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HOST PREFERENCE AND BIOLOGY OF TWO CRYPTIC SPECIES, *Planococcus citri* (Risso) AND *Planococcus minor* (Maskell) (HEMIPTERA: PSEUDOCOCCIDAE)

Preferência hospedeira e biologia de duas espécies crípticas de cochonilhas, Planococcus citri (RISSO) e Planococcus minor (MASKELL) (Hemiptera: Pseudococcidae)

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Abstract – The mealybugs *Planococcus minor* (Maskell) and *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) are cryptic species to be morphologically similar and share common hosts. However, some host preference has been observed. In addition to preference, evidence showed that host plants could affect the reproductive traits of mealybugs. Although there are few studies about the influence of these plants on development of *Planococcus* spp. In this work, we conducted laboratory studies to: (a) determinate the effect of original host cocoa, coffee and orange (leaves and fruits) on the preference of *P. minor* and *P. citri* using free choice assays; and, (b) investigate the nymph's development and female's oviposition of both *P. minor* and *P. citri* in cocoa, coffee and orange (fruits). *P. minor* before the preference and development assays was reared in cocoa fruits and *P. citri* was reared in coffee seedlings and orange fruits. In free choice assays, *P. minor* showed a great preference for cocoa fruits than *P. citri*. Rearing substrates did not affect the choice and development of *P. citri*. The preference of *P. minor* for cocoa fruits shows a possible food conditioning. All three tested hosts (original hosts: cocoa, coffee and orange) were accepted for this mealybug's species but the oviposition was strongly increased only on cocoa fruits, in both species *P. citri* and *P. minor*.

Keywords – Behavior, development, food preference, host plants, mealybugs.

Resumo – As cochonilhas-farinhentas *Planococcus minor* (Maskell) e *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) são consideradas espécies crípticas por apresentarem similaridade morfológica e, além disso, podem ocorrer na mesma planta hospedeira. No entanto, tem sido observada alguma preferência hospedeira por essas espécies de cochonilhas. Além da preferência, algumas evidências mostraram que as plantas hospedeiras podem afetar as características reprodutivas desses insetos. Contudo, existem poucos estudos sobre a influência dessas plantas no desenvolvimento de *Planococcus* spp. Neste trabalho, realizamos estudos de laboratório para: (a) determinar o efeito dos hospedeiros cacau, café e citros (folhas e frutos) sobre a preferência de *P. minor* e *P. citri*, utilizando ensaios de livre escolha; e, (b) investigar o desenvolvimento ninfal e a oviposição de fêmeas, de ambas as espécies, *P. minor* e *P. citri*, em cacau, café e citros (frutos). Antes de iniciar os ensaios de preferência e desenvolvimento, foram estabelecidas criações de ambas as espécies: as cochonilhas *P. minor* foram criadas em frutos de cacau e as cochonilhas *P. citri* foram criadas em mudas de café e frutos de citros. Nos ensaios de livre escolha, as cochonilhas *P. minor* mostraram uma grande preferência por frutos de cacau do que *P. citri*. Os substratos de criação não afetaram a escolha e o desenvolvimento de *P. citri*. A preferência de *P. minor* por frutos de cacau mostra um possível condicionamento alimentar. Todos os três hospedeiros testados (cacau, café e citros) foram aceitos por essas espécies de cochonilhas, mas a oviposição de *P. citri* e *P. minor*, foi significativamente maior apenas em frutos de cacau.

Palavras-chave – Comportamento, desenvolvimento, preferência alimentar, plantas hospedeiras, cochonilhas.

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INTRODUCTION

The mealybugs of the family Pseudococcidae (Hemiptera) are a group of insects that have soft bodied and sap-sucking behavior (MILLER; KOSZTARAB, 1979; COX, 1983). Many mealybugs, particularly those that infested citrus crops are considered serious pests, because their feeding activity can lead to reduced plant productivity and can cause infection by virus (MILLER and KOSZTARAB, 1979; COX, 1983).

