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Reproductive Mode and Male Mating Characteristic of *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae)

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Authors' Contribution
MSW designed the experiments and carried out most of the experimental work under the supervision of ZHS. AAZS, XC, QZ and ASSE carried out some experiments. MSW wrote the manuscript.

Key words
Pseudococcidae, *Phenacoccus solenopsis*, Biology, Mealybug, Mating capacity, Reproductive mode.

ABSTRACT

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), is an invasive, polyphagous pest species. Reproductive biology of this mealybug species is poorly known, which hinders the development of an effective management program. In this study, the reproductive mode, male's mating capacity, influence of female's age and density on male's mating capacity, as well as the influence of copulation on female's longevity were investigated under the laboratory condition. Our results demonstrated that *P. solenopsis* reproduce sexually. Males mated 1-2 times in one day and 3-6 times in their lifetime, and did not show mating preference for female age or density. Although a few unmated females produced ovisacs, while they neither produced eggs nor gave birth any crawlers in the ovisacs. Unmated females lived for longer durations than the mated ones. Sexual reproduction with short lived males may imply potential for control practices that target males; for example, application of sex pheromone for trapping and killing male, or spraying of chemicals during the time of male activity, may be an effective practice for the management of this pest.

INTRODUCTION

The cotton mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), a native of North America (Williams and Granara de Willink, 1992), is distributed over a wide range of agro-ecological zones in at least 24 countries (Wang *et al*., 2009). It has been recorded on more than 200 plant species belonging to over 55 families (Fand and Suroshe, 2015) as a polyphagous pest of many plants, causing huge economic losses to cotton and vegetables in the tropical and subtropical areas of the world (Dhawan *et al*., 2007; Jhala *et al*., 2008; Nagrare *et al*., 2009; Abbas *et al*., 2010; Arif *et al*., 2012). Estimated loss in seed cotton yield due to *P. solenopsis* was 40-50% (Nagrare *et al*., 2009). In China, it is distributed in 28 provinces (Wang *et al*., 2009).

Many studies relevant to the behavior, biology and ecology of *P. solenopsis* have been carried out in different parts of the world (Akintola and Ande, 2008; Dhawan *et al*., 2009; Fand *et al*., 2010; Nikam *et al*., 2010; Vennila *et al*., 2010; Tanwar *et al*., 2011; Ali *et al*., 2012; Asifa *et al*., 2012; Prasad *et al*., 2012; Suroshe *et al*., 2013).

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Phenacoccus solenopsis is reported to exhibit an ovoviviparous mode of reproduction with a very short egg incubation period ranging from a few minutes to a maximum of 2 h (Nikam *et al*., 2010; Vennila *et al*., 2010; Asifa *et al*., 2012; Prasad *et al*., 2012).

Phenacoccus solenopsis exhibits dimorphism, and sexes have distinct morphological differences. Male and female nymphs can be distinguished from their 3rd instar onwards (Nikam *et al*., 2010). Females have three nymphal instars before becoming adults. On the other hand, the immatures that are destined to develop as males construct a loosely-woven silky filamentous cocoon after the second moult and undergo two moultings inside the cocoon with prepupal and pupal stages, before emerging as winged adults (Dhawan *et al*., 2007; Vennila *et al*., 2010; Prasad *et al*., 2012; Fand *et al*., 2014). The adult males are short-lived and non-feeding (Silva *et al*., 2009), while the females are relatively long-lived depending on their reproduction status and temperatures (Vennila *et al*., 2010; Zhu *et al*., 2011; Asifa *et al*., 2012). Like a other mealybugs (Grasswitz and James, 2008; Cid *et al*., 2010), both males and females of the cotton mealybug have very limited range of movement.

Some scientists reported that this mealybugs was bisexual reproduction (Hodgson *et al*., 2008; Aheer *et al*., 2009; Prasad *et al*., 2012; Huang *et al*., 2013) whereas, some other scientists reported that parthenogenesis with ovoviviparity was dominant over the oviparous mode of reproduction (Nikam *et al*., 2010; Kumar *et al*., 2010; Vennila *et al*., 2010). These controversial reports definitively affect the selection of management strategy of the pest. If reproductive mode is truly bisexual, any mating absence and copulation delay may lead to destruction of the population since males have very limited range of movement. The obligate requirement of males for mating and production of offspring implies that a pheromone-based monitoring and management strategy for *P. solenopsis* would be the most practical. Therefore, understanding the reproductive attributes of the pest and the role played by males may generate ideas for amelioration of the pest management and for using this pest as a host in the multiplication of its natural enemies.

