EFFECT OF TEMPERATURE ON DEVELOPMENT AND SURVIVAL OF THE MEALYBUG COCHINEAL Pseudococcus longispinus (Targioni TOZZETTI, 1867) (Hemiptera: Pseudococcidae) IN COFFEE [1]

Lenira Viana Costa Santa-Cecilia[2], Ernesto Prado[3], Marcella Viana de Sousa[4], Ana Luiza Viana de Sousa[5], Lilian Roberta Baptist Correa[6]

(Received: September 4, 2009, accepted March 29, 2011)

ABSTRACT: The mealybug Pseudococcus longispinus (Targioni Tozzetti, 1867) (Hemiptera: Pseudococcidae) has been reported attacking coffee crops causing fruit fall in the State of Minas Gerais, Brazil. The full understanding of this pest is necessary to implement control measures. Its development was studied at temperatures of 15, 20, 25, 30 and 35 ° C. The insects were confined inside a Petri dish containing a foliar disc of 4 cm diameter of Coffea arabica L. cultivar ' Acaiá cerrado'. The temperature affected the P. longispinus development and survival. Few insects survived at temperatures of 15 and 30 ° C, and 100% mortality was obtained at 35 ° C. The duration of the nymphal stage was reduced when the temperature was increased from 20 to 25 º C, with a survival rate of 80% at both temperatures. The thermal parameters varied according to the development stage of the mealybug and the base temperature was fixed at 8.0 C for the nymphal stage of females, a thermal constant of 422.1 day degrees and number of generations increased with rising temperature. The optimal temperature for the insect development was 25 ° C.

Key words: biology, Coccoidea, thermal parameters, Coffea arabica.

1 INTRODUCTION

The unarmored scale insects of the family Pseudococcidae (Hemiptera), known as mealybug, have manifested in sporadic outbreaks in several coffee regions of the country, verifying unpredictable attacks of the roots and shoots (SANTA-CECILIA et al. 2007). Among the twelve species linked to coffee production in Brazil (CULIK; MARTINS, Gullane, 2006; WILLIAMS; GRANARA DE WILLINK, 1992), the mealybug Pseudococcus longispinus (Targioni Tozzetti, 1867), known as the long-tailed-white mealybug has been found colonizing the stalk region of the fruit, sucking the sap and causing its fall. Although it is a sporadic occurrence in the coffee plantations, the damage they cause can affect production (SANTA-CECILIA et al., 2007, Silva et al., 2010, Souza et al., 2008).

Aspects of the biology of this pest have been reported in some hosts, such as citrus and olive trees (Prado et al. 2003; RIPA, RODRIGUEZ, 1999), but information on coffee trees are still limited, given the scarcity of research involving biological studies on this insect in this culture, especially those related to development associated with different temperatures.

Considering that the development of P. longispinus may vary according to the host, temperature, along with other factors that may cause damage to the coffee, a better understanding of the biology and temperature requirements of this pest is of importance, as it will allow advances in understanding the population dynamics contributing to their management.

The objective of this study was to evaluate the development and survival of P. longispinus in coffee at different temperatures and to determine the thermal requirements of the development phases of this culture and the number of annual generations of this pest in laboratory conditions.

---

[1]Reasearch funded by the Brazilian Consortium for Coffee Research and Development

[2]Agronomist Engineer, D.Sc., IMA / EPAMIG-URESM-EcoCentro, Cx P. 176-37200-000-SE Brazil - Researcher FAPEMIG - scecilia@epamig.ufla.br 3

[3]Agronomist Engineer, D. Sc., EPAMIG / URESM-EcoCentro, Cx P. 176-37200-000-SE Brazil - Research and Scholarship CBPD & D / Café / EPAMIG - epradoster@gmail.com

[4]Agronomist Engineer, Ph.D., Department of Plant Pathology / DFP, Universidade Federal de Lavras / UFLA - Cx P. 3037-37200-000-SE Brazil - maviana@hotmail.com

[5]Biologist, MSc, Department of Entomology / DEN, Universidade Federal de Lavras / UFLA - Cx P. 3037-37200-000-SE Brazil - anhalu04@yahoo.com.br

[6]Biologist, Agronomist, Ph.D., Universidade Estadual Paulista, Faculty of Agriculture and Veterinary Sciences, Department of Plant Protection, Access Via Prof.. Paulo Donato Castellane, s / n, 14884-900, Jaboticabal, SP-robertaento @ yahoo.com.br

2 MATERIAL AND METHODS

The study was conducted at the Laboratory of Biological Control of Pests EPAMIG, URESM, EcoCentro, Lavras, MG.