The citrus mealybug *Planococcus citri* (Risso, 1813) and the passionvine mealybug *Planococcus minor* (Maskell, 1897) are poliphagous major pests affecting economic important crops throughout the world (COX, 1983). Both species are morphologically similar and share common hosts and distribution (WILLIAMS; GRANARA DE WILLINK, 1992; SANTA-CECÍLIA; REIS; SOUZA, 2002).

Host preference depends of insect previous experience that would influence in its development; this experience also can be passing to other generations (HEARD, 2000). In addition, host can affect growth, development and reproduction of mealybugs (BOAVIDA; NEUENSCHWANDER, 1995; TANGA et al., 2013). Studies of *Planococcus* species on cocoa hosts have shown preference of *P. minor* despite of the wide host range (COX; FREESTON, 1985; COX, 1989). On the other hand, *P. citri* and *P. minor* reared on coffee seedlings [*Coffea arabica* L. (Rubiaceae)], cocoa [*Theobroma cacao* L. (Malvaceae)] or sweet orange [*Citrus* sinensis L. Osbeck (Rutaceae)] transferred to leaves of the same hosts, revealed preference by coffee (CORREA et al., 2011). However, under natural conditions these mealybugs are found in fruits, and other parts of the plants; this preference for one of host plant part can affect their choice and development (KIRKPATRICK, 1953; BERNAYS; CHAPMAN, 1994).

In the present study, we tested the hypothesis that preference and development parameters of both mealybugs *P. minor* and *P. citri* shift in function of the original host cocoa, coffee and orange (leaves and/or fruits). To test this hypothesis, we conducted experiments to determinate if the original host affect the preference of *P. minor* and *P. citri* using free choice assays and we investigated the nymph's development and female's oviposition of mealybugs on original host. The obtaining this information is crucial to increase knowledge of the behavior and biology of these species involved in pest control programs.

MATERIAL AND METHODS

Insects - Insects were collected in Lavras, Minas Gerais, Brazil. *P. citri* was collected in coffee (*C. arabica* cv. Mundo Novo) and orange fruits (*C. sinensis* cv. Bahia). *P. minor* was collected in cocoa fruits (*T. cacao* cv. Comum). They were identified according with Cox (1981). The rearing of insects was realized under laboratory conditions (25 ± 1°C, 70 ± 10% R.H., 12L: 12D photoperiod) in the original host, i.e. cocoa and citrus fruits and coffee seedlings.

Preliminaries tests have shown that cocoa fruits did not provide enough number of *P. citri*. Similarly, citrus fruits and coffee seedlings did not lead to enough population density of *P. minor* to the tests.

Host-preference on leaves - The host preference of *Planococcus* spp. was investigated using free-choice assays. *P. citri* (reared on coffee seedlings or orange fruits) or *P. minor* (reared on cocoa fruits) nymphs were exposed for 72 hours to healthy leaves of cocoa, coffee or orange in a Petri dish (15 cm Ø). One leaf of cocoa, coffee and orange (5 cm Ø) were placed around of a circle paper (5 cm Ø) upon 5 mm of 1% agar/water (SANTA-CECÍLIA et al., 2008). Fifteen *P. citri* or *P. minor* nymphs were removed from the rearing substrate and released at the center of the arena (circle paper) and immediately covered with polythene film. Assays were carried out in laboratory to 25 ± 1°C, 70 ± 10% R.H., and darkness conditions. Number of insects present on each host was recorded after 24, 48 and 72 h. The experiment was a complete randomized design with nine treatments replicated seven times (Table 1).

