Prishanthini Muthulingam[1] and Muthuladchumy Vinobaba[2]

Life cycle of the cotton mealybug *Phenacoccus solenopsis* in shoe flower plants under the Laboratory conditions

(1), (2) Department of Zoology, Faculty of Science, Eastern University, Chenkalady, Sri Lanka.

Abstract: The cotton mealybug *Phenacoccus solenopsis* Tinsley (1989) is one of the invasive species recently introduced to Sri Lanka and nowadays it is wide spread among various parts of the country. The life cycle of *P.solenopsis* was studied under the laboratory conditions using *Hibiscus rosa-sinenesis* (Shoeflower) as host plant. This paper describes the lifecycle and discusses about the reproductive parameters of *P.solenopsis* under laboratory conditions relative to the appearance of symptoms on the host plant and the importance of making management interventions during the effective reproductive period of the insect.

Keywords: *Hibiscus rosa-sinenesis,* Neonate crawlers, *Phenacoccus solenopsis,* Reproductive period.

Introduction

The mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has a wide geographical distribution with its origin in Central America (Fuchs et al., 1991;Williams and Granara de Willink, 1992) followed by reports of the Caribbean and Ecuador (Ben-Dov,1994), Chile (Larrain, 2002), Argentina (Granara de Willink, 2003), Brazil (Mark and Gullan, 2005). *P.solenopsis* has been described as a serious and invasive pest of cotton in Pakistan and India (Hodgson *et al*., 2008) and on *Hibiscus rosa-sinensis* in Nigeria (Akintola and Ande, 2008). Latest report on the invasiveness of *P. solenopsis* has been from the Eastern region of Sri Lanka (Prishanthini and Vinobaba, 2009) on ornamentals, vegetable crops, and weeds, and in China (Wang et al. 2009; Wu and Zhang, 2009) on shoe flower. A detailed comparative study of few species of *Phenacoccus* including the Indian and Pakistan species, and details on the existence of seasonal morphological variations in *P.solenopsis* were provided by Hodgson *et al.* (2008).

Being a polyphagous pest, the P. solenopsis has been recorded to feed on a number of cultivated crops including weeds (Patel et al. 2009). According to the recent information provided by the authors (Prishanthini and Vinobaba, 2011) P.solenopsis has been reported from 28 host plant species comprising 10 families in Sri Lanka. In spite of its occurrence as a pest of several agricultural and horticultural crops since last few years, the information on its biology was scanty. Therefore, the present study on life cycle of *P. solenopsis* was carried out in the laboratory, so that the information generated may be used to formulate the management strategy of the pest.

Materials and Methods

Collection of insects

Studies on biology of *P.solenopsis* were conducted at the Zoology laboratory of Eastern University Sri Lanka using the population collected from unsprayed *Hibiscus rosa-sinenesis* plants in home gardens. Mealybug specimens of mealybugs used for the study were confirmed as *P.solenopsis* by the second author.

Rearing of insects

To establish initial culture of *P. solenopsis*, twigs of the host plants infested with adult females were brought to the laboratory individuals were separated and inoculated on shoe flower plants planted in the pots and reared in the laboratory. After about three days, the female mealy bugs settled on host leaves and stems and started egg laying. The crawlers emerged out and started feeding on the shoe flower plant.

The newly hatched crawlers were placed on shoe flower leaves with the help of fine camel hair brush. For that individual leaves with petioles of same size and maturity were collected from the shoe flower plants which did not exposed to any previous pesticide applications and free from mealybug infestation, were washed with tap water, shade dried and used as food source. The leaf petiole of the shoe flower leaves were wrapped with cotton wool dipped in water to keep the leaves turgid. Each leaf was infested with an adult female mealybug individual and was individually transferred to separate glass Petri plates (15 X 2 cm) each containing a shoe flower leaf. The study was conducted between May and June 2011 in the laboratory when maximum and minimum temperature and mean relative humidity of the study area ranged from 31.8 to 37.8 ° C and 23.8 to 27.5° C, and 53 to 81 % RH respectively.