To carry out this experiment, we collected colonies of P. longispinus in coffee plantations and then the infestation was made in pumpkins (Cucurbita maxima L.) cultivar Cabotchá, kept under controlled conditions of temperature, relative humidity and photoperiod (25.0 ± 1° C, 70±10 % and 12h photophase) aiming to obtain a population density sufficient for the development of the experiment.

Newly hatched nymphs of the mealybug, numbering 200, were collected in the laboratory and transferred to individual foliar discs (4 cm in diameter) of Coffea arabica L. cultivate Acaiá Savannah, arranged on a surface of approximately 5 mm of agar-water at 1%. These foliar discs were kept in Petri dishes (5 cm diameter) sealed with PVC plastic film, according to methodology developed by Santa Cecilia et al. (2008) and placed in climatic chambers at constant temperatures of 15, 20, 25, 30 and 35 ± 2 º C, 70 ± 10% RH and 12 hours photophase. For each temperature, we evaluated the development of 40 nymphs of P. longispinus, and the repetitions consisted of specimens of unknown sex, since at the first instar you can not make the distinction.

The plates were changed every five days, cutting the portion of the leaf where they were insects and transferring it to the new foliar disc, thereby avoiding the manipulation of insects and damage to the mouth stylets.

The development of mealybugs was observed daily under a stereomicroscope and the distinction of instars was made based on the presence of exuviae, considering the three nymphal stages of development for females and the four stages for males. The duration of the third and fourth instar of the male inside the cocoon was assessed by molting, which are externalized by the nymphs. We assessed the duration and mortality in each instar nymphal stage of males and females and their longevity. The longevity of adult males was not considered, since they exhibit a very short life span, with the only function of fertilizing the females.

The work was conducted in a completely randomized design and analysis of variance was performed with the five temperatures studied (15, 20, 25, 30 and 35 degrees C) for data on the duration of instars, nymphal stage and longevity. Only with the first instar was it possible to make comparisons of the measurements by Tukey test at 5% significance level, with data transformed in $\sqrt{x}$. In the other instars it was not possible to perform the analysis at 15, 30 and 35 ° C due to the reduced number of emerged insects, caused by high mortality. Thus, the low number of insects available for analysis in these treatments increased the margin of error of the experiment by not detecting the differences between the temperatures of 20 and 25 degrees C. In this case, we chose to use the Student test at 5% significance for comparison of means, not considering the treatments with high mortality. To compare the average mortality, we used the chi-square test at 5% significance.

Using the hyperbola method described by Haddad, Parra and Moraes (1999) determined the base temperature (Tb) in ° C and thermal constant (K) degree-day data of average duration of different instars and nymph phase. To estimate the number of generations per year we used the methodology developed by Parra (1981), through the use of thermal constants of the nymphal stage of females.

3 RESULTS AND DISCUSSION

Temperature influenced the development stages of immature and adult females of P. longispinus (Table 1), corroborating the observations found for other pseudococcídeos (CHONG; OETTINGER; IERSEL, 2003; COLEN et al., 2000, CORREA et al. 2008; GARCIA; ALAUZET; DECAZY, 1992; KIM, SONG, KIM , 2008).

In the first instar, there was a 70% reduction in the duration when the temperature rose from 15 ° to 25 ° C, finding on average 32.2 and 9.8 days, respectively. Similar results were reported by Correa et al. (2008), who investigated the development of the mealybug Planococcus citri (Risso, 1813) (Hemiptera: Pseudococcidae) in C. arabica cultivar Mundo Novo. These authors observed at 25 ° C an average of 8.5 days for the first instar, which corresponds to 70% reduction in the duration, the same temperatures mentioned above. Also in studies by Kim et al. (2008) in the laboratory on the development and fecundity of Pseudococcus cryptus Hempel, 1918 (Hemiptera:

Pseudococcidae) in citrus trees, reductions in duration were observed with increasing temperature from 16 to 28 ° C registering 10.4 days at 24 ° C.