We carried out four experiments, the first of which was designed to confirm the reproductive mode, then, to investigate the copulating capacity of male adult together with to evaluate influence of the female age and density on male copulating capacity, and finally to investigate the influence of copulation on female longevity.

MATERIALS AND METHODS

Mealybug and host plant

Tomato plants (*Solanum lycopersicum*) (Solanaceae) (Variety: Hezuo 903) were grown singularly with the field soil in 13-cm plastic pots in the greenhouse at 27 ± 1° C, 60–70% RH, and a 12:12 L:D photoperiod. All plants with 5–12 fully expanded true leaves were used in maintaining the colony of mealybugs in a climate room set at 27 ± 1° C, 60–70% RH, and a 12:12 L:D photoperiod. The detached tomato leaves from these plants were used in the experiments.

The male and female nymph of *P. solenopsis* were collected from the colony with Chinese calligraphy brush pen when they moulted from 2nd instar nymphs into 3rd instar, because at that time male nymphs have silky filamentous cocoons and the female nymph did not have. The isolated male and female nymphs were reared in clear plastic boxes (13×8×5 cm) with detached tomato leaves provided by petiole wrapped in water soaked cotton swab to prevent leaves from desiccation. There were less than 30 female nymphs on one leaf in one box. The box had holes covered with fine stainless steel mesh-covered holes on its top and walls for ventilation. The leaf was replaced as it looked not fresh with a new fresh one. Cotton swab was wetted with tap water as needed.

Eclosed male adults were collected every day, and reared with collecting date in a clear plastic box. Newly eclosed female adults (virgin females) were collected every day on the basis of exuviae after the complete moulting of 3rd instar into adult and reared on a detached tomato leaf in a clear plastic box labeled the collecting date. The collecting date was designated as the beginning day of their longevity.

Female's reproduction mode

Thirty isolated virgin adult females were maintained in a box on a detached tomato leaf at 30 ± 1° C, 60–70% RH, and a 12:12 L:D photoperiod and observed daily for ovisacs until death. Number of dead females was recorded daily. Any eggs in the ovisac produced by the female was moved into a clear plastic box using detached tomato leaf as host, and reared until crawlers developed into adult. Three replicates of 30 females each were carried out for this experiment.

Male's copulating capacity and influence of female's density

One male was confined with 5, 10 or 15 virgin females (3 days old) in a clear plastic box (13×8×5 cm) with a tomato leaf as described above for 1 day or for male's whole life (2.3±.5 days). subsequently, the confined females were individually reared using detached tomato leaves as host until they produced offspring or died. The number of females which produced ovisac with eggs was recorded. This experiment was repeated twenty times (males) for each female density category, and carried out at 27 ± 1° C, 60–70% RH, and a 12:12 L: D photoperiod (the same condition were used in the following experiments).

Male's copulating capacity and influence of female age

One male was confined with ten virgin females of three different ages (8, 16, and 25 days old) in a clear plastic box with a tomato leaf until the male's death. Then, the confined females were reared individually until they produced offspring or died. The number of females which produced ovisac with eggs was recorded. This experiment was repeated ten times (males) for each female's age.

Influence of copulation on female's longevity

Thirty mated females (Produced ovisacs with eggs) and thirty unmated (ovisac absent) females from the two male's copulating capacity experiments above were reared individually until their death. Death date was recorded to calculate female's longevity (from eclosion to death).

Statistical analysis

One-way analysis of variance (ANOVA) was carried out by using general linear model procedure PROC GLM (SAS Institute, 2009) to assess the male's copulating capacity, influences of female's density and age on the male's copulating capacity, while the longevity between mated female and unmated female was compared by using independent t-test.

