Pak. J. Sci. Ind. Res. 2009 **52**(5) 270-274

Effect of Different Humidity Levels on the Biology of Longtailed Mealy Bug *Pseudococcus longispinus* (Targioni and Tozzetti) (Homoptera: Pseudococcidae)

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(received April 16, 2009; revised May 28, 2009; accepted June 15, 2009)

Abstract. On determining the effects of different humidity levels on the biology of mealy bug *Pseudococcus longispinus* (Targioni and Tozzetti), it was found that the relative humidity (RH) at 35%, 55% and 75% had no effect on pre-adult development, adult longevity, life span and fecundity of *P. longispinus*. The survival of pre-adult stages was minimal at 35% RH. Sex ratio was male-biased at 35% RH and female-biased at 75% RH.

Keywords: Mealy bug; humidity levels; *Pseudococcus longispinus*

Introduction

Mealy bugs are mainly of tropical and subtropical origin and many of them have become established as pests. They attack a wide range of plants including fruits, vegetables and ornamentals. The very broad host range of mealy bugs in part explains their success. As sap feeders, they have the potential to be vectors of various viral diseases (Golino *et al*., 2002; Campbell, 1983; Harris, 1981) and some species are known to inject potent phytotoxins during feeding (Lema and Herren, 1985). Their direct damage takes the form of distortion, stunting and yellowing of foliage, early dropping of the flowers and fruits, sometimes followed by defoliation. Indirectly, their copious secretion of honeydew promotes the growth of sooty moulds which can detract from the aesthetic and economic value of the plants (Hattingh, 1993; Copland *et al*., 1985; Pritchard, 1949).

The longtailed mealy bug *Pseudococcus longispinus* (Targioni-Tozzetti) is widely distributed in tropical and sub-tropical regions and in glasshouses in the temperate zone. It is found in the Mediterranean basin, Africa, Southern Asia, Far East, Australia, New Zealand, Pacific Islands, USA and Central and South America (Pantoja *et al*., 2002; Anon, 1958). *P. longispinus* is polyphagous and has been recorded as an economic pest of varying importance on citrus, grapevines, avocado, palms, coffee, cocoa, peaches, plums and other horticultural field crops in various parts of the world, especially the southern USA, Australia and New Zealand (Charles, 1981). In Israel, the longtailed mealy bug was recorded on 36 host plants belonging to 29 different botanical families (Wysoki *et al.*, 1977). It was reported as one of the six mealy bug species from citrus pests in Mediterranean basin (Franco *et al*., 2004).

Clausen (1915) experienced considerable difficulty in measuring the rate of larviposition of *P. longispinus* due to the disturbing effect it had upon the female. He observed that the young remained clustered under the body of the parent for one or more days after birth. The time required for different stages was variable. Mating took place largely during the third instar and larviposition began within 10 to 15 days after the third moult. James (1937) found three nymphal instars in the female and four in the male. The sexes were indistinguishable externally in the first instar but sexual dimorphism was apparent in the second instar. Gullan (2000) provided identification guide to most of the immature stages of *P. longispinus* collected from citrus in Australia. A key and table based on microscopic features allow separation of different instars.

Browning (1959) showed that the number of long-tailed mealy bugs on irrigated orange trees in South Australia rose and fell in a fairly regular sequence throughout the year. Panis (1969) described *P. longispinus* as a viviparous species and showed that light, gravity and host plant quality had a great effect on adult orientation and distribution on plant leaves as well as on its sex ratio. Mating was obligatory for production of eggs and the development of ovaries. Males were capable of several matings.

Furness (1976) showed that dispersion of mealy bugs changed with age. The first-instar crawler dispersed over the whole tree; some second-instars were found in exposed positions but most second- and all third-instar larvae sought sheltered

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living sites. Adults reproduced in protected sites. El-Minshawy *et al*. (1974) found that the duration of all pre-adult stages was highly affected by temperature. In New Zealand, the longtailed mealy bug has three discrete generations on grapevines in a year (Charles, 1981).

A complete biological knowledge of a pest is the pre-requisite for its successful biological control. Very little information is available regarding longtailed mealy bug's bionomics. Flanders (1940) concluded that longtailed mealy bug is native to Australia. Furness (1976), El-Minshawy *et al*. (1974), Browning (1959) and James (1937) studied the biology of its different stages. In Auckland (New Zealand), the longtailed mealy bug has three discrete generations on grapevines in a year (Charles, 1981). Wakgari and Giliomee (2003) reported that the natural enemies of longtailed mealy bug were found on citrus. Mani and Krishnamurthy (2004) explained the role of predators in the control of this pest on temperate and tropical fruits. Recently, it was found that limonene, a citrus extract, has promising role in controlling the mealy bugs on tolerant plants (Hollingsworth, 2005).

