METHODOLOGY FOR BIOLOGICAL STUDIES OF MEALYBUGS (HEMIPTERA: PSEUDOCOCCIDAE)[1]

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ABSTRACT: Several methodologies have been used in biological studies of mealybugs (Pseudococcidae) in the laboratory. The objective of this work was to compare three methodologies in order to establish a pattern for development studies. The development nymphal period and mortality of the citrus mealybug, *Planococcus citri* (Risso, 1813), was evaluated in PVC clip cages of 10 and 30 mm in diameter attached to leaves of coffee plants (*Coffea arabica* L. cv. Acaia Cerrado), and on leaf sections placed over an agar film layer. Forty mealybug eggs were individually placed on the substrate and evaluated daily. The data was submitted to analysis of variance followed by the Test of Tukey (0.05 % significance). Differences were detected in citrus mealybug nymphal development period and mortality depending of the methodology. The shortest period and the lowest mortality were obtained using foliar sections maintained in agar-water which appears to be a viable methodology for mealybug studies in the laboratory. The excessive insect manipulation seems to be the main negative factor in mealybug development when using clip cages fixed to plant leaves.

Key words: Coccoidea, development, biology, clip cages, leaf sections.

METODOLOGIA PARA ESTUDOS BIOLÓGICOS DE COCHONILHAS (HEMIPTERA: PSEUDOCOCCIDAE)

RESUMO: *Várias metodologias têm sido empregadas em estudos de biologia de cochonilhas (Pseudococcidae) em laboratório e objetivou-se neste trabalho comparar três metodologias visando ao estabelecimento de um padrão. O desenvolvimento ninfal e a mortalidade da cochonilha* Planococus citri *(Risso, 1813) foram avaliados em gaiolas de PVC transparente de 10 mm e 30 mm de diâmetro fixadas em folhas de cafeeiro (*Coffea arabica *L. cv. Acaia Cerrado), e em secções foliares mantidas em ágar-água. Ovos da cochonilha foram individualizados sobre o substrato e seu desenvolvimento avaliado diariamente. Os dados foram submetidos à análise de variância, seguidos pelo Teste de Tukey a 5% de significância. Foram constatadas diferenças na duração do período ninfal e na mortalidade da cochonilha em função das três metodologias testadas. A menor duração do desenvolvimento e a menor mortalidade foram obtidas usando secções foliares mantidas em ágar-água, demonstrando que essa é uma metodologia viável para estudos de biologia de cochonilhas-farinhentas em laboratório. A excessiva manipulação do inseto parece ter sido o principal fator negativo no desenvolvimento da cochonilha, quando foram utilizadas gaiolas (10 e 30 mm de diâmetro) fixadas nas folhas das plantas de cafeeiro.*

Palavras-chave: Coccoidea, cochonilhas-farinhentas, biologia, gaiola, secções foliares.

1 INTRODUCTION

Mealybugs are sap sucking insects noxious to several crops including coffee (*Coffea* spp.) plants. These insects can be found in roots forming galls restricting water and nutrient absorption. They also remove sap from floral buttons and fruits, resulting in empty and dry berries (SANTA-CECILIA et al., 2002, 2007). Few studies have been made on mealybugs in spite of the damages caused to coffee plants, probably due to their unpredictable occurrence. Recent studies have improved their knowledge in Brazil (CULIK et al., 2006; SANTA-CECILIA et al., 2002, 2007) but further studies are needed to establish a management program on coffee crop ecosystems.

Laboratory studies on mealybug biology have been accomplished using plant leaves containing nymphs inside test tubes (ITO, 1938), clip cages

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(SANTA-CECILIA et al., 2004), potato sprouts (GHOSE, 1983; NAKANO, 1972), leaves in nutritive solution (MENEZES, 1973), leaf sections (COLEN et al., 2000), germinated broad bean seeds (NARAI & MURAI, 2002) and foliar sections in agar-water (CORREA et al., 2005).

