea* spp.), where may impair their production. Despite attacking many plants, frequent outbreaks are only found in certain plants. In this work, we tested the hypothesis that *P. citri* and *P. minor* show a better performance, i.e., greater longevity and higher reproductive rate, as well as higher survival in a given coffee species and it exists preference for volatiles of certain coffee species. We studied in the laboratory the food preference of these mealybugs through a free choice test and their development and reproduction in *Coffea arabica* L. cv. Mundo Novo-IAC-379-19 and *Coffea canephora* (Pierre & Froenher) cv. Conilon 213. The responsiveness of these insects to the volatiles emitted by these coffee plants was analyzed using an olfactometer. The data showed that mealybugs preferred Conilon coffee plants over the Mundo Novo and, despite completing their development in both of them, most of the biological evaluated parameters were affected by the host species. In the olfactometer assays, the volatiles emitted by the coffee plants cv. Mundo Novo and Conilon were equally attractive to these mealybugs. It is concluded that, although there is no discrimination of odors between coffee plants, Mundo Novo and Conilon, both species of mealybugs show food preference for Conilon, having their development and reproduction favored in these plants.

**Key words:** *Coffea* spp.; Food preference; Olfactory responses; Performance; White mealybug.

## 1 INTRODUCTION

The citrus mealybug, *Planococcus citri* (Risso) and the passionvine mealybug *Planococcus minor* (Maskell) (Hemiptera: Pseudococcidae) are important pests to the coffee crop in Brazil, attacking *Coffea arabica* L. and *Coffea canephora* (Pierre & Froenher). Nymphs and adult females of both species colonize the aerial part of plants, mainly the flower and fruit buds, as well as new shoots, and as a result sap sucking, fruits dry and fall, limiting crop yield (Santa-Cecília; Souza, 2014; Santa-Cecília et al., 2020).

These mealybugs are capable of colonizing various host plants, belonging to 84 and 73 botanical families for *P. citri* and for *P. minor* respectively (García Morales et al., 2016). Despite colonizing several plant species, outbreaks are more frequent in certain hosts, which can be attributed to several factors, including biological performance (Santa-Cecília et al., 2018).

This performance is related to the reproductive success depending on their ability to avoid and/or overcome the plant defenses present at the cuticle, epidermis, mesophyll and phloem levels. As an example, plant surface presents hair, thorns, trichomes and thick cuticles, which may exert some action preventing mealybug settling (Renard, 1993; Le Rü et al., 1995; Santa-Cecília; Prado; Souza, 2016).

Food quality and quantity are fundamental for insect performance (Slansky; Rodriguez, 1987; Parra, 1991; Awmack; Leather, 2002). Ingested food directly influences the distribution and abundance of insects, affecting biological

parameters such as fecundity, longevity, development and behavior (Silveira-Neto et al., 1976). As phloem sap sucking insect, mealybugs (Pseudococcidae) are greatly influenced by the quality of the phloem sap (Santa-Cecília; Prado; Souza, 2016).

Considering the importance of coffee culture for the economy of Brazil, and the frequency of attacks and damages of *P. citri* and *P. minor* to these plants, the objective of this work was to know some aspects about the colonization and establishment of these insects in *C. arabica* and *C. canephora*. We tested the hypothesis that *P. citri* and *P. minor* show a better performance, i.e., greater longevity, higher reproductive rate, as well as higher survival in a given coffee species and have an olfactory preference for volatiles in between coffee species.

We used the choice test to evaluate the food preference that, involves morphological characteristics of plant tissues such as the cuticle, cells, intercellular space, and phloem sap composition. The development, survival, and fecundity were evaluated on both coffee species considering that different hosts may affect parameters of their life history (Amarasekare et al., 2008; Qin et al., 2011; Tanga et al., 2013; Costa et al., 2016; Sousa et al., 2018). Attractiveness and repellency were tested using olfactometry (Santa-Cecília et al., 2018).

With the results obtained, a better understanding is expected of the interaction of these mealybugs and coffee trees, which could explain the most frequent outbreaks of these pests on certain coffee species.

## 2 MATERIAL AND METHODS

**Mealybugs:** *P. citri* was collected in coffee trees and *P. minor* from cocoa plants (*Theobroma cacao* L.). Insect rearing was established in laboratory on pumpkins (*Cucurbita maxima* L.) cv Cabotchá (Lepage, 1942), kept in Flanders batteries (0.42 x 0.63 x 0.39 m) in total scotophase under  $25 \pm 1^\circ\text{C}$  and  $70 \pm 10\%$  RH, according to the methodology described by Santa-Cecília, Prado and Oliveira (2013).