A temperature of 35 ° C caused the death of all the nymphs, making it impossible to evaluate subsequent instars.

In the second and third instars and nymphal stage of females of P. longispinus, there were reductions of 32% by increasing temperature from 20 to 25 ° C, where we observed an average duration of 10.0 and 6.8 days (second instar), 11.9 and 8.1 days (third instar), and 35.5 and 24.1 days (nymphal stage). Similar results were found by Correa et al. (2008) for the mealybug P. citri, who observed, for the same aforementioned temperatures, durations of 10.2 and 7.1 days, 9.5 and 8.1 days and 35.2 and 23.2 days, respectively.

For the second instar of male nymphs, temperatures of 15 and 35 ° C caused the death of all insects. This may indicate a greater sensitivity to extreme temperatures in the range tested in this study. However, when compared to temperatures of 20 and 25 ° C, the average duration was not affected in the second and fourth instars (Table 1). It is noteworthy that as the development of male nymphs from the later stage of the second instar occurs inside the cocoon of waxy filaments secreted by the nymph, it was expected that the temperature would not affect the duration of subsequent instars. However, in the third instar, eventhough the analysis detected a difference, the values ranged from 4.2 to 2.3 days, which were not representative of the variation.

In adult females, there were no significant differences in temperatures of 20 and 25° C, which did not affect their longevity. These results are similar to those observed for virgin females of Phenacoccus madeirensis Green, 1923 (Hemiptera: Pseudococcidae) and in the studies of Chong and Oetting Iersel (2003), on chrysanthemum.

Analyzing the mortality of the mealybug in the five temperatures studied (Table 2) revealed that 35 ° C resulted in the death of all insects. However, Chong and Oetting Iersel (2003) failed to establish colonies of P. madeirensis from 30 ° C.

We emphasize that it is common to see the occurrence of P. longispinus under high temperatures, but in the field, these insects live in colonies inside the coffee buds, which provide a microclimate more favorable to their development. In the same manner, under natural conditions, insects are not subject to constant temperatures, but instead are subject to temperature fluctuations, especially those occurring between day and night.

Temperatures of 20 and 25°C provided the lowest mortality rates in the first and second instars and the nymphal stage (Table 2). For the third instar, the number of dead insects did not differ at temperatures of 20, 25 and 30°C, with the lowest values recorded. For the nymphal stage, the survival of this mealybug was 80% at temperatures of 20 and 25°C. Similar results were found by Garcia et al. (1992) who studied the development of the Dysmicoccus cryptus mealybug (Hempel, 1918) (Hemiptera: Pseudococcidae), at different temperatures and humidities, maintained in potato tubers, observed low mortality (2.7%) at 25° C and 70% RH, registering that at 20 and 25°C chances of survival were above 90%. Also Correa et al. (2008) found the temperature 25 ° C as the most favorable for the development of P. citri in coffee. However, different results have been verified by Colen et al. (2000), who studied the effect of temperature on the biology of the mealybug Dysmicoccus brevipes (Cockerell, 1893) (Hemiptera: Pseudococcidae), in pineapple, and obtained high percentages of mortality of these insects, including 25 ° C, which was attributed to methodologyused.

Analyzing the effect of temperature on the duration of instars and mortality during the nymphal stage found that the temperature of 25 º C showed higher survival and shorter duration, indicating that it was the most suitable for the development of this mealybug. At 15 º C, we observed a slower development, accompanied by high mortality, a condition unfavorable to the biological cycle of this insect. This result is attributed to the fact that low temperatures may lead to a slowdown in the rate of metabolism, resulting in longer development periods, as reported in studies for other insect species (Aguiar-Valgôde; Milward-DE-AZEVEDO, 1992; CARDOSO et al. 2007; Milward-DE-AZEVEDO et al., 1995). The constant temperature of 30 ° C was not the most suitable for the cochineal, because of the high rate of mortality (85% in the nymphal stage), while at 35 ° C showed no development.