Results are not comparable due to the different methodologies and are often quite variable each one having advantages and disadvantages. The use of cages involves much manipulation, negatively influencing insect development and survival since the nymphs are very delicate. On the other hand, cutting leaves could eventually cause physiological changes and therefore modify insect responses (GREEN & RYAN, 1972). However, confined insects is the only way to avoid them escaping. This work compares three methodologies commonly used in biological studies in the laboratory.

2 MATERIALS AND METHODS

The experiment was carried out at the Biological Pest Control Laboratory CTSM/ EcoCentro/EPAMIG in a room kept at 25±1ºC, 70±10% RH and 12 hour photophase. Coffee plants cv. Acaia Cerrado were grown in greenhouse and used in the experiments when they had from four to six pair of leaves.

Insects. A stock culture of *Planococcus citri* (Risso) was kept in the laboratory in pumpkin (*Cucurbita maxima* L.) cv. Cabotcha.

Treatments. Nymph development and mortality were compared using the following three methodologies:

1- transparent PVC clip cages of 10 mm diameter and 11 mm height, closed with 0.2 mm mesh nylon screen. Cages were fixed to coffee leaves by means of metal clips. Cages and leaves were taken to a stereomicroscope for evaluations with augmentation ranged from 10x to 30x.

2- transparent PVC clip cages of 30 mm diameter and 11 mm height, closed with the same nylon screen. Evaluations were made using a 10x pocket glass magnifying lens, without manipulation of the plants.

3- agar-water in 5 cm diameter Petri dishes containing 4 cm diameter foliar sections. Agar-water at 1% was disposed in a 5 mm layer and dishes were sealed with PVC plastic film. Mealybugs were transferred to new dishes every five days by cutting off a small leaf area around the mealybugs in the old leaves, and carefully moved to the new foliar section. So, insects drew off stylets from the old leaf and moved to the new leaf section. Evaluations were made under a stereomicroscope with augmentation ranged between 10x and 30x.

Eggs from the stock culture were transferred to the plants by means of a small brush and every cage or leaf section received one egg. Only females were considered for analysis, males were discarded because they stop feeding early during the cocoon stage and data obtained from females should be sufficient to show the effects of treatments.

Experimental design. Plants and Petri-dishes were disposed in a completely randomized design with 30 to 40 replicates per treatment, where each insect represents one replicate. Nymphal development period for each ínstar was assessed only in those insects reaching the next stage (dead insect not considered).

Evaluations. Mealybug development stage and mortality were observed daily and the duration of the nymph stage and mortality recorded. Data was submitted to the analysis of variance followed by the Tukey´s multiple range test. To assess the differences, mortality data was grouped in three or four lots of 10 insects, depending on the available number of insects.

3 RESULTS AND DISCUSSION

The shortest nymphal stage duration and lowest mortality were obtained in foliar sections on agar. This methodology produced less interference in mealybugs rearing when compared to clip cages fastened to leaves. Insects did not escape from the leaf section to the agar, and fixing in the new leaf sections is performed without manipulation. The use of a 10 mm diameter clip cage showed a prolonged development period and the highest mortality, with intermediated values obtained when 30 mm diameter cages were used. Small clip cages means a lot of manipulation and nymphs, especially those of the first instar, easily escape from the cages. Handling impact was previously reported as detrimental for mealybug laboratory studies and should be avoided (ITO, 1938).

The use of leaf sections in agar showed to be the best among the tested methodology. This technology has also shown good results in studies with aphids (SAMPAIO et al., 2001; SOGLIA et al., 2002).

Table 1 – Female development of *Planococcus citri* according to the methology.

Methodology	Nymphal stage duration (days)	Nymph mortality (%) *
Clip cage (10 mm diameter)	44.6 ± 3.2 a (n = 8)	77.5 ± 6.3 a (n = 4)
Clip cage (30 mm diameter)	24.6 ± 1.6 b (n = 14)	50.8 ± 6.5 ab (n = 3)
Foliar sections in agar	20.2 ± 0.5 c (n = 24)	40.0 ± 10.8 b (n = 4)
Anova p value	< 0.001	0.031

Means within columns followed by the same letter are not statistically different according to the Tukey´s multiple range test (P > 0.05).
n = number of evaluated insects.
* n = grouped data in 10 nymphs.

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