RESULTS

Reproductive mode

Virgin females did not produce eggs. Some deaths of these virgin females were observed on day 27, but no female survived until day 34. The average female's longevity (from eclosion to death) was 30.5±1.8 days. However, we observed empty ovisacs in the 7 out of 90 unmated female abdomen's posterior ends.

Male's copulating capacity and influence of female's density

One male could mate with one to two females in 1 day, and mated with three to six females in the male's lifespan (Table I). No difference has been found in the numbers of mated females among the three female's densities confined either for 1 day or during the male's lifespan (F=0.26; df=2, 57; P= 0.77 for 1 day, F=1.84, df=2, 57, P=0.17 for male's whole lifespan).

Table I.- Numbers of females (mean±SD) which produced ovisacs at different densities after confinement with one male.

Female's density	Confined time	
	1 day	Whole male's life
5	1.70±0.47 a	3.80±0.41a
10	1.75±0.44a	3.95±0.39a
15	1.80±0.41a	4.10±0.64a

Means (calculated from 20 repetitions) within a column followed by the same letters are not significantly different at 0.05 levels (Fisher LSD test).

Table II.- Numbers of females (mean±SD) that produced ovisacs with eggs at different ages after confinement with one male for the male's lifespan.

Female's age (days)	No. of copulated female
8	4.00±0.47a
16	3.80±0.42a
25	3.90±0.56a

Means (calculated from 10 repetitions) within a column followed by the same letters are not significantly different at 0.05 levels (Fisher LSD test).

Male's copulating capacity and influence of female' age

The numbers of females which produced ovisac are shown in Table II. One male could mate with three to six females during its whole life. No effects were observed for the females age wether it was young or old. No significance difference (F=0.64; df=2, 27; P= 0.53) was found at the number of mated females among the three female age categories.

Influence of copulation on female's longevity

Figure 1 depicted that copulation had a negative impact on the survival of *P. solenopsis* Tinsley. Copulation significantly reduced the female longevity (N = 100, t-test, df=98, t=35.3, P <0.001) (Fig. 1). The mated females survived 19.3±1.3 days while unmated ones survived 30.5±1.8 days.

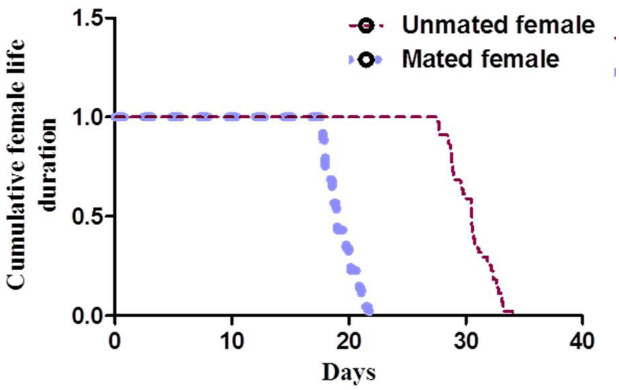

Fig. 1. Effect of copulation on the female longevity.

DISCUSSION

Mode of reproduction

In the present study, only mated female *P. solenopsis* were able to produce offspring while unmated adult females were unable to do so. However, empty ovisacs were observed in the 7 out of 90 unmated female abdomen's posterior ends. Our results unequivocally confirmed previous reports of sexual reproduction (Hodgson *et al.*, 2008; Aheer *et al.*, 2009; Prasad *et al.*, 2012; Huang *et al.*, 2013) but were different from earlier descriptions of obligate parthenogenesis (Kumar *et al.*, 2010; Nikam *et al.*, 2010; Vennila *et al.*, 2010). Although obligate and facultative parthenogenesis has been reported in scale insects, most of them reproduce sexually (Normark, 2003). The congeneric of *Phenacoccus madeirensis* Green also has a sexually reproductive mode (Chong *et al.*, 2003). Three mealybug species, *Pseudococcus viburni* (Signoret), *Pseudococcus calceolariae* (Maskell) and *Planococcus citri* (Risso), which were previously controversial on parthenogenesis traits were demonstrated to be obligate amphimictic (Silva *et al.*, 2010). The controversial of reproductive mode in cotton mealybug maybe resulted from unintentional incorporations of males which were staying concealed during much of the daytime (Huang *et al*., 2013), or perhaps it is due to environmental variations such as host plant, and biological variations like female mating status (Nur, 1971). For example, *Ferrisia virgata* (Cockerell) showed a parthenogenetic mode of reproduction on the cocoa plant (*Theobroma cocao*) (Padi, 1997), while on the cotton plants (*Gossypium hirsutum*) it reproduced sexually (Oliveira *et al*., 2014). Thus, the differentiation in the reproductive mode of *P. solenopsis* between China and India may be caused by some factors which have not been taken into account so far (Huang *et al*., 2013). Probably they are different biotypes, cryptic species, or originated from different host plants. To make sure this, enormous information was needed to interpret the difference on reproductive modes reported by scientists. Silva *et al*. (2010) also found that more than 50% of virgin females of *P. viburni* and *P. calceolariae* formed ovisacs but only less than 16% of them laid some (1 to 8) infertile eggs. A similar phenomenon was reported also by Waterworth *et al*. (2011) on *P. viburni*. Silva *et al*. (2010) suggested that oviposition and the secretion of the ovisac were independent processes.