TABLE 1 – Average length (± SE) (days) of instars of the nymphal stage of males and females and longevity of females of Pseudococcus longispinus (Targioni Tozzetti, 1867) in Coffea arabica L. Acaiá Cerrado grown at different temperatures. 70 ± 10% RH and 12 hour photophase.

Instar/Fase (sex)	Temperature (ºC)					
	15	20	25	30	35	Value P
1° instar(F/M)	32,2 ± 2,7a (n=15)	13,1 ± 0,7b (n=37)	9,8 ± 0,2c (n=40)	13,0 ± 1,0b (n=19)	--	<0,001
2° instar (F)	15,6 *(n=7)	10,0 ± 0,5a (n=14)	6,8 ± 0,4b (n=17)	11,0 *(n=2)	--	<0,001
2° instar (M)	--	9,7 ± 0,9 (n=18)	8,9 ± 0,4 (n=15)	12,8 *(n=4)	--	0,404
3° instar (F)	12,0 *(n=3)	11,9 ± 0,8a (n=14)	8,1 ± 0,5b (n=17)	7,0 *(n=2)	--	<0,001
3° instar (M)	--	4,2 ± 0,7a (n=18)	2,3 ± 0,1b (n=15)	3,0 *(n=4)	--	0,021
4° instar (M)	--	3,6 ± 0,4 (n=18)	2,9 ± 0,2 (n=15)	3,3 *(n=4)	--	0,109
Nymph Fase (F)	60,0 *(n=3)	35,5 ± 1,4a (n=14)	24,1 ± 0,5b (n=17)	30,0 *(n=2)	--	<0,001
Nymph Fase (M)	--	31,0 ± 1,3a (n=18)	24,4 ± 0,5b (n=15)	31,3 *(n=4)	--	<0,001
adult Fase (F)	13,0 *(n=3)	89,2 ± 6,1 (n=14)	76,5 ± 7,0 (n=17)	32,0 *(n=2)	--	0,190

Means followed by same letter in the first line, do not differ by Tukey test at 5% significance level. Means followed by same letter in rows 2 to 9, do not differ by Student test at 5% significance. * Inability to conduct statistical analysis of these data due to the small number of repetitions. F = female, M = male, n = number of specimens evaluated

TABLE 2 – Mortality Mean (%) in instars and nymphal phase of Pseudococcus longispinus (Targioni Tozzetti, 1867) in Coffea arabica L. Acaiá Cerrado grown at different temperatures. 70 ± 10% RH and 12 hours photophase.

Instar/Fase(sex)	Temperature (°C)*					
	15°	20°	25°	30°	35°	Valor P
1° instar(F/M)	62,50b (n=40)	7,50c (n=40)	0,00c (n=40)	52,50b (n=40)	100,00a (n=40)	<0,001
2° instar (F/M)	53,33b (n=15)	10,81c (n=37)	20,00c (n=40)	68,42b (n=19)	100,00a (n=40)	<0,001
3° instar (F/M)	57,14b (n=7)	3,03c (n=33)	0,00c (n=32)	0,00c (n=6)	100,00a (n=40)	<0,001
Nymphal Fase (F/M)	92,50ab (n=40)	20,00c (n=40)	20,00c (n=40)	85,00b (n=40)	100,00a (n=40)	<0,001

*Means followed by the same letter on line do not differ by Chi-Square at 5% significance. F = female, M = male, n = number of specimens evaluated

The estimated values for the lower thermal limit for development (Tb) obtained by regression analysis, preceded by adjustments in the second degree equations to describe the effect of temperature on the duration of instars and the nymphal stage of P. longispinus was possible only for females, since the data for males did not fit the model of the hyperbolic method. The biological significance is that the development threshold was exceeded, showing an inverse relationship of temperature increase with the decrease in development time.

The results obtained for thermal requirements of females of P. longispinus from first to third instar, estimated that the lower thermal limit of theoretical development (Tb) at 10.2 C, 7.4 C and 5.0 º C, respectively, and 8.0 C for the nymphal stage (Table 3). Thus, there was tolerance of P. longispinus at low temperatures and the third instar females, which showed more tolerance for having the lowest threshold temperature.