Host-preference on fruits - In a plastic tray (43 x 30 x 9 cm) were placed one cocoa fruit, one coffee branch with fruits and one whole orange around of a circle paper (20 cm Ø) equidistant one each other. Fifteen *P. citri* (reared on coffee seedlings or orange fruits) or *P. minor* (reared on cocoa fruits) nymphs were removed from their rearing substrate and released in the center of the arena (circle paper). Assays were carried out in laboratory to 25 ± 1°C, 70 ± 10% R.H., and darkness conditions. The number of insects present on each host was recorded after 24, 48 and 72 h. The experiment was a complete randomized design with nine treatments replicated seven times (Table 1).

Mealybug's development - *P. citri* (reared on coffee seedlings or orange fruits) or *P. minor* (reared on cocoa fruits) eggs were transferred from the rearing to the fruits of cocoa, coffee and orange. In the case of cocoa and orange fruits were placed clip cages (1.5 cm height and 3.0 cm Ø) upon the eggs. Elastic bands were positioned around the fruits pressed down the cage. To coffee were placed two fruits in a Petri dish (5 cm Ø) upon 5 mm of 1% agar/water (SANTA-CECÍLIA et al., 2008). Petri dishes were covered with polythene film.

The experiment was carried out in a climate chamber at 25 ± 1°C, 70 ± 10% R.H., and 12L: 12D photoperiod in the laboratory of Biological Control in EPAMIG/Ecocentro. The nymphal development and mortality were evaluated only for female nymphs because they feed during prolonged periods allowing a better assessment of the host effect on their development.

When females reached adulthood, they were transferred individually to new fruits (cocoa, orange and coffee). To ensure the mating one male adult (still inside the cocoon) was introduced in the clips cages to cocoa and orange. To coffee, also one male adult as transferred in a Petri dish. Finally, the eggs numbers in each host were recorded. The experiment was a complete randomized design with nine treatments replicated 30 times. One insect represented a replicate (Table 1).

Statistical analysis - The host preference in leaves and fruits of mealybugs was analyzed using a generalized linear model (GLM) with a binomial distribution and a logit-link function. The statistical model included the main effects of rearing substrates, original hosts, time and their interactions (Statistical Analysis Software, SAS® 9.3).

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The duration of nymphal stage and eggs number were square-root transformed ($\sqrt{X}$) before analysis to satisfy the homogeneity of variance assumption for ANOVA. Means values were compared using multiple comparison Tukey test ($P \leq 0.05$). Mean proportion mortality of nymphs was arcsine square-root transformed ($\sqrt{X/100}$) and analyzed using Chi-square test (χ^2) (Statgraphics® Centurion XVII).

Table 1. Treatments of free-choice assays and development of *Planococcus* spp. (Hemiptera: Pseudococcidae).

Mealybug species and rearing substrates	Free-choice assays	
	Leaf substrates	Fruit substrates
P. minor (cocoa fruits)	Mixed cocoa, coffee and orange	Mixed cocoa, coffee and orange
P. citri (coffee seedlings)	Mixed cocoa, coffee and orange	Mixed cocoa, coffee and orange
P. citri (orange fruits)	Mixed cocoa, coffee and orange	Mixed cocoa, coffee and orange

Mealybug species and rearing substrates	Mealybug's development		
P. minor (cocoa fruits)	Cocoa fruits	Coffee drupelets	Orange fruits
P. citri (coffee seedlings)	Cocoa fruits	Coffee drupelets	Orange fruits
P. citri (orange fruits)	Cocoa fruits	Coffee drupelets	Orange fruits

RESULTS AND DISCUSSION

Host-preference on leaves - There was no effect of rearing substrates ($F = 0.14$, $df = 2$, $P = 0.8721$) or original hosts ($F = 1.62$, $df = 2$, $P = 0.2079$) on mealybugs host preference. There was also no significant difference of rearing substrates × original hosts ($F = 0.3$, $df = 4$, $P = 0.8735$), rearing substrates × time ($F = 0.75$, $df = 4$, $P = 0.5570$) and rearing substrates × original hosts × time ($F = 1.87$, $df = 8$, $P = 0.0726$) on host preference. However, there was significant effect of time ($F = 5.74$, $df = 2$, $P = 0.0043$) and original hosts × time ($F = 2.87$, $df = 4$, $P = 0.0265$) on mealybugs preference (Table 2).