Keeping in view the extensive importance, the effect of different levels of relative humidity (RH) on the biology of longtailed mealy bug has been investigated.

Materials and Methods

The experiments were carried out in the Department of Environment, Wye College, University of London. The studies were conducted in plastic boxes (27 × 15 × 10 cm) containing one butternut squash, at a constant temperature of 27 °C in an incubator with a continuous photoperiod and light intensity of 7.5 watts/m^2. Three different RH levels, 35%, 55% and 75%, were produced in these boxes by placing saturated salt solutions of magnesium chloride, magnesium nitrate and sodium chloride, respectively, in small plastic boxes of 75 × 45 × 20 mm dimension, covered with mesh cloth lids. At each RH level, five reproducing female mealy bugs were transferred, one to each butternut squash, within a 25 mm diameter ring cage fastened to the squash with a rubber band. These mealy bugs were removed after 24 hs. The crawlers laid by them were allowed to develop within the cages and their positions were noted. After the first moult, differentiation between the male and female became evident. Observations were made every 24 hs to ascertain the time of moulting and the longevity of each instar.

Ten virgin females were isolated soon after the third moult and each was confined with a male for fertilisation. These fertilised females were released singly on butternut squashes in the ring cages to study the pre-larviposition and the larviposition periods, fecundity total life span. For sex ratio, ten fertilised, newly-emerged 4th-instar females were released singly in the cages on butternut squashes. Their progeny was raised until the determination of gender. Number of males and females was determined after the fourth moult of males. The experiments were conducted in a randomised complete block design with ten replications. The results obtained for developmental stages, fecundity, survival and the total life span were analysed statistically through one-way ANOVA; means were compared and ranked through Fisher's test at 5% level. The data for sex ratio was pooled separately into the males and females, which had emerged at different humidity levels for chi-squared tests.

Results and Discussion

The female passed through three and the male through four pre-adult instars. The data (Table 1) showed that RH had no effect on the developmental time of any of the pre-adult stages of male *P. longispinus* and on the overall total developmental period of all stages ($P > 0.05$, Table 1). Because of the larviposition behaviour of *P. longispinus*, no observations could be made on the egg incubation period.

Table 1. Effect of three different relative humidity levels on the development of pre-adult stages of male *P. longispinus*

Humidity level	Mean developmental period in days ± S.E. at 27 ºC				
	1st instar	2nd instar	3rd instar	4th instar	Total pre-adult period
75%	13.49±0.15	9.34±0.15	4.41±0.12	3.42±0.15	30.66±0.54
	n=33	n=33	n=33	n=33	n=33
55%	13.47±0.32	9.41±0.14	4.45±0.10	3.43±0.12	30.76±0.56
	n=37	n=37	n=37	n=37	n=37
35%	13.60±0.14	9.26±0.14	4.54±0.09	3.56±0.14	30.96±0.41
	n=40	n=40	n=40	n=40	n=40
$P_{5\%}$value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

ANOVA one-way Fisher's test showed no significant difference at $P \leq 0.05$; n = number of individuals studied

RH had no significant effect on the duration of pre-adult stages of the female. *P* values had no significant effect on the overall total developmental period of female *P. longispinus* ($P > 0.05$, Table 2).

RH showed no significant effect on the pre-larviposition and the larviposition periods, fecundity and life span of the female ($P > 0.05$, Table 3).

RH seems to have a significant effect on the sex ratio (Table 4). Primary sex ratio for *P. longispinus* is normally 1:1. The proportion of female significantly increased with the increasing RH (Table 4). At RH 70% the female proportion was significantly more than the expected proportion ($c^2 = 135.08$, $P < 0.001$, Table 4) and at RH 35%, significantly less than the expected value ($c^2 = 66.02$, $P < 0.001$, Table 4).

RH had a significant effect on the survival of all pre-adult stages (pooled sexes) of *P. longispinus* ($P < 0.05$, Fig. 1). The number of individuals which survived at 75% relative humidity level was significantly higher than those which survived at RH 55% and 35%. The lowest survival was observed at RH 35% ($P < 0.05$, Fig. 1).

