



Survival, development and reproduction of *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) on kaolin-treated cotton

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Received: November 6, 2018 – Accepted: August 12, 2019 – Distributed: November 30, 2020

Abstract

The mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is a cotton pest widespread in several cotton growing regions of Brazil, particularly in the semi-arid region of southwestern Bahia. The impact of kaolin on survival, development and reproduction of *P. solenopsis* was evaluated in the laboratory. The experiment was developed in a completely randomized design with two treatments: immature or newly emerged adults of *P. solenopsis* sprayed with kaolin and fed with cotton leaf discs treated with kaolin suspension (with kaolin) (T1) and immature or newly emerged adults of *P. solenopsis* sprayed with distilled water and fed with cotton leaf discs treated with distilled water (without kaolin) (T2). The kaolin suspension shortens the life cycle, increases the reproductive potential and population growth of the cotton mealybug, *P. solenopsis* and, therefore, it should be used with caution on cotton plants in regions with a history of occurrence of this pest.

Keywords: biological traits, *Gossypium hirsutum*, mealybug, particle film, stimulatory effects.

Sobrevivência, desenvolvimento e reprodução de *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) em algodão tratado com caulim

Resumo

A cochonilha *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) é uma praga de algodão, amplamente, difundida em várias regiões produtoras do Brasil, particularmente, na região semiárida do sudoeste da Bahia. O impacto do caulim na sobrevivência, desenvolvimento e reprodução de *P. solenopsis* foi avaliado em laboratório. O delineamento experimental foi inteiramente casualizado com dois tratamentos: imaturos ou adultos recém-emergidos de *P. solenopsis* alimentados com discos foliares de algodão tratados com caulim (com caulim) e pulverizadas com este produto (T1) e imaturos ou adultos recém-emergidos de *P. solenopsis* alimentados com discos foliares de algodão tratados com água destilada (sem caulim) e pulverização dos insetos com água destilada (T2). A suspensão do caulim encurtou o ciclo de vida, aumentou o potencial reprodutivo e o crescimento populacional da cochonilha-do-algodoeiro, *P. solenopsis* e, portanto, deve ser utilizado com cautela em plantas de algodão em regiões com histórico de ocorrência desta praga.

Palavras-chave: características biológicas, *Gossypium hirsutum*, cochonilha, filme de partículas, efeito estimulante.

1. Introduction

Cotton plants host a high boll, leaf, root, square and stem pest complex (Allen et al., 2018). The mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) is a major cotton pest in several countries (Nagrare et al., 2016). This insect sucks cotton sap, injects toxins and secretes “honeydew” stimulating the development of black sooty moulds which adversely affect photosynthesis (Joshi et al., 2010).

In Brazil, *P. solenopsis* severely infests cotton plants in Southwest and mid São Francisco, Bahia State (Silva,

2012). Heavy infestation of the cotton mealybug may result from the absence of natural enemies of this invasive pest (Noureen et al., 2016). Parasitoids (Aslam et al., 2017; Karmakar and Shera, 2017; Joodaki et al., 2018) and predators (Arif et al., 2012; Zazycki et al., 2015; Aslam et al., 2017; Lima et al., 2018) can control this mealybug. However, chemical control, with highly toxic insecticides to natural enemies, is the main method to reduce populations of this mealybug (Huang et al., 2012; Karmakar and Shera, 2017).

New insecticide formulations have been developed with effective physicochemical properties against *P. solenopsis* including natural products with potential to substitute conventional chemical insecticides (Silva and Ramalho, 2013; Silva and Silva, 2015). These products need to be biodegradable and selective, characteristics largely found in plant extracts or in minerals.

Kaolin, a white clayey mineral that dissolves to form a white syrup in water, has been successfully used to protect cotton plants against pests, including the boll weevil, *Anthonomus grandis* (Boheman, 1843) (Coleoptera: Curculionidae) (Silva and Ramalho, 2013); cotton leafworm, *Alabama argillacea* (Hübner, 1818) (Lepidoptera: Noctuidae) (Gonçalves et al., 2015); cotton bollworm, *Helicoverpa armigera* (Hübner, 1809) (Lepidoptera: Noctuidae) (Alavo et al., 2011); beet armyworm, *Spodoptera exigua* (Hübner, 1808) (Lepidoptera: Noctuidae) (Showler, 2003); pink bollworm, *Pectinophora gossypiella* (Saunders, 1844) (Lepidoptera: Gelechiidae) (Sisterson et al., 2003) and aphid, *Aphis gossypii* (Glover, 1877) (Homoptera: Aphididae) (Alavo et al., 2011), although adverse effects of its application were reported (Showler and Armstrong, 2007).