There was higher preference of both species *P. citri* and *P. minor* nymphs on cocoa leaves than coffee and orange leaves at 24 hours (Figure 1A). To orange leaves there were significant preference of mealybugs nymphs up to 48 hours and there was no significant difference on preference of mealybugs on cocoa and coffee leaves at 24, 48, and 72 hours (Figure 1B).

Host-preference on fruits - There was no effect of rearing substrates ($F = 0.24$, $df = 2$, $P = 0.7869$) or time ($F = 0.32$, $df = 2$, $P = 0.7283$) on mealybugs host preference. There was also no significant difference of rearing substrates × time ($F = 0.15$, $df = 4$, $P = 0.9617$), original hosts × time ($F = 1.23$, $df = 4$, $P = 0.3006$) and rearing substrates × original hosts × time ($F = 0.47$, $df = 8$, $P = 0.8758$) on host preference. However, there was significant effect of original hosts ($F = 12.43$, $df = 2$, $P < 0.001$) and rearing substrates × original hosts ($F = 8.00$, $df = 4$, $P < 0.001$) on mealybug preference (Table 3).

P. minor nymphs from cocoa (rearing substrate) showed significant preference for cocoa than coffee and orange fruits. *P. citri* nymphs from coffee seedlings (rearing substrate) showed no preference for any original host (cocoa, coffee and orange fruits). However, *P. citri* nymphs from orange (rearing substrate) showed significant preference for cocoa and orange than coffee fruits (Figure 2B).

The original host cocoa was more preferred by *P. minor* nymphs from cocoa fruits (rearing substrate) than *P. citri* nymphs reared in coffee seedlings and orange fruits (Figure 2A).

Coffee fruits (original host) were most preferred by *P. citri* nymphs reared in coffee seedlings than *P. minor* nymphs reared in cocoa fruits and *P. citri* nymphs reared in orange fruits. On the other hand, orange fruits (original host) were most preferred by *P. citri* nymphs from orange fruits and coffee seedlings (rearing substrate) than *P. minor* reared in cocoa fruits (Figure 2A).

Development of P. citri and P. minor - *P. minor* nymphs reared in cocoa fruits developed faster in cocoa fruits (original host) than coffee and orange fruits. Eggs number of *P. minor* females from cocoa fruits were higher (up to 6-fold approx.) in cocoa fruits (original host) than coffee and orange fruits. While nymphal mortality there was no significant difference in any original host (Table 4).

P. citri nymphs from coffee seedlings did not show significant difference on the original hosts in their development and mortality. Eggs number of *P. citri* (rearing in coffee seedlings) were higher (up to 5.3-fold approx.) in cocoa fruits (original host) than coffee and orange fruits (Table 4).

On the other hand, *P. citri* nymphs rearing in orange fruits showed faster development in orange fruits (original host) than cocoa and coffee fruits. Eggs number of this mealybug were higher (up to 6.4-fold approx.) in cocoa fruits (original host) than coffee and orange fruits. While nymphal mortality was similar in all treatments regardless of the rearing substrate or original host (Table 4).

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Table 2. Preference of third instar nymphs of *Planococcus* spp. for cocoa, coffee and orange healthy leaves as assessed during free-choice assay.

Host preference	Effects	*df* [a]	*F*	*P*
Leaves	Rearing substrate	2, 162	0.14	0.8721
	Original hosts	2, 162	1.62	0.2079
	Time	2, 162	5.74	**0.0043**
	Rearing substrate x Original hosts	4, 162	0.3	0.8735
	Rearing substrate x Time	4, 162	0.75	0.557
	Original hosts x Time	4, 162	2.87	**0.0265**
	Rearing substrate x Original hosts x Time	8, 162	1.87	**0.0726**

[a]: Degrees of freedom – numerator, denominator (error).