**Plants:** The treatments consisted of leaves taken from two coffee species, *C. arabica* cv. Mundo Novo-IAC 379-19 and *C. canephora* cv. Conilon 213. Leaves free from pests, diseases and injuries were collected in the middle third of the coffee plants, approximately four years old, growing at the Coffee Culture Studies Center - NECAF, Campus of the Federal University of Lavras, MG.

**Choice test for nymphal food preference:** The free choice test was conducted to determine the food preference of mealybugs. The arena consisted of a Petri dish (0.15 m diameter) containing a filter paper (0.05 m diameter) in the center and both coffee-leaf discs (0.05 m diameter) kept on a layer of 1% agar with the abaxial surface facing up. Ten third instar nymphs of each mealybug species, were taken from the rearing and fasted for one hour. Soon after, the insects were released in the middle placed on the circle of filter paper (Correa; Souza; Santa-Cecília, 2008; Santa-Cecília; Prado; Oliveira, 2013). So, both mealybugs species were allowed to choose between Conilon 213 and Mundo Novo leaves.

The plates were sealed with polyvinyl chloride plastic film (PVC) to prevent the mealybugs from escaping, kept at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH and covered with black fabric to avoid possible phototropic effect. The evaluations were performed at 24, 48 and 72 hours, counting the number of mealybugs present on each leaf substrate. Mealybugs found outside leaf discs (on agar and/or paper circle) were not counted. Results obtained at 72 hours of exposure were considered as the definitive preference.

A completely randomized experimental design was adopted for each bioassay, with two treatments (Mundo Novo - IAC 379-19 and Conilon 213) and ten replications, making a total of 100 nymphs for each mealybug species.

**Nymphal development and reproduction of mealybugs:** Newly hatched nymphs were placed on leaf discs of both hosts (abaxial surface) kept on a layer of 1% agar (0.005 m) in Petri dishes (0.05 m diameter). These Petri dishes were sealed with PVC plastic film to prevent mealybugs from escaping and placed in two climate chambers at  $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH and scotophase.

Nymphal development was monitored daily until they reached adulthood, using a stereomicroscope (30x). Soon after, couples were formed by placing a male, still in the cocoon with a female in Petri dishes (Santa-Cecília; Prado; Oliveira, 2013).

Nymphal period, mortality and the number of viable eggs (considering the number of nymphs emerged) were recorded.

The experimental design was completely randomized, using two treatments (Mundo Novo-IAC 379-19 and Conilon 213) and each Petri dish was considered a replicate. Ninety replicates were performed for each treatment and mealybug specie. Only females were considered for this analysis.

**Olfactory response:** The olfactory response of *P. citri* and *P. minor* mealybugs was studied using a four-arms olfactometer with purified air flow (modified from Vet et al., 1983).

The experiments consisted of leaf odors of Mundo Novo-IAC 379-19 and Conilon 213 for each mealybug species, kept inside a 400 cc glass container, which were randomly placed on the olfactometer. Two-arms were used for each odor. Air flux was calibrated to  $1.2 \text{ L}\cdot\text{min}^{-1}$  so each arm received  $0.3 \text{ L}\cdot\text{min}^{-1}$  of air. A stream of purified air carried the volatiles emitted by the coffee leaves to the center of the arena, where mealybug nymphs were released and exposed to these odors for 15 minutes. Thirty fasting nymphs (first instar) of each species were tested. A choice for an odor was defined when the insect surpassed a mark located at 0.02 m from the releasing point toward one arm. At the end of 15 minutes the arm choice was computed and considered as a definitive choice. The time of permanence in each arm of olfactometer was also registered (Santa-Cecília et al., 2018).

After 10 tests, the leaves were replaced and the odor tubes were washed with detergent, rinsed in running water and cleaned with 70% alcohol to avoid possible contamination in subsequent tests. The tests were conducted in an environmental room without external interference and visual stimulation.