The main basic action of kaolin particle film against pest insects is due to its interference during the host plant location and acceptance process by the insect. Indeed, the white particle films of kaolin on plants alters the visual and tactile recognition and smell inducing repellence from treated foliage and a consequent feeding and oviposition by insects (Glenn and Puterka, 2005). The kaolin may affect the moisture of the cotton leaf surface and, consequently, the feeding and oviposition behavior of *P. solenopsis* on its leaves. However, kaolin has specific action against each pest (Silva and Ramalho, 2013) and, therefore, understanding its impact on sucking insects can minimize failures when used against these organisms.

The objective of the present study was to assess kaolin effects on the survival, development and reproduction of *P. solenopsis* on cotton plants.

2. Material and Methods

2.1. Site and insect

Experiments were conducted at Embrapa Algodão Entomology Laboratory, Campina Grande, Paraíba State, Brazil in climatic chambers with a temperature of 25 ± 1 °C, relative humidity of $60 \pm 10\%$ and photophase of 12 hours.

The *P. solenopsis* specimens were collected from an experimental cotton crop of the Embrapa Algodão.

2.2. Biology and life table

One hundred adult *P. solenopsis* were transferred from the stock-rearing unit to leaves of the cotton cultivar BRS 286 for oviposition and obtainment of first instar nymphs of this pest. The nymphs were collected forty-eight hours after transferring the females of this mealybug to the cotton leaves.

The experiment was developed in a completely randomized design with two treatments: immature or newly

emerged adults of *P. solenopsis* sprayed with kaolin and fed with cotton leaf discs treated with kaolin suspension (with kaolin) (T1) and immature or newly emerged adults of *P. solenopsis* sprayed with distilled water and fed with cotton leaf discs treated with distilled water (without kaolin) (T2). Fifty replications were used. A manual pre-compressed sprayer (Guarany Indústria e Comércio Ltda, Itu, SP, Brazil) with a 1.2 L volume capacity and empty cone nozzle was used to spray the newly emerged immature and adult *P. solenopsis*. The kaolin particle film was applied at a concentration of 60g of kaolin/L of water based on previous studies with sap-sucking cotton pests (Showler and Armstrong, 2007).

Fifty-five *P. solenopsis* newly emerged first instar nymphs were individually maintained per rearing unit of PVC tubes (2.5 cm x 2.0 cm) sealed at the base. A moistened filter paper was inserted inside the tubes and a cotton leaf disc (2.4 cm diameter) collected from the cotton leaf ventral side. The rearing units were covered with a transparent plastic film and maintained in climatic chambers until the conclusion of observations.

Biological observations were performed daily at 8:00 A.M. and 04:00 P.M. with stereoscopic microscope. The development, duration and survival of each instar and of the nymph stage, besides the pre-oviposition, oviposition and post-oviposition periods, longevity, fecundity and number of eggs/day and of eggs/female/day of *P. solenopsis* were evaluated. The adults of this insect were sexed based on sexual dimorphism and separated into couples with the newly emerged males and females individualized in pairs in new arenas. The fertility and sex ratio of the progeny on the cotton leaf discs, with and without kaolin, were determined. The number of *P. solenopsis* eggs in these arenas was quantified daily at 04:00 P.M.

2.3. Data analysis and statistics

Development data of *P. solenopsis* males and females were submitted to homoscedasticity tests and to the analysis of variance (ANOVA) and the means compared by LSD.

The *P. solenopsis* reproductive data were organized for life table calculations (Silveira et al., 1976). The reproductive rate net values ($R_0 = \sum mx.lx - mx$: total eggs/female number; lx : alive individuals/total), gross reproductive, also expressed in units of number of females per female ($GRR = \sum_{x=0}^n mx$ generation time ($T = mx.lx.x/mx.lx \Sigma$), intrinsic rate ($r_m = \log R_0/T.0.4343$), and finite ($\lambda = \text{antilog } r_m$) rate of increase were calculated. The confidence intervals of the means were estimated with these parameters determined using the SAS computer program (SAS Institute, 2008) applying the Jackknife resampling technique (Maia et al., 2000).

3. Results

The survival of immature *P. solenopsis* had no interactions with the treatment duration (Table 1) but differed between its stages. The survival of *P. solenopsis* second and third

instars and of the pupa stage was highest and that of the first instar lowest in both treatments (Table 2).

The duration of the *P. solenopsis* immature stage showed no interaction with the treatments (Table 2), but it differed between treatments and immature stages. The duration of second and third instar and of the egg to adult period of females was longer with distilled water (without kaolin) and shorter with kaolin.

The duration of pre-oviposition and oviposition periods of *P. solenopsis* were, respectively, shorter and longer with kaolin, but that of the post-oviposition was similar between treatments (Table 3). The number of eggs per female and their fecundity were higher with kaolin, but that of eggs per day was similar between treatments.