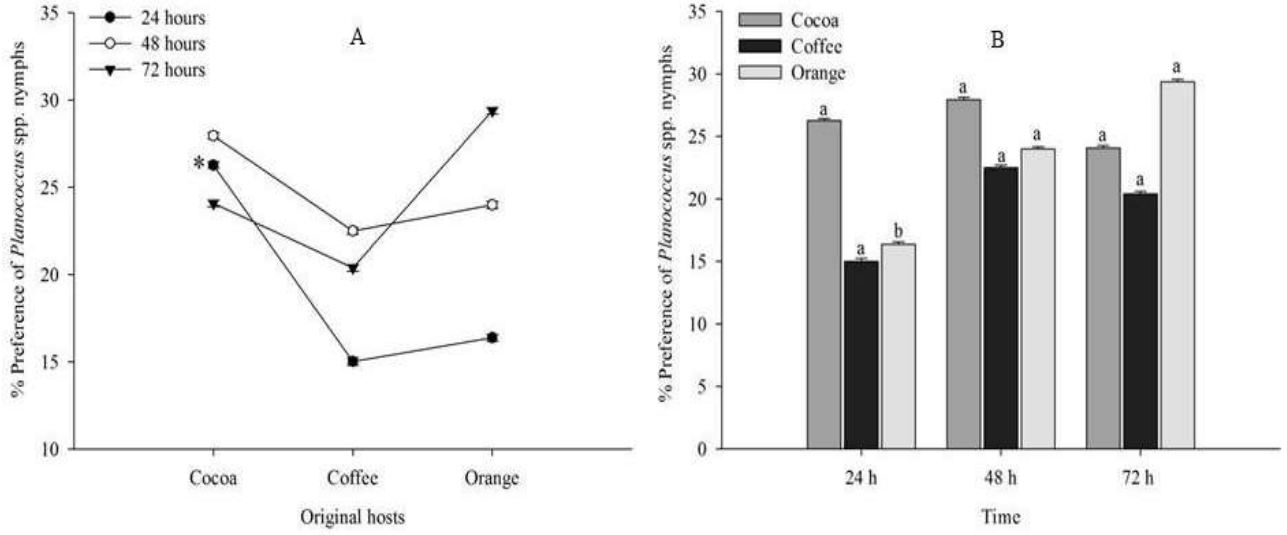

Figure 1. (%) Preference of third instar nymphs of *Planococcus* spp. for cocoa, coffee and orange healthy leaves as assessed during free-choice assay.A: * significant difference (Tukey Test). B: Bars with different letters were significantly different (P<0.05) based on the Tukey test.