Effect of female density and age on male's mating capacity

Female density did not affect male's mating capacity. This result is not consistent with the citrophilus mealybug, *P. calceolariae* and the citrus mealybug, *P. citri* (Silva *et al*., 2013). The inconsistency in results is possibly due to the different species and methodologies used. In the present study, each male was exposed to female density at 5, 10 or 15 individuals, which already exceeded the mating capacity since males of the cotton mealybug mate only about 4 times on average with a range of 3-6 times (Table I). Whereas in Silva *et al*. (2013), each male was exposed to female density at 1, 2, 4, 8, or 16 individuals, the first three densities were apparently lower than the mating capacity since in 1 h of exposure to females each male of *P. calceolariae* could mate 3.7 times on average with a maximum of 8 times, and each male of *P. citri* could mate 1.6 times on average with a maximum of 4 times.

Female age did not affect male's mating potential. This result supported the finding on *P. calceolariae*, but did not support their report on *P. citri*, where mating performance decreased with increased female age (Silva *et al*., 2013). They suggested that pheromone emission was inversely proportional to the age of females, with an eventual cessation of pheromone emission once females pass a certain age, and that this decrease resulted in a decreased rate of copulation with the males. However, since the virgin female mealybugs usually continue growing during their life, the larger body size may tradeoff the reduced pheromone emission with age by means of visual attraction (Franco *et al*., 2009). Meanwhile, the test time may be another reason for our difference. In Silva's study, males were paired with females only for 1 h (Silva *et al*., 2013), whereas in our study the male was exposed to females for the male's entire life. During shorter mating periods, males may not meet the female as frequently as compared to longer mating periods where the male may meet the female repeatedly, leading to increased opportunity for copulation.

Effect of copulation on the female longevity

Unmated *P. solenopsis* females live longer than mated females in our experiment (Fig. 1). This result is consistent with earlier report by Prasad *et al*. (2012), where the longevity of virgin female lasted about a month and that of mated females survived only about 20 days. Unmated striped mealybug *Ferrisia virgata* live 19 days more than mated females (Oliveira *et al*., 2014). This phenomenon has also been found in *Callosobruchus chinensis* (Linn.) (Yanagi and Miyatake, 2003) and *Photinus obscurellus* Legonte (South and Lewis, 2012) (Coleoptera), *Ephestia kuehniella* Zeller (Xu, 2010) and *Ostrinia nubilalis* (Hübner) (Fadamiro and Baker, 1999) (Lepidoptera), *Gryllus bimaculatus* De Geer (Green and Tregenza, 2009) (Orthoptera) and other many insects.

CONCLUSIONS

Male adults of *P. solenopsis* start to find their sexual partner immediately after they emerge from their cocoons, since they have a very short adult lifespan. They mate 3-6 times in their lifetime, can mate with young and old females, without displaying preference for females of any age range. For this species, only mated females are able to produce offspring, whereas unmated females are unable to do so despite living for much longer than mated females and often producing empty ovisacs. These results suggest that developing male targeted killing techniques, for example, applying the synthetic pheromones and Citrullus colocynthis extracts (Gulzar *et al*., 2017; Tabata and Ichiki, 2016) or spraying insecticides at the peak times of male activity, may be an effective practice for the management of this pest.