Phenacoccus solenopsis longevity was similar between treatments ($F_{1,33} = 1.64, P = 0.21$) and differed between sex of this insect ($F_{1,33} = 180.62; P < 0.01$), with females always outliving the males.

The duration of one generation (DG) and the period for the *P. solenopsis* to double its population in numbers (DT) did not differ between treatments (Table 4). The positive r_m and λ values show that *P. solenopsis* populations tend to grow, exponentially, in both treatments. On the other hand, the gross (GRR) and net (Ro) reproductive rates and the intrinsic (r_m) and finite (λ) rate of increase differed with higher values with the kaolin suspension, indicating that the particle film increased this mealybug biotic potential.

Table 1. Summary of the effects of the treatments* and immature stages on the development and survival period** of the immature stages of *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) using the analysis of variance (ANOVA).

Source	Model	DF	F	P
Development (days)	Treatment (T)	1	15,33	< 0,01
	Immature stage (IS)	5	444,45	< 0,01
	T × IS	5	1,36	> 0,05
	Residue	144	-	-
Survival (%)	Treatment (T)	1	0,03	> 0,05
	Immature stage (IS)	5	17,96	< 0,01
	T × IS	5	0,14	> 0,05
	Residue	212	-	-

*Treatments: spraying the immature and newly emerged adult *P. solenopsis* and immersing cotton leaf discs with kaolin suspension in 60 gL⁻¹ distilled water or with distilled water (control); **Survival: data transformed into square root of $x + 0.5$. DF = degrees of freedom; F = Fisher test; P = probability.

Table 2. Survival (%) and development (mean ± standard error) of the immature stages and of the egg to adult of *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) on the cotton leaf disks treated with kaolin suspension and distilled water (control).

Stages	Treatments					
	Ind.	Sur. (%)	With kaolin	Ind.	Sur. (%)	Without kaolin
1° instar	50	40.0 a*B**	6.50 ± 0.19 aB	50	44.0 aB	6.75 ± 0.28 aB
2° instar	18	90.0 aA	3.92 ± 0.10 bC	22	100.0 aA	4.80 ± 0.40 aC
3° instar	12	100.0 aA	5.25 ± 0.11 bC	10	90.0 aA	6.17 ± 0.14 aC
Pupa	06	100.0 aA	7.83 ± 0.28 aA	10	90.0 aA	7.95 ± 0.46 aA
Egg-Adult female	12	37.0 aA	15.75 ± 0.28 bB	10	40.0 aA	17.50 ± 0.42 aB
Egg-Adult male	06	35.0 aA	17.92 ± 0.32 aA	10	40.0 aA	19.50 ± 0.90 aA

Means followed by the same lowercase letter per row* or upper** case per column do not differ by the F- LSD test (P<0.05). Ind. = number of individuals per stages and treatment; Sur. = Survival.

Table 3. Reproductive characteristics (mean ± standard error) of *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) on the cotton leaf disks treated with kaolin suspension and distilled water (control).

Variables	With kaolin	Control
Pre-oviposition period (days)	9.50 ± 0.34 b*	10.83 ± 0.28 a
Oviposition period (days)	22.67 ± 2.11 a	15.17 ± 1.38 b
Post-oviposition period (days)	1.50 ± 0.94 a	2.50 ± 1.28 a
Number of eggs per female	24.61 ± 4.66 a	18.81 ± 3.05 b
Number of eggs per day	29.32 ± 5.24 a	22.19 ± 4.74 a
Fecundity	642.00 ± 71.53a	374.84 ± 117.21b
Sex rate	0.67	0.47

*Means followed by the same lower case letter per line do not differ by the F- LSD test (P< 0.05).

Table 4. Parameters of the fertility life table of *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) on the cotton leaf disks treated with kaolin suspension and distilled water (control).

Parameters	With kaolin	Control
Gross reproduction rate (GRR)	443.00 a*	194.78 b
Net reproductive rate (R_0)	79.38 a	44.27 b
Duration of a generation (DG)	20.50 a	20.47 a
Intrinsic rate of increase (r_m)	0.21 a	0.18 b
Finite rate of increase (λ)	1.24 a	1.20 b
Period for the population to double in numbers (TD)	3.24 a	3.67 a

*Means followed by the same lower case letter per line do not differ by the F- LSD test ($P < 0.05$).

4. Discussion

Kaolin did not affect *P. solenopsis* immature stage survival. However, the second and third instar and *P. solenopsis* egg to adult period for females on cotton leaf discs with kaolin was shorter than without this particle film. The higher nymph vulnerability of this instar to the kaolin suspensions and water is due to the thinner wax layer of their bodies compared to older individuals. Additionally, the removal of these nymphs from the female ovisac may decrease their survival. Wax on the coccids forms a physical or chemical barrier against parasitoids, predators and the external environment (Foldi, 1983; Zhang et al., 2012). The ovisac represents a physical barrier against predatory insects and its hydrophobic properties enable the mealybug females to keep their interior morphological structure dry (Zhang et al., 2012).