Adopting 8.0 º C as the lower thermal limit for development of the nymphal stage, 422.1 degree-days (DD), are required for P. longispinus to complete development and reach adulthood. These values are close to those reported by Martinez-Ferrer, and Garcia-Mari-Ripollés- Moles (2003) for the mealybug P. citri in citrus groves, and given the lower threshold temperature of 8.3 º C and 562.4 DD for the constant heat.

Considering the temperature range studied and also the lower thermal threshold and thermal constant obtained, it was found that with the rise of the isotherms, there was an increase in the number of annual generations of P. longispinus (Table 4). Nevertheless, the calculation would be inappropriate to extrapolate the number of generations obtained in laboratory studies by uniform temperature and humidity, such as what happens in the field, given that, under these conditions, the temperature and microclimate around insects vary during the day / night and the season, affecting the development of the insect. Thus, the number of generations in the field should be studied under natural conditions. However, the results are a reference for the establishment of laboratory creations, as well as to estimate the optimum and extreme temperatures that the insect can tolerate.

In regions of Chile that show relatively lower temperatures and humidities, the long-tailed-white mealybug, may present up to four generations per year, as its lower thermal limit is 12.5 ° C (Prado et al. 2003). Under laboratory conditions, Correa et al. (2008) obtained an estimate of 10.2 generations per year for females of P. citri in coffee, when adopting a temperature of 20 ° C. whereas, Colen et al. (2000) calculated 10.55 annual generations for females of D. brevipes in pineapple, when the temperature was raised to 27 ° C.

TABLE 3 – Lower heat Limit development or base temperature (Tb), thermal constant (K), the equations of development speed and coefficient of determination (R^2) of Pseudococcus longispinus (Targioni Tozzetti, 1867) in Coffea arabica L. cultivate Acaiá Cerrado.

Instares/fase (sex)	Tb (ºC)	K (DD)	Equations (l/D)	R^2
First (F,M)	10,2	139,80	- 0,072934+0,007151x	0,975
Second (F)	7,4	121,36	- 0,060950+0,008240x	0,996
Third (F)	5,0	170,82	- 0,029269+0,005854x	0,959
nymphal Fase (F)	8,0	422,10	- 0,018980+0,002369x	0,999

F = female, M = male

TABLE 4 – Number of possible annual generations of females of Pseudococcus longispinus (Targioni Tozzetti, 1867) in function of base temperature of 8.0 ° C and thermal constant of 422.1 DD in four temperatures studied and found in coffee growing regions of Brazil.

Average temperature	Generations per year
15°C	6,1
20°C	10,4
25°C	14,7
30°C	19,0

4 CONCLUSIONS

The development of P. longispinus in coffee is influenced by temperature, verifying the greatest survival of the nymphal stage at 20 and 25 ° C and shorter at 25 ° C.

Extreme and constant temperatures at 15 and 35 ° C did not allow the development of P. longispinus. The lower thermal limit for development of the nymphal stage of P. longispinus is 8.0 º C and thermal constant of 422.1 DD.

5 ACKNOWLEDGMENTS3

To the Coffee Research Consortium for funding and research grants. To the laboratory technician, Fabiana R. do Nascimento, for collaboration in conducting the experiment. To FAPEMIG for the scientific initiation scholarship granted.

6 REFERENCES

AGUIAR-VALGODE, M.; MILWARD-DE-AZEVEDO, E. M. V. Determination of thermal requirements of *Stomoxys calcitrans* (L.) (Diptera, Muscidae), under laboratory conditions. **Memórias do Instituto Oswaldo Cruz**, Rio de Janeiro, v. 87, p. 11-20, 1992.

CARDOSO, A. C. et al. Exigências térmicas de estágios imaturos de *Galleria mellonella* L. (Lepidoptera: Pyralidae). **Neotropical Entomology**, Vacaria, v. 36, n. 5, p. 657-661, 2007.

CHONG, J.; OETTING, R. D.; IERSEL, M. W. van. Temperature effects on the development, survival, and reproduction of the Madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), on chrysanthemum. **Annals of the Entomological Society of America**, College Park, v. 96, n. 4, p. 539-543, 2003.