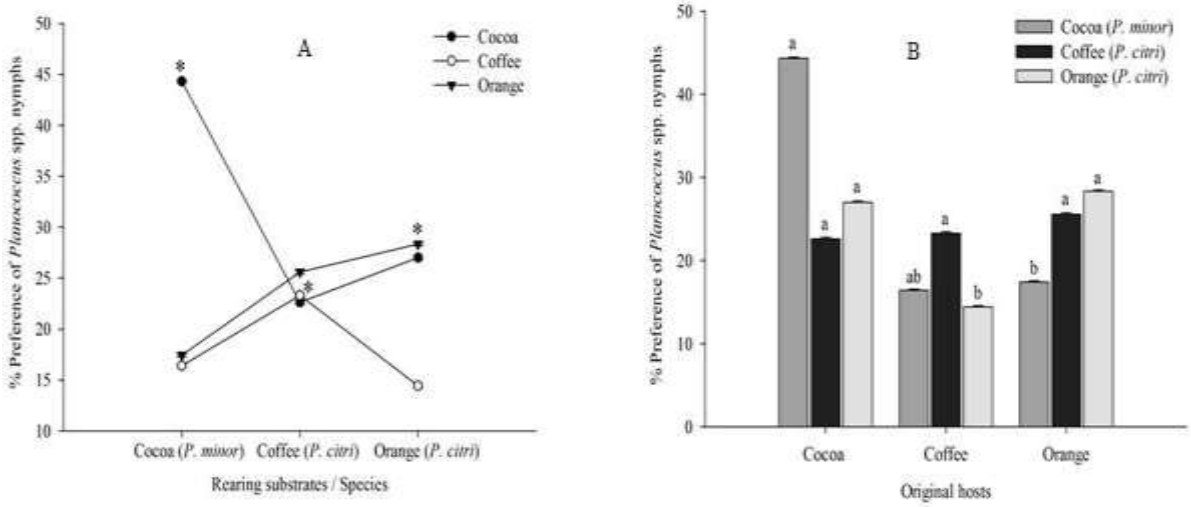

Figure 2. (%) Preference of third instar nymphs of *Planococcus* spp. for cocoa, coffee and orange fruits as assessed during free-choice assay**.** A: * significant difference (Tukey test). B: Bars with different letters were significantly different (P<0.05) based on the Tukey test.

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Table 3. Preference of third instar nymphs of *Planococcus* spp. for cocoa, coffee and orange healthy fruits as assessed during free-choice assay.

Host preference	Effects	*df* [a]	*F*	*P*
Fruits	Rearing substrate	2, 162	0.24	0.7869
	Original hosts	2, 162	12.43	**< 0.001**
	Time	2, 162	0.32	0.7283
	Rearing substrate x Original hosts	4, 162	8.00	**< 0.001**
	Rearing substrate x Time	4, 162	0.15	0.9617
	Original hosts x Time	4, 162	1.23	0.3006
	Rearing substrate x Original hosts x Time	8, 162	0.47	0.8758

[a]: Degrees of freedom – numerator, denominator (error).

Table 4. Mean (±SE) of development parameters of *Planococcus* spp. (Hemiptera: Pseudococcidae) distributed according with different rearing substrates and original hosts plant combinations.

Rearing/ original hosts	Duration of nymphal stage [1] (days)	Eggs number/ female[1]	(%) Nymphal mortality (n=30)
P. minor			
Cocoa/cocoa fruits	20.9 ± 0.23 b (n=24)	301.5 ± 24.1 a (n=24)	20.0
Cocoa/coffee fruits	23.7 ± 0.35 a (n=19)	59.8 ± 9.1 b (n=19)	33.3
Cocoa/orange fruits	22.8 ± 0.21 a (n=20)	49.4 ± 5.3 b (n=20)	33.3
P value	**< 0.001[a]**	**< 0.001[a]**	0.406[b]
P. citri			
Coffee/cocoa fruits	21.2 ± 0.23a (n=23)	260.4 ± 28.8 a (n = 23)	23.3
Coffee/coffee fruits	20.9 ± 0.26a (n=23)	60.4 ± 6.8 b (n = 23)	23.3
Coffee/orange fruits	20.7 ± 0.12a (n=24)	48.7 ± 5.5 b (n = 24)	20.0
P value	0.182[a]	**< 0.001[a]**	0.937[b]
P. citri			
Orange/cocoa fruits	22.1 ± 0.23 a (n=20)	238.7 ± 22.2 a (n = 20)	33.3
Orange/coffee fruits	22.0 ± 0.44 a (n=21)	37.3 ± 4.2 b (n = 21)	30.0
Orange/orange fruits	20.8 ± 0.25 b (n=26)	57.4 ± 5.5 b (n = 26)	13.3
P value	**0.008[a]**	**< 0.001[a]**	0.142[b]

[1] Data transformed to $\sqrt{X}$ prior to ANOVA. Means followed by different letters within columns are significantly different according with ANOVA followed by the Tukey Test of multiple comparisons (α=0.05). n = specimen numbers. [a] *P* value Analysis of variance ANOVA. [b] *P value* χ^2 test.