Data Collection

When the newly emerged crawlers settled for feeding on shoe flower leaves, the crawlers were marked by drawing a circle around them. The crawlers thus marked were observed daily in the morning till they attained adult stage for further aspects of biology.The eggs laid by females of *P. solenopsis* were examined under binocular microscope for colour, shape and size. The adult female of mealy bug were picked up and placed individually on shoe flower leaf with the help of fine camel hair brush. The leaves were kept turgid for longer period as described earlier. The individual leaf was kept in glass petridish and observed daily under microscope till egg laying.

The time of egg laying was noted. Freshly laid eggs were counted and transferredto fresh shoe flower leaves. Time taken for egg hatching was recorded to obtain the incubation period. Hatching percentage of eggs was calculated from the number of eggs hatched out of total number of eggs kept under observation. The freshly emerged nymphs were marked individually on shoe flower leaves and observed daily under microscope to note moulting process The moulting was confirmed by the presence of exuivum on the leaf or on the posterior end of nymphs. The colour, shape and size of each nymphal instar were critically observed.

Adult females emerged after the last moult was observed for colour and shape. Measurements of the females were made by using measuring scale. Similarly, adult males emerged out from the silken cocoons were observed under microscope to study their colour, shape and size. Freshly emerged females were reared separately on shoe flower leaves to study their pre-oviposition, oviposition and post-oviposition periods. Since the female laid their eggs in shoe flowery sac located at posterior end of its abdomen, the ovisacs were collected during the oviposition period and counted the number of eggs in each ovisac for calculating fecundity. Longevity of male and female was assessed separately i.e. days of survival from emergence to the death of adults. Total 150 newly hatched crawlers were reared on shoe flower leaves up to third instar to determine the sex ratio. The third instar stage forming cocoons were separated as male and female and sex ratio was worked out. Total life cycle of female and male was calculated from the egg laying to the death of adult stage.

Data Analysis

Data were statistically analysed using statistical software Minitab 15.0

Results and Discussion

To understand the mode and degree of its population growth of an insect pest, it is important to understand the environmental conditions of the crop. Since a study of the life history and pattern of biological activities are difficult under field conditions because of the interference of biotic and abiotic factors, laboratory studies have become essential. Studies conducted in the laboratory using shoe flower leaves placed in Petri plates with detailed observations of reproductive and developmental stages of *P. solenopsis* formed the basis for the present study. Shoe flower leaves collected from the same position on the plant provided a similar food source for developing mealybugs thus avoiding any variation in food quality. Since individual leaves could be placed in Petri plates, they were easily observed under the microscope.

The *P. solenopsis* female laid their eggs in cottony ovisac located at posterior part of abdomen. The eggs

were smooth translucent, light creamy yellow in colour and oblong in shape with tapering ends (Fig 2). *P. solenopsis* exhibited variation in males and females at immature stages itself. The female nymphs moulted three times and males four times. Freshly emerged first instar nymphs were oblong in shape, dorsally convex, light yellow in colour with three pairs of legs and a pair of seven segmented filiform antennae. Body colour of newly hatched nymphs changed to pale white within two days after hatching from eggs. The newly emerged nymphs (Fig 2) crawled over to leaf surface for some time in search of suitable place for feeding and then settled down.

