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Biological development of *Saccharicoccus sacchari* (Cockerell, 1895) (Hemiptera: Pseudococcidae) on sugarcane in different temperatures

Desenvolvimento biológico de *Saccharicoccus sacchari* (Cockerell, 1895) (Hemiptera: Pseudococcidae) em cana-de-açúcar em diferentes temperaturas

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Abstract

The pink sugarcane mealybug *Saccharicoccus sacchari* (Cockerell, 1895) (Hemiptera: Pseudococcidae) occurs practically in all sugarcane producing regions *Saccharum* spp. (Poaceae) causing damage. Little information is available about insect's biology under Brazilian conditions. In this work, biological development of pink sugarcane mealybug was studied at temperatures of 23 °C ± 2 °C and 28 °C ± 2 °C without photophase and relative humidity of 80%. Number and viability of eggs, incubation time, duration of the last oviposition, duration of each nymphal instar, viability of the nymphs, start of oviposition and longevity of the females were recorded. Biological development of insects was stipulated by the SAS University Edition software, version 9.4. There were differences in the life cycle of the pseudococcid at both temperatures evaluated. Females of *S. sacchari* had three nymphal instars and reproduce asexually. Asexual reproduction occurs in the field and under controlled conditions. By increasing the temperature increases, insect lived longer and the presence of the winged male in Brazil indicates the possibility of sexual reproduction of the species.

Keywords: life cycle, population growth, instars, fertility parameters, fertility life table.

Resumo

A cochonilha-rosada-da-cana-de-açúcar *Saccharicoccus sacchari* (Cockerell, 1895) (Hemiptera: Pseudococcidae) ocorre praticamente em todas as regiões produtoras de cana-de-açúcar *Saccharum* spp. (Poaceae) causando prejuízos. Poucas informações estão disponíveis sobre a biologia do inseto nas condições brasileiras. Neste trabalho foi estudado o desenvolvimento biológico da cochonilha-rosada-da-cana-de-açúcar nas temperaturas de 23 °C ± 2 °C e 28 °C ± 2 °C sem fotofase e umidade relativa de 80%. Diariamente foi registrado o número e viabilidade dos ovos, tempo de incubação, duração da última oviposição, duração de cada ínstar ninfal, viabilidade das ninfas, início da oviposição e longevidade das fêmeas. O desenvolvimento biológico dos insetos foi estipulado pelo software SAS University Edition, versão 9.4. Houve diferenças no ciclo de vida do pseudococcídeo nas duas temperaturas avaliadas. As fêmeas de *S. sacchari* apresentaram três instares ninfais e reproduzem assexuadamente. A reprodução assexuada ocorre em campo e em condições controladas. Ao se aumentar a temperatura o inseto viveu mais e a presença do macho alado no Brasil indica a possibilidade da reprodução sexuada da espécie.

Palavras-chave: ciclo de vida, crescimento populacional, ínstares, parâmetros de fertilidade, tabela de vida de fertilidade.

1. Introduction

The pink sugarcane mealybug *Saccharicoccus sacchari* (Cockerell, 1895) (Hemiptera: Pseudococcidae), probably originated in New Guinea, is now reported from almost all sugarcane-growing areas in the world (Watson and Sirisena, 2022). It is found primarily on sugarcane and its wild relatives *Saccharum* spp. (Poaceae) such as *Cortaderia* spp., *Cymbopogon caesius* (Hook. and Arn.), *Holcus* spp., *Imperata cylindrica* (L.), *Miscanthus* spp., *Oryza sativa* L., *Phragmites* spp. and *Sorghum* spp., but has also been recorded on *Cocos nucifera* L. (Arecaceae) and *Carica papaya* L. (Caricaceae) (García Morales et al., 2016). However, it is on sugarcane considered one of the main pests, causing direct and indirect damage (Monteiro et al., 2021).

In Brazil, *S. sacchari* has been recorded in the states of Amazonas, Pará, Paraíba, Pernambuco, Minas Gerais, Mato Grosso, Rio de Janeiro, São Paulo, Santa Catarina and Rio Grande do Sul (Silva et al., 1968; Monteiro et al., 2019; Sturza et al., 2021). In São Paulo state, it has been considered a common and emergent pest, however little information about its population is available (Cruz et al., 2019; Monteiro et al., 2021).