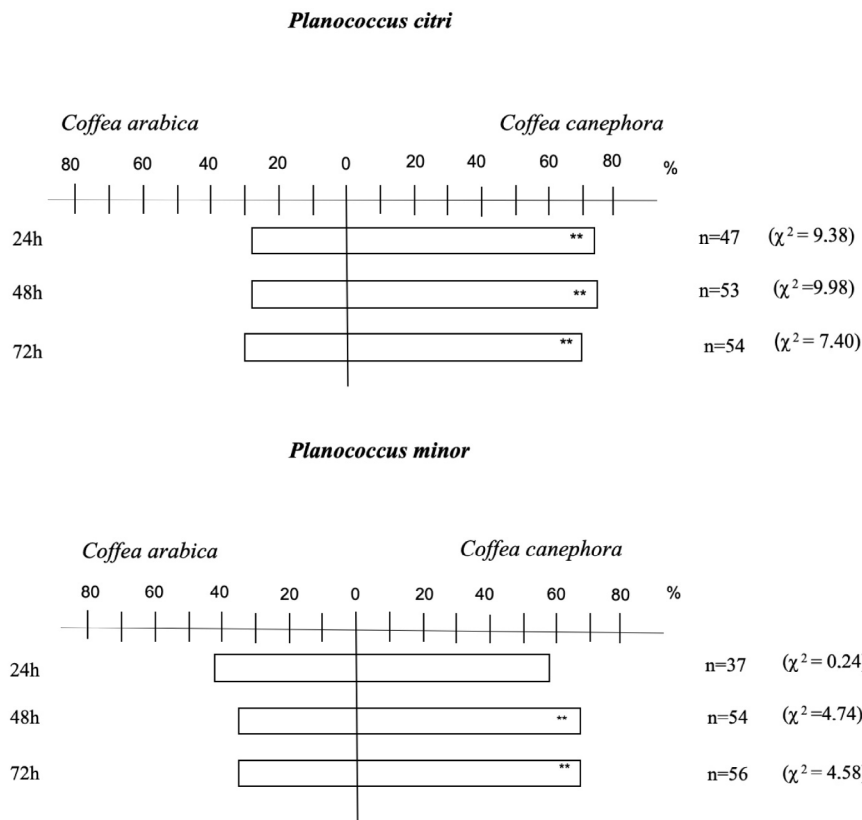
**Data analysis:** The Chi-Square test ( $\chi^2$ ) was used in the choice test and the final choice the olfactometry, considering the observed and expected frequencies. Data from nymphal mortality (%) was analyzed by the Chi square test ( $\chi^2$ ). The Student test was used for the duration of the nymphal period, number of viable eggs/female and the permanence time.

We also recorded the number of nymphs that did not respond to odors and remained in the neutral zone of the olfactometer, which were considered “non-responsive”.

## 3 RESULTS AND DISCUSSION

**Free choice – *P. citri*** showed a preference for Conilon coffee leaves within 24 hours, while for *P. minor* this preference occurred within 48 hours. Although they settled on both coffee species, they showed preference for Conilon. At 72 hours, about 70% and 62% of the *P. citri* and *P. minor* nymphs, respectively, were found on Conilon (Figure 1).

**Development and reproduction of mealybugs:** Mortality, nymphal period and oviposition of *P. citri* were influenced by the food substrate, presenting higher mortality, longer nymphal development and fewer eggs/female on *C. arabica* (Table 1).



**Figure 1:** Free choice test of *Planococcus citri* and *Planococcus minor* nymphs in leaves of *Coffea arabica* cv. Mundo Novo-IAC 379-19 versus *Coffea canephora* cv. Conilon 213. Differences according to the  $\chi^2$  test with 1 g.l ( $p=3.84$ ) ( $n$  = number of insects with choice).

**Table 1:** Biological parameters of *Planococcus citri* on leaves of coffee species ( $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH, total scotophase).

| Hosts                                | Nymph mortality (%)   | Female nymph period (days)     | Number of viable eggs/fertile female |
|--------------------------------------|-----------------------|--------------------------------|--------------------------------------|
| <i>Coffea arabica</i> cv. Mundo Novo | 85.7 A<br>( $n=49$ )  | $34.3 \pm 1.9$ A<br>( $n=7$ )  | $131.3 \pm 17.6$ B<br>( $n=7$ )      |
| <i>Coffea canephora</i> cv. Conilon  | 56.3 B<br>( $n=48$ )  | $24.4 \pm 1.1$ B<br>( $n=21$ ) | $176.0 \pm 7.8$ A<br>( $n=21$ )      |
| p value                              | 0.003<br>( $\chi^2$ ) | $\leq 0.001$<br>(Student)      | 0.01<br>(Student)                    |

Means followed by different letters in columns differ from each other by the  $\chi^2$  and Student test.  $n$  = number of observed insects.

Only survival of *P. minor* nymphs was affected by the host, showing a higher mortality in *C. arabica*. The developmental period and the number of eggs were not influenced by food substrates (Table 2).

### 3.1 Olfactometry

About 30% of nymphs of both species of mealybugs showed no response to odors ( $n=60$ ). Both mealybug species did not discriminate volatiles from Conilon and Mundo Novo (Table 3, Figure 2).

The nymphs of the two species of mealybugs showed no olfactory preference for the tested coffee species. Thus, odors do not seem to be important in host selection. Under

field conditions, mealybugs do not face a choice situation due to their limited mobility, thus olfactory stimuli are not supposed to be relevant in host selection.