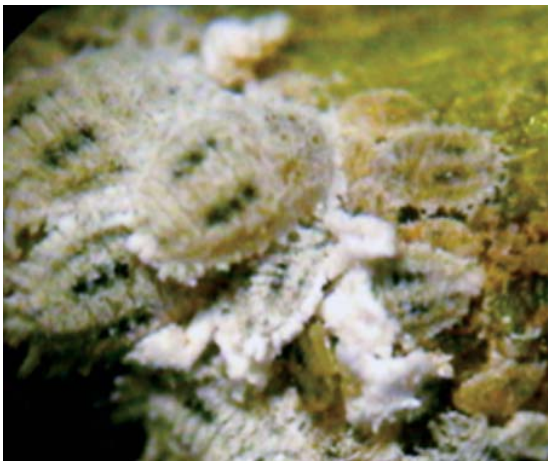

Figure1: Colony of adult female of *P.solenopsis*

Duration of first instar nymphs lasted for 4 to 6 days with an average of 3.24 ± 2.11 days (Table 1). After first moult, the second instar nymphs found to be oblong and yellow in colour. The second instar nymphs were similar to that of first instar nymphs in general appearance and morphological features, except in size. The antennae showed a marked increase in size but remained seven segmented. They secreted white waxy powder and waxy fibres on dorsal side after about 24 hours of first moult. The exuvium of the instar was seen near the posterior end of the abdomen. Duration of second instar nymphs ranged from 3 to 7 days with an average of 4.75 ± 3.28 days (Tab 1). Male and females of *P. solenopsis* nymphs can be distinguished from third instar onwards. The male nymphs formed a white silken cocoon after their third moult, but no such phenomenon in females. They continued to moult for remain in juvenile stage still. Male cocoons were cylindrical in shape and white in colour. Duration of male lasted for 5 to 7 days with an average of 6.06 ± 4.03 days (Tab 1).

Third instar nymphs of females were oblong in shape with yellow in colour (Fig 2). There were two pairs of dark black coloured spots with number of prominent glassy fibres of wax on dorsal surface of its body. Female bears a pair of prominent compound eyes, a pair of 7 segmented filiform antennae and three pairs of well-developed thoracic legs.

Duration of third instar nymphs ranged from 4 to 6 days with an average of 5.20 ± 0.45 days (Tab1). Adult males of *P. solenopsis* (Fig 2)were delicate, slender and elongated in shape. The colour of head, thorax, antennae and legs was yellowish-brown, whereas abdominal region pale yellow. A pair of well-developed metathoracic milky white wings and three pairs of well-developed legs could be seen easily. The antennae were ten segmented and found to be much longer than that of female antennae. It was as long as the total body length of males. Two pairs of waxy filaments were present at anal end of body of which the inner pair was long while the outer pair was short or to an extend half of the inner pair. Longevity of males ranged from 1 to 2 days with an average of 1.5±0.5 days and total life cycle ranged from 23 to 30 days with an average of 27.41 ± 1.10 days (Tab 1)

Female adults of *P. solenopsis* were oblong in shape and light to dark in colour having two pairs of black spots/strips on dorsal side of body region. Females were apterous, soft bodied, well distinguished segmented and body covered with white dusty secretion. It also possessed a pair of brownish, short, eight segmented filiform antennae and three pairs of red coloured legs. Longevity of female ranged from 32-55 days with an average of 34.3 ± 2.64 days. Total life cycle lasted for 55 to 60 days with an average of 58.3 ± 2.64 days (Tab 1). Observations on preoviposition, oviposition, and post oviposition periods of *P. solenopsis* revealed that it varied from 2 to 8, 12 to 18 and 7 to 9 days with an average of 8.56 ± 0.61, 16.73 ± 0.57 and 9.33 ± 0.47 days, respectively (Tab 1). Total