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Previous studies demonstrated influence of constant temperature on the development, survival, and fecundity of *S. sacchari* (Hafez and Salama, 1969; Atiqui, 1987; Rae and De'ath, 1991; Rae, 1993). Oviposition period at 20 °C was 31 days in Australia (Rae and De'ath, 1991). In India, under 26 °C, it was between 20 and 29 days, and 42 days was the maximum time for it complete its development (Atiqui, 1987). Under natural conditions in China, first instar nymphs remain in this period for two and three days (Qin et al., 2017). However, in Egypt, Hafez and Salama (1969) observed the nymphal period was 82 days when it was reared at 16 °C, and longevity was 84 days, under 30 °C, instar period was 15 days and longevity, 51 days, for both temperatures, four to five generations were observed per year.

After hatching, *S. sacchari*, through instars, will transform into an adult individual by hemimetabolous metamorphosis (Uichanco and Villanueva, 1932; Beardsley, 1962; Hafez and Salama, 1969; Rae, 1993). Uichanco and Villanueva (1932) described that, in the Philippines, there are five instars to the female to reach maturity. However, other authors, such as Beardsley (1962) in Hawaii and Hafez and Salama (1969), recorded only four instars, three being nymphal instars and adult. Upon reaching adult stage, the species can reproduce and oviposit (Uichanco and Villanueva, 1932; Inkerman et al., 1986). Uichanco and Villanueva (1932) suggested that the species can reproduce asexually by thelytocal parthenogenesis or sexually, with parthenogenetic form being the most common. Inkerman et al. (1986) observed in Australia, both forms, but due to large number of males found, it is understood the sexual form is the most common, as well as in Egypt (Hafez and Salama, 1967).

Regarding information on the morphology, Rae (1993) proposed differentiation between immature instars and adult female based on the measure in length and width of the body, antennae length and number of antennal segments.

It is understood in different locations, the same species showed differences in life cycle when raised under different temperatures and in number of instars. Knowing the survival and reproduction strategies is essential to implement insect population control actions that make up integrated pest management in sugarcane crops.

Therefore, the objective of this study was to evaluate the biological development of *S. sacchari* under different temperatures.

2. Material and Methods

The experiments were conducted at the Hemiptera Biosystematic Laboratory (LABHEM) of the Department of Agricultural Production Sciences, Plant Health Sector of the Faculty of Agricultural and Veterinary Sciences (FCAV), Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Campus of Jaboticabal, São Paulo, Brazil.

2.1. Collection, identification and deposit of voucher species of Saccharicoccus sacchari

Fifty gravid females of *S. sacchari* were collected under sugarcane plants, cultivar RB867515, from a rural location, coordinates: -21°13'22" S, -48°16'81" W and altitude of 605 m, located in Jaboticabal, São Paulo, Brazil.

To confirm the species, samples were mounted on permanent slides using the technique by Granara de Willink (1990). Identification occurred under an optical microscope through morphological characteristics using the work of Williams and Granara de Willink (1992).

Voucher species was deposited in the Insects and Mites Reference Collection (CRIA) of the Department of Agricultural Production Sciences, Plant Health Sector of FCAV/UNESP, Jaboticabal, São Paulo, Brazil.

2.2. Rearing of Saccharicoccus sacchari in laboratory conditions under different temperatures

In the same location, ten sugarcane stalks, cultivar RB857515, were collected. The culms were transported to LABHEM, sectioned into 40 ten-centimeter internodes. They were sterilized with 70% alcohol and previously dried using disposable paper and exposed to a natural environment for complete drying. To delay the loss of sucrose, internodes were waxed at their ends (Beardsley, 1962).

Each fragmented internode was infested with a gravid mealybug and then individualized in 40 Petri dishes.

Petri dishes were kept in two BODs. The first maintained 20 Petri dishes under temperature condition of 23 °C ± 2 °C, and the other half in the second BOD at 28 °C ± 2 °C, both with photoperiod 24 h darkness and relative humidity of 80% (Hafez and Salama, 1969; Atiqui, 1987; Rae and De'ath, 1991).

2.3. Assessment of the biological development of Saccharicoccus sacchari

Daily oviposition was recorded, measuring the number of viable and non-viable eggs through hatching of nymphs, egg incubation time, duration of the adult's last oviposition until death, number of nymphs hatching, duration of each nymphal instar, mortality nymphs for each stage, duration of the pre-oviposition period and adult longevity.

Observations of the incubation, nymphal, pre-oviposition, oviposition and post-oviposition periods of females were conducted under a stereoscopic microscope.