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Statement of conflicts of interest

No potential conflict of interest was reported by the authors.

REFERENCES

Abbas, G., Arif, M.J., Ashfaq, M., Aslam, M. and Saeed, S., 2010. Host plants, distribution and overwintering of cotton mealybug (*Phenacoccus solenopsis*) Homoptera: Pseudococcidae. *Int. J. Agric. Biol.,* **12**: 421-425.

Aheer, G.M., Shah, Z. and Saeed, M., 2009. Seasonal history and biology of cotton mealy bug, *Phenacoccus solenopsis* Tinsely. *J. agric. Res.,* **47**: 423-431.

Akintola, A.J. and Ande, A.T., 2008. First record of *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on *Hibiscus rosa-sinensis* in Nigeria. *Agric. J.,* **3**: 1-3.

Ali, A., Asif, H., Saleem, M., Khalil, N. and Saeed, M., 2012. Effect of temperature and relative humidity on the biology of cotton mealybug (*Phenacoccus solenopsis* Tinsley). *J. agric. Res.,* **50**: 89-101.

Arif, M.J., Gogi, M.D., Arshad, M., Ashraf, A., Suhail, A., Zain-ul-Abdin, Wakil, W. and Nawaz, A., 2012. Host-plants mediated population dynamic of cotton mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) and its parasitoid, *Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae). *Pak. Entomol.,* **34**: 179-184.

Asifa, H., Aziz, M.A. and Aheer, G.M., 2012. Impact of ecological conditions on biology of cotton mealybug, *Phenacoccus solenopsis* (Hemiptera: Pseudococcidae) in laboratory. *Pakistan J. Zool.,* **44**: 685-690.

Chong, J.H., Oetting, R.D. and Iersel, M.W.V., 2003. Temperature effects on the development, survival, and reproduction of the madeira mealybug, *Phenacoccus madeirensis* Green (Hemiptera: Pseudococcidae), on chrysanthemum. *Annls. entomol. Soc. Am.,* **96**: 539-543. https://doi.org/10.1603/0013-8746(2003)096[0539:TEOTDS]2.0.CO;2

Dhawan, A.K., Kamaldeep, S.A. and Saini, S., 2009. Distribution of mealybug *Phenacoccus solenopsis* Tinsley in cotton with relation to weather factors in South-Western districts of Punjab. *J. entomol. Res.,* **33**: 59-63.

Dhawan, A.K., Singh, K., Saini, S., Mohindru, B., Kaur, A., Singh, G. and Singh, S., 2007. Incidence and damage potential of mealybug, *Phenacoccus solenopsis* Tinsley on cotton in Punjab. *Indian. J. Ecol.,* **34**: 110-116.

Cid, M., Pereira, S., Cabaleiro, C. and Sequra, A., 2010. Citrus mealybug (Hemiptera: Pseudococcidae) movement and population dynamics in an Arbor-trained vineyard. *J. econ. Ent*., **103**: 619-630. https://doi.org/10.1603/EC09234

Fadamiro, H.Y. and Baker, T.C., 1999. Reproductive performance and longevity of female European corn borer, *Ostrinia nubilalis*: Effects of multiple mating, delay in mating, and adult feeding. *J. Insect Physiol.,* **45**: 385-392. https://doi.org/10.1016/S0022-1910(98)00137-1

Fand, B.B., Gautam, R.D., Chander, S. and Suroshe, S.S., 2010. Life table analysis of the mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) under laboratory conditions. *J. entomol. Res.,* **34**: 175-179.