Host quality could only be identified after plant degustation, that is, after sap ingestion, which was verified through the free choice test. The free choice test primarily indicates the condition of the feeding site, namely phloem sap quality, stimulating moving or remaining on the feeding site. Even if a plant is accepted as a host in a preference test, *C. canephora* in this study, it does not mean a complete suitability of the plant for colonization. The nutritional characteristics of the plant may not be totally favorable for reproduction (Le Rü et al., 1995), restricting the size of the colony.

**Table 2:** Biological parameters of *Planococcus minor* on leaves of coffee species ( $25 \pm 1^\circ\text{C}$ ,  $70 \pm 10\%$  RH, total scotophase).

| Hosts                                | Nymph mortality (%)   | Female nymph period (days) | Number of viable eggs/fertile female |
|--------------------------------------|-----------------------|----------------------------|--------------------------------------|
| <i>Coffea arabica</i> cv. Mundo Novo | 90.0 A<br>(n=50)      | $28.6 \pm 0.7$ A<br>(n=5)  | $142.8 \pm 18.4$ A<br>(n=5)          |
| <i>Coffea canephora</i> cv. Conilon  | 74.5 B<br>(n=47)      | $28.5 \pm 1.5$ A<br>(n=11) | $168.9 \pm 18.8$ A<br>(n=11)         |
| p value                              | 0.044<br>( $\chi^2$ ) | 0.951<br>(Student)         | 0.412<br>(Student)                   |

Means followed by different letters in columns differ from each other by the  $\chi^2$  test and Student test. n = number of observed insects.

**Table 3:** Final choice of 1<sup>st</sup> instar nymphs of *Planococcus citri* and *Planococcus minor* submitted to the olfactometry test (n=30).

| Insect                   | Olfactory Response                        |   |                        | $\chi^2$ Test |
|--------------------------|---|---|------------------------|---------------|
|                          | <i>Coffea canephora</i><br>(arms 1 and 3) | <i>Coffea arabica</i><br>(arms 2 and 4) | Absence of<br>Response |               |
| <i>Planococcus citri</i> | 9   | 10                                      | 11 (36.7%)             | 0.06 ns       |
| <i>Planococcus minor</i> | 9   | 14                                      | 7 (23.3%)              | 1.08 ns       |

ns = not significant.

**Figure 2:** Relative permanence (%) of 1<sup>st</sup> instar nymphs of *Planococcus citri* and *Planococcus minor* in olfactometry test (Student values  $p=0.4559$ ,  $n=20$  and  $p=0.4117$ ,  $n=24$ , respectively). Data analyzed with arcsine transformation  $\sqrt{x}/100$ ,  $n$ =number of insects that responded to odors.

Development and reproduction were favored in Conilon coffee, agreeing with the results obtained in the free choice test. These results indicate that, in this plant, mealybugs are capable of forming larger colonies, which seems to agree with a situation found in the field.

It can be inferred that Conilon coffee shows greater adaptive value for these mealybugs, producing lower mortality in both mealybug species.

Although *P. citri* and *P. minor* have been registered for some years in Brazilian coffee growing (Von Ihering, 1897; Williams; Granara de Willink, 1992) are restricted the researches on the biology of these species in cultivars of coffee *C. canephora* cultivated under Brazilian conditions. Santa-Cecília et al. (2009) evaluated some biological aspects of the nymphal phase of *P. citri* in *C. canephora* cultivars, however, research showing aspects related to reproduction in these coffee plants is unknown for both scale insects. Thus,

the discussion of the results obtained in the present study is limited in view of the scarce existing literature.

In *C. arabica*, several researches on the development and reproduction of these insects were carried out in Brazil (Correa et al., 2011; Santa-Cecília et al., 2009; 2018; Santa-Cecília; Prado; Oliveira, 2013; Sousa et al., 2012; 2018; Costa et al., 2016). Different periods of development and reproduction of both mealybug species in these coffee plants are found in these literature but laboratory results are difficult to compare due to different experimental conditions, manipulation and above all, the host plant and the plant organ used.

Dissimilar biological performance among coffee species explains why mealybugs are found more frequently and in higher populations in *C. canephora*. These results are important, mainly because they are mealybugs species involved in pest control programs in coffee crops and

for contributing to a better understanding of mealybugs and coffee plant species interactions. In addition, this information consequently contributes to increase knowledge in the area of entomology and integrated management of coffee pests.

## 4 CONCLUSIONS

- *Planococcus citri* and *P. minor* show preference between coffee species presenting higher population growth potential in *C. canephora* cv. Conilon 213.

- *Planococcus citri* and *P. minor* do not discriminate odors emitted by *C. canephora* cv. Conilon 213 and *C. arabica* Mundo Novo-IAC 379-19.

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