number of eggs laid by a single female during its entire life period ranged from 212 to 772 eggs with an average of 574 ± 82 eggs. The sex ratio of *P. solenopsis* in laboratory culture revealed that out of 330 third instar nymphs, 272 were females and 58 were males. Thus female to male ratio was 1: 0.21. The present study is first report on detailed reproductive biology of *P. solenopsis* from Sri Lanka. However, majority of observations match with the biological features of *P. solenopsis* on *Hibiscus rosa-sinenesis*as explained by Akintola and Ande (2008) from Nigeria and with the observations of Vennila *et al* (2010) in cotton plants in India. Shoe flower and cotton and some of the other preferred hosts which are agricultural crops are of Family Malvaceae. Therefore the results would be comparable to other crops and weeds act as host of *P.solenopsis*. The present study would lead a better understanding of incidence and spread of mealybug, *P. solenopsis* in shoe flower and alternate hosts which may be used in drafting management strategies. Lower numbers and shorter life span of males suggested that they have a minor role in reproduction, although under field conditions sexual reproduction also could be a possibility. In relation with the biology of *P.solenopsis* it is quite clear that the longevity of the adults, and their larger size with increased waxy coating, and higher food requirement, result in visibility of the pest and symptoms on the crop. Therefore, with the initial notice of *P. solenopsis* infestation on few plants it is essential to monitor the plants regularly for at least 14 to 20 days, which is when reproduction by females occurs, to make management decisions for using insecticidal sprays. Higher mortality of the crawlers, the longer effective reproductive period and increased longevity of adult females along with the expected natural mortality factors such as predation, parasitization and action of abiotic factors on crawlers and adults under natural field conditions, suggest that management interventions should be focused against reproducing adult females rather than crawlers to prevent the multiplication and spread of the pest. Therefore bioassay studies should use adult females instead of crawlers to determine an efficacious management scheme.

Table 1:
Lifecycle and reproductive parameters of *P.solenopsis* reared in shoe flower plants

Stage	n	Duration (days)	
		Range	Mean± S.D
Eggs	50	32-75	48.34 ± 5.67
Nymph			
1st instar	25	4-6	3.24 ± 2.11
2nd instar	25	3-7	4.75 ± 3.28
3rd instar	25	4-6	5.20 ± 0.45
Cocoon (Male)	25	5-7	6.06 ± 4.03
Adult			
Male	25	10-17	14.52 ± 2.92
Female	25	12-21	16.32 ± 7.76
Female			
Pre oviposition	25	2-8	4.88 ± 1.22
Oviposition	25	12-18	15.56 ± 3.42
Post oviposition	25	7-9	8.01 ± 2.18
Total life cycle			
Male	25	23-30	27.41 ± 1.10
Female	25	55-61	58.3 ± 2.64
Male after maturity	25	1-2	1.5 ± 0.10
Fecundity	25	212-772	574.4 ± 82.0

n- Number of observations

Longer developmental duration of males compared to females was due to an additional moulting and prepupal processes.. While the longer developmental period of the 2nd instar of males along with their high mobility could be the reason for their lower survival, it was not observed in the fourth instar due to the scarce population of males, together with the difficulty of *observation of any sex related differences during early* crawler stages.Akintola and Ande (2008) studied *P. solenopsis* on *H. rosa-sinensis* and found progressively increasing developmental periods of 6, 8 and 10 days for the 1st, 2nd and 3rd instars, respectively.