Fand, B.B., Henri, E.Z.T., Kumar, M., Kamble, A.L. and Bal, S.K., 2014. A temperature-based phenology model for predicting development, survival and population growth potential of the mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae). *Crop Prot.,* **55**: 98-108. https://doi.org/10.1016/j.cropro.2013.10.020

Fand, B.B. and Suroshe, S.S., 2015. The invasive mealybug *Phenacoccus solenopsis* Tinsley, a threat to tropical and subtropical agricultural and horticultural production systems –A review. *Crop Prot.,* **69**: 34-43. https://doi.org/10.1016/j.cropro.2014.12.001

Franco, J.C., Zada, A. and Mendel, Z., 2009. Novel approaches for the management of mealybug pests. In: *Biorational control of arthropod pests: Application and resistance management* (eds. I. Ishaaya, A.R. Horowitz). Springer, Dordrecht, Netherlands, pp. 233-278. https://doi.org/10.1007/978-90-481-2316-2_10

Grasswitz, T.R. and James, D.G., 2008. Movement of grape mealybug, *Pseudococcus maritimus*, on and between host plants. *Ent. Exp. Appl.*, **129**: 268-275. https://doi.org/10.1111/j.1570-7458.2008.00786.x

Green, K. and Tregenza, T., 2009. The influence of male ejaculates on female mate search behaviour, oviposition and longevity in crickets. *Anim. Behav.,* **77**: 887-892. https://doi.org/10.1016/j.anbehav.2008.12.017

Gulzar, A., Maqsood, A., Ahmed, M., Tariq, M., Ali, M. and Qureshi, R., 2017. Toxicity, antifeedant and sub-lethal effects of *Citrullus colocynthis* extracts on cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Pakistan J. Zool.,* **49**: 2019-2026.

Hodgson, C., Abbas, G., Arif, M.J., Saeed, S. and Karar, H., 2008. *Phenacoccus solenopsis* Tinsley (Sternorrhyncha: Coccoidea: Pseudococcidae), an invasive mealybug damaging cotton in Pakistan and India, with a discussion on seasonal morphological variation. *Zootaxa,* **1913**: 1-35.

Huang, F., Zhang, J.M., Zhang, P.J. and Lu, Y.B., 2013. Reproduction of the solenopsis mealybug, *Phenacoccus solenopsis*: Males play an important role. *J. Insect Sci.,* **13**: 137. https://doi.org/10.1673/031.013.13701

Jhala, R.C., Bharpoda, T.M. and Patel, M.G., 2008. *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae), the mealy bug species recorded first time on cotton and its alternate host plants in Gujarat, India. *Uttar Pradesh J. Zool.,* **28**: 403-406.

Kumar, R., Jat, S.L., Pal, V. and Chauhan, R., 2010. Biology of the mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Psuedococcidae) in India. *Entomon,* **34**: 189-192.

Nagrare, V.S., Kranthi, S., Biradar, V.K., Zade, N.N., Sangode, V., Kakde, G., Shukla, R.M., Shivare, D., Khadi, B.M. and Kranthi, K.R., 2009. Widespread infestation of the exotic mealybug species, *Phenacoccus solenopsis* (Tinsley) (Hemiptera: Pseudococcidae) on cotton in India. *Bull. entomol. Res.,* **99**: 537-541. https://doi.org/10.1017/S0007485308006573

Nikam, N.D., Patel, B.H. and Korat, D.M., 2010. Biology of invasive mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on cotton. *Karnataka J. agric. Sci.,* **23**: 649-651.

Normark, B., 2003. The evolution of alternative genetic systems in insects. *Annu. Rev. Ent.,* **48**: 397-423. https://doi.org/10.1146/annurev.ento.48.091801.112703

Nur, U., 1971. Parthenogenesis in Coccids (Homoptera). *Am. Zool.,* **11**: 301-308. https://doi.org/10.1093/icb/11.2.301

Oliveira, M.D., Barbosa, P.R.R., Silva-Torres, C.A.S. and Torres, J.B., 2014. Performance of the striped mealybug *Ferrisia virgata* Cockerell (Hemiptera: Pseudococcidae) under variable conditions of temperature and mating. *Neotrop. Ent*., **43**: 1-8. https://doi.org/10.1007/s13744-013-0171-z

Padi, B., 1997. Parthenogenesis in mealybug occurring in cocoa. In: *Proc. 1st International Cocoa Pest and Diseases Seminar,* 6-10 November 1995. Cocoa Research Institute, Accra Ghana, pp. 242-248.