In the laboratory studies, we demonstrated that: (1) the host preference on leaves (cocoa, coffee and orange) of both species *P. citri and P. minor* was influenced by time; (2) the host preference on fruits (cocoa, coffee and orange) of *P. citri* from orange fruits and *P. minor* from cocoa fruits was influenced by original host; (3) cocoa fruits were more suitable for both species *P. citri* and *P. minor* females to lay their eggs; and, (4) same rearing substrate and original host have positive influence on the nymphal development of mealybugs.

The preference of mealybug species *P. minor* and *P. citri* on leaves of different hosts (cocoa, coffee and orange) has variability during the time, because mealybugs at 24 hours preferred cocoa leaves than coffee and orange leaves, but at 72 hours the preference of these mealybugs shift to orange leaves. This host change can be due to high mortality when mealybugs species feed on only one host for a long time. For example, one study showed that both species *P. minor* and *P. citri* had 93% of nymphal mortality when feeding with cocoa leaves (CORREA et al., 2011). Other reason to change host can be associated with the

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preference of specific nutrients and allelochemicals present in host plants (VET; DICKE, 1992, PANIZZI; PARRA, 2009) as well as the physical and chemical characteristics of the plant tissues (SANTA-CECÍLIA; PRADO; OLIVEIRA, 2013).

Regarding to fruits preference, free-choice assays showed that *P. citri* nymphs did not has host preference associated with the rearing substrate. Results indicated that they could shift host easily without any effect on their biology. *P. minor* nymphs reared only on cocoa fruits preferred the original substrate cocoa fruits than coffee and orange fruits. This behavior indicates a possible food conditioning (SANTA-CECÍLIA; PRADO; OLIVEIRA, 2013). These results are consistent with previous studies showing that host preference depends of insect previous experience (COX; FREESTOM, 1985; HEARD, 2000; SANTA-CECÍLIA; PRADO; OLIVEIRA, 2013). Despite of the preference of *P. minor* for cocoa fruits, this specie is also able to colonize the other crops (COX; FREESTON, 1985; SANTA-CECÍLIA; REIS; SOUZA, 2002).

Cocoa fruits are more suitable host substrate for both species *P. minor* and *P. citri* in terms of eggs production. Cocoa fruits are heavy infested by mealybug's population under natural conditions while leaves support fewer individuals. How the fruit sap composition affect development and fecundity of sucking insects is still unknown, but has been suggested a fruit "sink effect" that increase the sap flow and nutrients accumulation in this structure (VALLE; BOGGIO; HELDT, 1998). A different composition and access to sap of orange and coffee fruits could be a cause of the low eggs number of mealybugs in these hosts.

Despite of the significant differences in the nymphal development of *P. minor* and *P. citri* on original hosts, mean values in all treatments had a small variation (Table 4). This variation was due to both species of mealybugs have a great adaptive capacity and development on new hosts because of their limited mobility (SANTA-CECÍLIA; PRADO; OLIVEIRA, 2013). In addition to this variation on nymphal development, mortality did not show any difference in all treatments (Table 4). Low mortality rates (< 30%) have been reported previously for *P. citri* reared on orange leaves (CORREA et al., 2005) and coffee cultivars (SANTA-CECÍLIA et al., 2009). In contrast to these findings, recent studies about the biology of *P. minor* and *P. citri* reared on fruits and leaves of cocoa, coffee and orange suggested that these hosts have a strong influence on nymphal survival with mortalities from 16 to 50% in fruits (SOUSA et al., 2012), and 18 to 93% in leaves (CORREA et al., 2011). Thus, is clear that neither nymphal development nor percentage mortality of *Planococcus* spp. is affected by original substrates.

CONCLUSIONS

P. citri no showed a defined host preference, while *P. minor* showed a great preference for cocoa fruits.

All three tested hosts (original hosts: cocoa, coffee and orange) were accepted for this mealybug's species but the oviposition was strongly increased only on cocoa fruits, in both species *P. citri* and *P. minor*.

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