2.4. Differentiation of the phases of the female Saccharicoccus sacchari

For each instar observed, samples were mounted on permanent slides using the technique described by Granara de Willink (1990).

The differentiation of female phases was carried out under an optical microscope using morphological characteristics: body length and width, antennal length and number of antennal segments (Rae, 1993).

2.5. Obtaining and depositing a voucher specimen of a winged male of Saccharicoccus sacchari

A winged specimen was found in the field together with the population.

The individual was captured and mounted on a permanent slide using the technique described by Granara de Willink (1990). Identification was carried out using an optical microscope using morphological characteristics (Beardsley, 1960).

The voucher specimen was deposited in the Insects and Mites Reference Collection (CRIA) of the Department of Agricultural Production Sciences, Plant Health Sector, of FCAV/UNESP, Jaboticabal, São Paulo, Brazil.

2.6. Statistical procedures

After obtaining data, fertility life table and insect's longevity were calculated using the SAS® University Edition program, version 9.4.

Life table was created using the LIFETABLE.sas procedure developed by Maia et al. (2000), through estimates of net reproduction rate (R_0), intrinsic increase rate (r_m), finite increase ratio (λ), average time between generations (T) and population doubling time (TD).

Biological development of the insect was elaborated through a completely randomized design (DIC), with two treatments, referring to rearing temperatures in laboratory conditions and 20 replications. The data, time of each stage and longevity, were submitted to the Bartlett test to verify homoscedasticity (PROC GLM) and the Cramer von Mises test for normality (PROC UNIVARIATE). The data showed normality, so analysis of variance (PROC ANOVA) was conducted. The means (PROC MEANS), when significant, were compared using the Student test ($p < 0.05$) (Everitt and Hothorn, 2005).

3. Results

There were differences in the biological development of the insect under different temperatures and the finding of three instars for the female.

During the work, a winged male of *S. sacchari* was obtained under sugarcane plants.

3.1. Biological development of Saccharicoccus sacchari at different temperatures

Population of *S. sacchari*, when raised at 23 °C, demonstrates an adult individual, during oviposition period, added approximately 23 females that reached maturity. Intrinsic increase rate was 0.123 pseudococcidia per day. Every day, a female that completes her biological development was added to population. At this temperature, insects began oviposition 25 days after birth. Approximately five weeks is estimate to population double (Table 1).

In relation to population established at 28 °C, an adult female added an average of 34 females that completed their biological development. Intrinsic increase rate was 0.128 insects per day. One female was added per day, which reached the post-oviposition period. Under these conditions, after hatching, pseudococcids took approximately 27 days to begin oviposition. It takes around five weeks to this population double (Table 2).

Egg incubation time and third nymphal stage did not show significant differences at both temperatures. There were significant differences in duration of the first and second nymphal stages and adult stage, at 28 °C lasting the longest in these stages (Table 3).

Longevity of the pseudococcid presents a significant difference. Insects, at 28 °C, showed greater longevity, with an average of 52.0 days (Table 4).

Table 1. Population growth parameters of *Saccharicoccus sacchari* in sugarcane, cultivar RB867515, under 23 °C ± 2 °C, 24 h darkness and relative humidity of 80%.

R_0 (females)	r_m (females/females/day)	λ (females/day)	T (days)	TD (weeks)
23.34	0.123	1.13	25.49	5.61

Note. R_0 = net reproduction rate; r_m = intrinsic increase rate; λ = finite increase ratio; T = average time between generations; TD = population doubling time.

Table 2. Population growth parameters of *Saccharicoccus sacchari* in sugarcane, cultivar RB867515, under 28 °C ± 2 °C, 24 h darkness and relative humidity of 80%.

R_0 (females)	r_m (females/females/day)	λ (females/day)	T (days)	TD (weeks)
34.62	0.128	1.13	27.48	5.37

Note. R_0 = net reproduction rate; r_m = intrinsic increase rate; λ = finite increase ratio; T = average time between generations; TD = population doubling time.

Table 3. Time in hours for the egg phase, and in days for the nymphal and adult stage of *Saccharicoccus sacchari* under different temperatures.