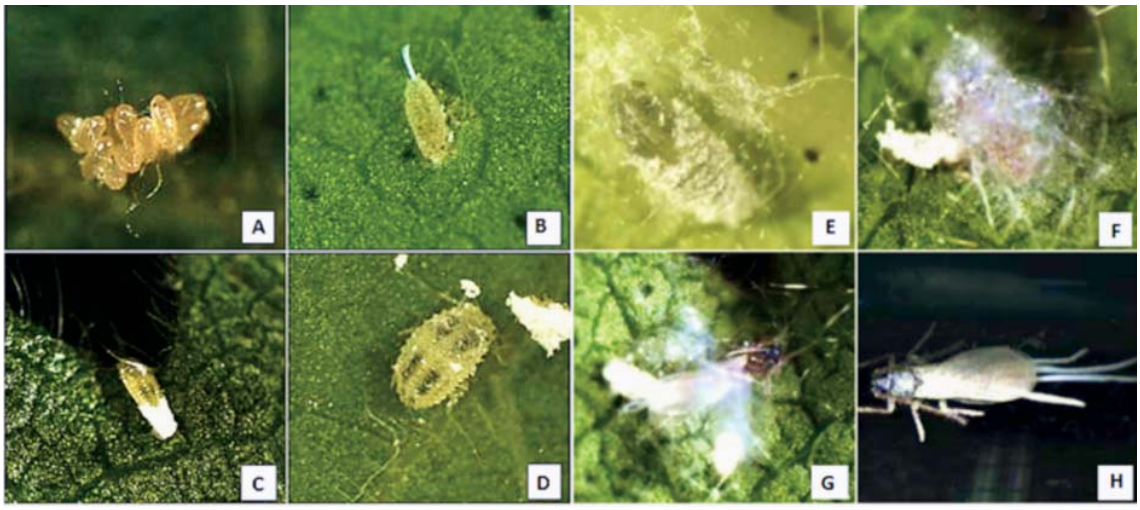

Figure 2:
Female life stages : A. Egg, B. First instar, C. Second instar D. Third instar and Male life stages: E. Third moult, F. Fourth moult, G. Adult ready for emergence H. Adult.

However *P. solenopsis* under laboratory conditions had longer developmental periods for the 2nd instar over the other two instars, indicating the influence of ecological zone with the associated weather conditions as well as host plants that could influence *P.solenopsis* development. The total developmental duration of a closely related species *Phenacoccus madeirensis* reared under constant temperatures of 25, 20 and 15^0 C was reported to be 30, 46 and 66 days respectively (Chong et al. 2003). This suggested that *P. solenopsis* has become acclimatized to a tropical environment that may have allowed its rapid spread across widely differing agro climatic zones of the country.

Further studies are required to determine developmental rates at different constant temperatures in growth chambers, ability of *P. solenopsis* to multiply, survive and spread across regions among many host plants, and the continuing molecular studies on the variations in their populations would be able to resolve and strengthen the species identity, biology and effect of environmental factors.

References

Akintola AJ, Ande AT. 2008. First Record of Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) on Hibiscus rosa-sinensis in Nigeria. Agricultural Journal (Medwell Journals, Pakistan) 3(1): 1-3.

Ben-Dov Y. 1994 A systematic catalogue of the mealybugs of the world, p.686. Intercept Limited, Andover, UK.

Chong JH, Oetting RD, Iersel MW Van. 2003. Temperature effects on the development, survival and reproduction of the Madeira Mealybug, Phenacoccus madeirensis Green (Hemiptera: Pseudococcidae), on Chrysanthemum. Annals of Entomological Society of America 96 (4): 539-543.

Fuchs TW, Stewart JW, Minzenmayer R, Rose M. 1991. First record of Phenacoccus solenopsis Tinsley in cultivated shoe flower in the United States. South western Entomologist 16(3): 215-221.

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

Larrain SP. 2002. Insect and mite pes incidence on sweet pepinos Solanum muricatum (Ait.) cultivated in the IV Region, Chile. Agricultura-Tecnica 62(1): 15-26.

Mark PC, Gullan PJ. 2005. Anew pest of tomato and other records of mealybugs (Hemiptera: Pseudococcidae) from Espirito Santo, Brazil. Zootaxa 964: 1-8.

Patel HP, Patel AD. and Bhatt NA. 2009, Record of coccinellids predating on mealy bug, Phenacoccus solenopsis Tinsley in Gujarat. Insect Environ. 14(4): 179. http:/www.ncipm.org.in.

Prishanthini M. and Vinobaba M. 2009 ,First record of new exotic Mealybug species, Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae), its Host range and abundance in the Eastern Sri Lanka, Journal of Science 6(1),88-100

Prishanthini M and Vinobaba M.2011, A survey of mealybugs and their natural enemies in home gardens of Batticaloa, Sri Lanka, Proceedings of 10th Annual Research Session.2011,Eastern university, Sri Lanka, 1-2 December 2011, 202-208

Vennila S, Deshmukh AJ, Pinjarkar D, Agarwal M, Ramamurthy VV, Joshi S, Kranthi KR, Bambawale OM. 2010. Biology of the mealybug, Phenacoccus solenopsis on cotton in the laboratory. Journal of Insect Science 10:115 available online: insectscience.org/10.115

Williams DJ, Granara de Willink MC. 1992. Mealybugs of Central and South America, p.635. CAB International.