COLEN, K. G. F. et al. Efeitos de diferentes temperaturas sobre a biologia da cochonilha pulverulenta *Dysmicoccus brevipes* (Cockerell, 1893) (Hemiptera: Pseudococcidae). **Revista Brasileira de Fruticultura**, Jaboticabal, v. 22, n. 2, p. 248-252, 2000.

CORREA, L. R. B. et al. Efeito de diferentes temperaturas e exigências térmicas da cochonilha-branca *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) em cafeeiro. **Arquivos do Instituto Biológico**, São Paulo, v. 75, n. 1, p. 53-58, 2008.

CULIK, M. P.; MARTINS, D. dos S.; GULLAN, P. J. First records of two mealybug species in Brazil and new potential pests of papaya and coffee. **Journal of Insect Science**, Elmsford, v. 6, n. 23, p. 540-546, 2006.

GARCIA, A.; ALAUZET, C.; DECAZY, B. Biologie de la cochonilhe racinaire du caféier *Dysmicoccus cryptus* (Hempel, 1918) comb. n. (Homoptera: Pseudococcidae). **Café Cação Thé**, Paris, v. 36, n. 1, p. 35-44, 1992.

HADDAD, M. L.; PARRA, J. R. P.; MORAES, R. C. B. **Métodos para estimar os limites térmicos inferior e superior de desenvolvimento de insetos.** Piracicaba: FEALQ, 1999. 29 p.

KIM, S. C.; SONG, J.; KIM, D. Effect of temperature on the development and fecundity of the cryptic mealybug, *Pseudococcus cryptus*, in the laboratory. **Journal of Asia Pacific Entomology**, Southampton, v. 11, p. 149-153, 2008.

MARTINEZ-FERRER, M. T.; GARCIA-MARI, F.; RIPOLLES-MOLES, J. L. Population dynamics of *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) in citrus groves in Spain. **Integrated Control in Fruit Crops**, Chicago, v. 26, n. 6, p. 149-161, 2003.

MILWARD-DE-AZEVEDO, E. M. V. et al.Desenvolvimento ontogenético, potencial reprodutivo e elongevidade de *Chrysomya megacephala* (Fabricius) (Diptera, Calliphoridae), em condições de laboratório. **Revista Brasileira de Entomologia**, São Paulo, v. 39, p. 623-632, 1995.

PARRA, J. R. P. **Biologia comparada de *Perileucoptera coffeella* (Guerin-Meneville, 1842) (Lepidoptera: Lyonetiidae), visando ao seu zoneamento ecológico no Estado de São Paulo.** 1981. 96 f. Tese (Livre-Docência) – Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, 1981.

PRADO, E. et al. **Plagas del olivo, sus enemigos naturales y manejo**. Santiago: Centro Regional de Investigatión, 2003. 74 p. (Coleccion Libros INIA, 8).

RIPA, R.; RODRIGUEZ, F. **Plagas de cítricos, sus enemigos naturales y manejo.** Santiago: Instituto de Investigaciones Agropecuárias, 1999. 151 p. (Colección Libros INIA, 3).

SANTA-CECÍLIA, L. V. C. et al. **Cochonilhas-farinhentas em cafeeiros**: **bioecologia, danos e métodos de controle.** Belo Horizonte: Epamig, 2007. 40 p. (Boletim técnico, 79).

_____. Methodology for biological studies of mealybugs (Hemiptera: Pseudococcidae). **Coffee Science**, Lavras, v. 3, n. 2, p. 152-155, jul./dez. 2008.

SILVA, R. A. et al. Sintomas de injurias causadas pelo ataque de pragas em cafeeiro. In: GUIMARÃES, R. G.; MENDES, A. N. G.; BALIZA, D. P. (Ed.). **Semiologia do cafeeiro:** sintomas de desordens nutricionais, fitossanitárias e fisiológicas. Lavras: UFLA, 2010. p. 107-142.

SOUZA, B. et al. Cochonilhas-Farinhentas (Hemiptera: Pseudococcidae) em cafeeiros (*Coffea arabica* L.) em Minas Gerais. **Coffee Science**, Lavras, v. 3, n. 2, p. 104-107, jul./dez. 2008.

WILLIAMS, D. J.; GRANARA DE WILLINK, M. C. **Mealybug of Central and South America**. Walingford: CABI, 1992. 629 p.