Treatments	Egg	Nymph 1	Nymph 2	Nymph 3	Adult
	(Hours)	(Days)			
23 °C ± 2 °C	1.9 ± 0.30a*	5.5 ± 0.15b	8.8 ± 0.13b	13.3 ± 0.26a	16.0 ± 0.27b
28 °C ± 2 °C	2.25 ± 0.36a	6.0 ± 0.19a	9.5 ± 0.13a	13.3 ± 0.28a	25.0 ± 0.66a

*Mean ± standard deviation followed by the same letter in the same column do not differ according to the Student test ($p > 0.05$); Egg in hours ($F = 0.39$; $df = 1$; $p > 0.5337$); Nymph 1 in days ($F = 4.80$; $df = 1$; $p > 0.0347$); Nymph 2 in days ($F = 15.11$; $df = 1$; $p > 0.00004$); Nymph 3 in days ($F = 0.02$; $df = 1$; $p > 0.8977$); Adult in days ($F = 159.65$; $df = 1$; $p < .0001$).

3.2. Differentiation of the phases of the female Saccharicoccus sacchari

Five growth periods were obtained: an egg phase, three nymphal stages and adult phase, resulting in three molting for the mature insect.

Egg is 0.36 mm length and 0.18 mm width. First instar nymph is 0.45 mm length, 0.22 mm width and antennal length of 0.16 mm. Second instar nymph is 0.87 mm length, 0.34 mm width and antennal length of 0.17 mm and third instar nymph is 1.25 mm length, 0.68 mm width and antennal length of 0.24 mm. Adult female is 4.07 mm length, 3.34 mm width and antennal length of 0.37 mm. All nymphal stages have six antennal segments, and adult has seven (Table 5).

3.3. Obtaining a winged male of Saccharicoccus sacchari in Brazil

The winged specimen is 0.73 mm length, 0.23 mm width, antennae with ten segments and antennal length of 0.34 mm, abdomen with a pair of cluster pores, forming the tail of filaments, associated with long setae to the ninth segment; apex of the penile sheath slightly expanded and surrounded with a width of 9 µm and a height of 22 µm; dermal discs with four peripheral locules; less than ten seta present on the sides of the abdomen; well-developed thorax; antennae covered with digital seta of maximum 18 µm in length.

4. Discussion

Biological development showed differences under two temperatures.

At 23 °C, insects began oviposition 25 days after birth, while at 28 °C, adult maturity began on the 28th day of life. These data corroborate those of Beardsley (1962) who observed under natural conditions, pseudococcid oviposition period is between 28 and 36 days after birth.

Table 4. Longevity in days of *Saccharicoccus sacchari* under different temperatures.

Treatments	Days
23 °C ± 2 °C	41.7 ± 1.33b*
28 °C ± 2 °C	52.0 ± 1.79a

*Mean ± standard deviation followed by the same letter in the same column do not differ according to the Student test ($p > 0.05$); Days (F = 23.39; df = 1; p <.0001).

However, Hafez and Salama (1969) show that, under 24 °C, the beginning of oviposition is from 21 °C the day after birth and, when temperature increases to 27 °C, maturity is from 16 °C days after the first instar nymph hatches.

In this work, insects raised at 23 °C added 23 females to population that reached maturity, and those raised at 28 °C added 34 females. At both temperatures, it was possible to add, each day, a female that reached maturity. Rae and De'ath (1991) reported insects raised under 25 °C added 69 individuals to population that reached maturity, and only 20 females reached maturity when raised under 30 °C; they also reported that breeding in laboratory conditions reduces fecundity of the species.

For both temperatures, during this study, time for generation to double is approximately five weeks. Ebieda Ahmed et al. (2020) described in Egypt, under natural conditions, population of *S. sacchari* can also double every five weeks, and, in the field is possible to observe four to seven generations.

Egg incubation time did not differ between the temperatures analyzed, with an average of two hours. Atiqui (1987) also reports incubation time of eggs is a few hours. However, Hafez and Salama (1969) and Uichanco and Villanueva (1932) mention incubation variations of six to ten minutes.

Under 28 °C, duration of first and second nymphal stages differed significantly, but there was no difference for the third nymphal stage, with six, 9.5 and 13 days required to complete each nymphal instar, consecutively. These results are similar to Atiqui (1987) who recorded, under natural conditions, five to seven days for the first nymphal stage, nine days for the second nymphal stage and 12 days for the last nymphal stage.

Adult stage, in this study, showed significant differences between treatments, with the longest duration being obtained at 28 °C, approximately 25 days, and at 23 °C, 16 days. This result was similar to Hafez and Salama (1969), who reported 16 days in this phase for insects raised at 27 °C. However, Atiqui (1987) recorded duration up to 29 days under natural conditions for this phase. Beardsley (1962) recorded, under natural conditions, 21 to 77 days for adult stage.