Prasad, Y.G., Prabhakar, M., Sreedevi, G., Ramachandra, R.G. and Venkateswarlu, B., 2012. Effect of temperature on development, survival and reproduction of the mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on cotton. *Crop Prot.,* **39**: 81-88. https://doi.org/10.1016/j.cropro.2012.03.027

SAS, 2009. *SAS online Doc 9.2*. SAS Institute Inc., Cary, NC. Available online: http://support.sas.com/documentation

Silva, E.B., Mendel, Z. and Franco, J.C., 2010. Can facultative parthenogenesis occur in biparental mealybug species? *Phytoparasitica,* **38**: 19-21. https://doi.org/10.1007/s12600-009-0069-9

Silva, E.B., Mouco, J., Antunes, R., Mendel, Z. and Franco, J.C., 2009. Mate location and sexual maturity of adult male mealybugs: Narrow window of opportunity in a short lifetime. *Int. Org. BioContr. West Palearctic Regional Section Bull.,* **4**: 3-9.

Silva, E.B.D., Branco, M., Mendel, Z. and Franco, J.C., 2013. Mating behavior and performance in the two cosmopolitan mealybug species *Planococcus citri* and *Pseudococcus calceolariae* (Hemiptera: Pseudococcidae). *J. Insect Behav.,* **26**: 304-320. https://doi.org/10.1007/s10905-012-9344-6

South, A. and Lewis, S.M., 2012. Effects of male ejaculate on female reproductive output and longevity in *Photinus* fireflies. *Can. J. Zool.,* **90**: 677-681. https://doi.org/10.1139/z2012-031

Suroshe, S.S., Gautam, D.R. and Fand, B.B., 2013. Natural enemy complex associated with the mealybug, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) infesting different host plants in India. *J. Biol. Contr.,* **27**: 204-210.

Tabata, J. and Ichiki, R.T., 2016. Sex pheromone of the cotton mealybug, *Phenacoccus solenopsis*, with an unusual cyclobutane structure. *J. chem. Ecol*., **42**: 1193-1200. https://doi.org/10.1007/s10886-016-0783-y

Tanwar, R.K., Jeyakumar, P., Singh, A., Jafri, A.A. and Bambawale, O.M., 2011. Survey for cotton mealybug, *Phenacoccus solenopsis* (Tinsley) and its natural enemies. *J. environ. Biol.,* **32**: 381-384.

Vennila, S., Deshmukh, A.J., Pinjarkar, D., Agarwal, M., Ramamurthy, V.V., Joshi, S., Kranthi, K.R. and Bambawale, O.M., 2010. Biology of the mealybug, *Phenacoccus solenopsis* on cotton in the laboratory. *J. Insect Sci.,* **10**: 1-9. https://doi.org/10.1673/031.010.11501

Wang, Y.P., Wu, S.A. and Zhang, R.Z., 2009. Pest risk analysis of a new invasive pest, *Phenacoccus* *solenopsis* to China. *Chinese Bull. Ent.,* **46**: 101-106.

Waterworth, R.A., Wright, I.M. and Millar, J.G., 2011. Reproductive biology of three cosmopolitan mealybug (Hemiptera: Pseudococcidae) species, *Pseudococcus longispinus*, *Pseudococcus viburni*, and *Planococcus ficus*. *Annls. entomol. Soc. Am.,* **104**: 249-260. https://doi.org/10.1603/AN10139

Williams, D.J. and Granara de Willink, M.C., 1992. *Mealybugs of Central and South America*. CAB International, Wallingford, UK, pp. 635.

Xu, J., 2010. *Reproductive behaviour of* Ephestia kuehniella *Zeller (Lepidoptera: Pyralidae)*. A Ph. D. thesis, Massey University, Palmerston North, New Zealand.

Yanagi, S. and Miyatake, T., 2003. Costs of mating and egg production in female *Callosobruchus chinensis*. *J. Insect Physiol.,* **49**: 823-827. https://doi.org/10.1016/S0022-1910(03)00119-7

Zhu, Y.Y., Huang, F. and Lu, Y.B., 2011. Bionomics of mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) on cotton. *Acta entomol. Sin.,* **54**: 246-252.