The shorter duration of the second and third instar and of the egg to adult period of *P. solenopsis* females with kaolin can be explained by the absorption of moisture from kaolin on the cotton leaf disk surface due to its hygroscopic properties, associated with an ambient temperature of 25 °C. Water shortage, like any other stress, has the potential to change the plant suitability for insect development, colonization, oviposition, and offspring survival (Blaney and Simmonds, 1988; Ramaswamy, 1988; Torres and Ruberson, 2006). These modifications in plant quality may favor or not insect pest damage (White, 1969; Mattson Junior and Haack, 1987; Bostanian and Racette, 2008; Markó et al., 2008; Showler and Armstrong, 2007), especially by sapsucking species (White, 1969; Miles et al., 1982; Oliveira et al., 2014). Likewise, the combination between high temperatures with low moisture content may shorten the insect development. This has been demonstrated for the *Cephalcia arvensis* (Panzer, 1805) (Hymenoptera: Pamphiliidae) larval development (Battisti, 1993). However, the longer duration of the egg to adult period of males compared to females seems to be common for this mealybug genus (Vennila et al., 2010; Prasad et al., 2012; Tok et al., 2016).

The longer oviposition period and the higher fecundity and number of eggs per *P. solenopsis* female with kaolin indicate a positive influence of this mineral particle film on the reproduction of this mealybug. This is probably due to the moisture reduction in the cotton leaf disk surfaces from kaolin water absorption. Variability in environmental

moisture can also affect insect egg morphology, embryo physiology and oviposition. Low moisture content may stimulate egg cooling by evaporation, thereby protecting them from short thermal shocks and maintaining their viability (Potter, 2010). Host quality, food, light intensity and relative humidity (Vennila et al., 2010; Asifa et al., 2012; Fand et al., 2014) in addition to the kaolin particle film may affect insect fecundity. The longer longevity of females compared to males is due to the fact that males of this mealybug family present sexual dimorphism and shorter life span because they do not ingest food due to their nonfunctional and fragile mouthparts (Miller, 1991; Williams, 1991). Female longevity was 38.50 ± 2.23 days compared to the 3.50 ± 0.33 days for males, similar to the 35.6 days and 3.2 days for virgin female and mated male *P. solenopsis* with cotton leaves, respectively (Prasad et al., 2012).

The intrinsic rate of population increase (r_m) links R_0 and DG and demonstrates the biotic potential of the species (Price, 1997). The shorter time required for *P. solenopsis* to double its population (TD) as well as its higher crude (TBR) and net (R_0) reproductive rate with and without kaolin, demonstrates the favorable conditions from both treatments for this mealybug to increase its population.

The population growth of *P. solenopsis* sprayed with kaolin suspension, fed on cotton leaf discs treated with kaolin suspension, was higher. Kaolin apparently reduces the cotton foliar surface moisture and consequently, the duration of the life cycle while increasing mealybug reproduction. Kaolin also increased the population density of *Aphis gossypii* (Glover, 1877) (Homoptera: Aphididae) on cotton (Showler and Armstrong, 2007), and *Archips argyrospila* (Walker, 1863) (Lepidoptera: Tortricidae), *Cydia pomonella* (Linnaeus, 1758) (Lepidoptera: Tortricidae), *Dysaphis plantaginea* (Passerini, 1860) (Homoptera: Aphididae), *Lyonetia clerkella* (Linnaeus, 1758) (Lepidoptera: Lyonetiidae), *Phyllonorycter blancardella* (Fabricius, 1781) (Lepidoptera: Gracillariidae), *Phyllonorycter elmaella* (Doganlar & Mutuura, 1980) (Lepidoptera: Gracillariidae), *Phytomyza heringiana* (Hendel, 1922) (Diptera: Agromyzidae), and *Quadraspidiotus perniciosus* (Comstock, 1881) (Homoptera, Diaspididae) on apple orchards (Markó et al., 2008; Bostanian and Racette, 2008). This demonstrates the need to evaluate the kaolin effects with different environmental conditions and cotton cultivars. Kaolin particles should be used with caution

on cotton plants in regions with a history of *P. solenopsis* occurrence, particularly those in the semi-arid regions of northeastern Brazil.

5. Conclusion

The kaolin suspension shortens the life cycle, increases the reproductive potential and population growth of the cotton mealybug, *P. solenopsis* and, therefore, it should be used with caution on cotton plants in regions with a history of occurrence of this pest.

Acknowledgements

To “Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)” and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)”, for financial support. Dr. Phillip John Villani (University of Melbourne, Australia) revised and corrected the English language used in this manuscript.

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