Longevity showed significant difference, averaging 41 days when reared at 23 °C and 52 days at 28 °C. Atiqui (1987) reports the insect raised at 26 °C has life cycle between 42 and 58 days. According to Ebieda Ahmed et al. (2020), under controlled conditions of 30 °C, insect's longevity is 30 days, at 23 °C, 44 days.

Table 5. Metric parameters of *Saccharicoccus sacchari* female instars.

Instar	Lenght (mm)	Width (mm)	Antennal length (mm)	Number of antennal segments
Egg	0.36	0.18	-	-
First instar nymph	0.45	0.22	0.16	6
Second instar nymph	0.87	0.34	0.17	6
Third instar nymph	1.25	0.68	0.24	6
Adult female	4.07	3.34	0.37	7

Rae and De'ath (1991) report at 20 °C biological development is 107 days; under 30 °C, 25 days; and, under natural conditions, pseudococcids develop faster at low temperatures when they are present at high latitudes. However, these authors point out that, in all geographic features, it is possible to observe the same reproductive rate when temperature exceeds 27 °C. Under natural conditions, Beardsley (1962) reports biological development as 42 days, Latha and Bautista (2020) as 30 days in Belize, and Hafez and Salama (1969) mention development varies from 52 to 138 days, however, if raised at 30 °C, the longevity is 51 days.

Five development phases of the female *S. sacchari* were obtained, with each stage presenting different metric parameters.

It was observed: an egg stage, three nymphal stages and an adult stage. This result is similar to Beardsley (1962), Atiqui (1987) and Rae (1993) who report three nymphal instars. However, Uichanco and Villanueva (1932) are the only authors who mentioned five instars for the female. They probably made an interpretative error when observing the instars, as the general rule for insects in this family is presenting only three nymphal instars (Beardsley, 1962; Atiqui, 1987; Rae, 1993).

Metric parameters for each phase during this study, were: egg 0.36 mm length and 0.18 mm width; first instar nymph 0.45 mm length, 0.22 mm width and 0.17 mm antennal length; second instar nymph 0.87 mm length, 0.34 mm width and 0.17 mm antennal length; third instar nymph 1.25 mm length, 0.68 mm width and 0.24 mm antennal length; and adult female measuring 4.07 mm length, 3.34 mm width and 0.37 mm antennal length. In all nymphal stages, six antennal segments were recorded, and in adult, seven.

These results are similar to previous studies (Beardsley, 1962; Atiqui, 1987; Rae, 1993). According to Atiqui (1987), egg is 0.37 mm length and 0.19 mm width; first instar nymph is 0.50 mm length and 0.15 mm width; second nymphal stage is 0.80 mm length and 0.30 mm width; and third instar nymph is 1.12 mm length and 0.48 mm width. According to the author, in all phases there are six antennal segments. Rae (1993) mentions the first nymphal stage there is 0.61 mm length, 0.22 mm width and 0.17 mm antennal length; second nymphal stage the length is 0.87 mm, width is 0.43 mm and antennal length is 0.18 mm; the third instar nymph there are 1.26 mm length, 0.61 mm width and 0.23 mm antennal length, with six antennal segments for all phases. According to Beardsley (1962), a small adult female is 1.25 mm length, but Rae (1993) reports at this stage around 1.93 mm length, 0.92 mm width and 0.28 mm antennal length with seven antennal segments.

A winged individual was observed, based on the identification key for male mealybugs by Beardsley (1960), understanding that sexual reproduction is also possible in national territory. According to Uichanco and Villanueva (1932), there are few winged males in the Philippines. Atiqui (1987) also reports low number of males in India. Beardsley (1962) and Rae (1993) report winged and apterous males occur in Hawaii and Australia, respectively. And Hafez and Salama (1967) mention in Egypt a largest polymorphic quantity of males, and in laboratory rearing winged male is more numerous than apterous or brachyapterous.

Saccharicoccus sacchari, under controlled temperature conditions, presents same reproductive behavior as observed in natural conditions, with asexual reproduction occurring through thelitotic parthenogenesis. As ambient temperature increases, duration of each phase increases, demonstrating an ideal rearing at 28 °C ± 2 °C.

The record of winged male indicates the possibility of sexual reproduction in the country.

Future studies should be based on monitoring life cycle of winged male and analyzing biological development based on sexual reproduction in different locations.

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