In Russia, Oganesyan and Babayan (1979) observed that the host, temperature and RH had a marked effect on the egg viability and duration of embryonic development of *Pseudococcus comstocki* (Kuw.) but in the present study, RH was found to have no significant effect on the developmental periods of either male or female. However, RH affected the survival and the sex ratio. The proportion of females was greater at higher RH compared to lower RH probably due to

Table 2. Effect of three different humidity levels on the development of pre-adult stages of female *P. longispinus*

Humidity level	(Mean developmental period in days ± S.E. at 27 °C)			
	1st instar	2nd instar	3rd instar	Total pre-adult period
75%	13.27±0.14 n=66	9.35±0.14 n=66	8.47±0.12 n=66	31.09±0.34 n=66
55%	13.40±0.16 n=43	9.50±0.14 n=43	8.61±0.11 n=43	31.51±0.37 n=43
35%	13.42±0.10 n=20	9.56±0.13 n=20	8.66±0.11 n=20	31.64±0.30 n=20
$P_{5\%}$ value	> 0.05	> 0.05	> 0.05	> 0.05

ANOVA one-way Fisher's test showed no significant difference at $P \leq 0.05$; n = number of individuals studied

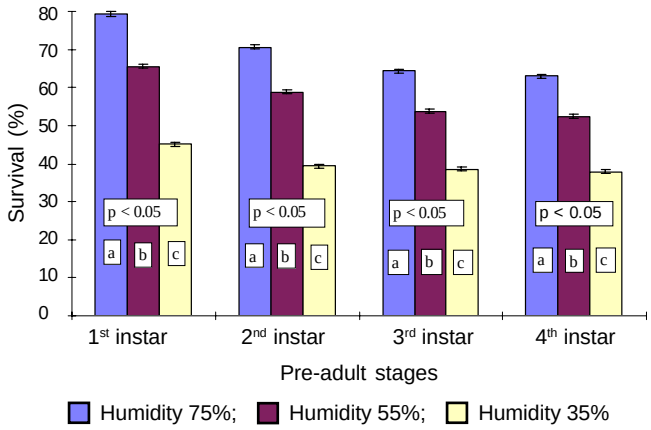

Fig. 1. Effect of three different relative humidity levels on the age specific survival of pre-adult stages (pooled sexes) of *P. longispinus* at 27 °C. [within a group, bars with different letters are significantly different at 5% level (ANOVA)].

Table 3. Effect of three different relative humidity levels on pre-larviposition and larviposition periods, fecundity and lifespan of female *P. longispinus*

Humidity level	Pre-larviposition period (days)	Larviposition period (days)	Fecundity (crawlers)	Total life-span (days)
75%	23.88±0.68 n=10	26.23±0.80 n=10	165.0±0.93 n=10	81.20±0.60 n=10
55%	23.57±0.55 n=10	25.79±1.02 n=10	162.4±1.38 n=10	80.87±0.64 n=10
35%	23.62±0.55 n=10	25.84±1.02 n=10	163.5±2.01 n=10	81.10±0.65 n=10
$P_{5\%}$ value	> 0.05	> 0.05	> 0.05	> 0.05

ANOVA one-way Fisher's test showed no significant difference at $P \leq 0.05$; n = number of individuals studied; values are mean ± S.E. at 27 °C

Table 4. Effect of three different relative humidity levels on the sex ratio of *P. longispinus*

Humidity level	75%	55%	35%
No. of adults emerged	1041	850	618
No. of males	333	378	410
No. of females	708	472	208
Sex ratio (M : F)	1:2.13	1:1.24	1:0.50
χ^2 value	135.08	10.39	66.02
$P_{5\%}$ value	< 0.001	0.001	< 0.001
df	1	1	1

reduced survival of females at low RH. The survival of *P. longispinus* (pooled sexes) was significantly higher at higher RH in all the stages and lowest at lower RH. The findings agree with those of Gordon (1984) who stated that the first-instar of insects were more susceptible to lower RH as compared to other stages.

The effect of RH on insect development cannot be described by general rules similar to those that seem to govern temperature effects. However, variations in the RH can have marked effect on the life cycle of insects (Gordon, 1984). Some species show RH optima, from as low as 30% or less to as high as 90%, occasionally with a narrow range permitting high survival. Others show little effect until RH falls below a critical level that is temperature-dependent. The egg and pupal stages are often unaffected by humidity over a very wide range, except at temperature extremes, in contrast to the first-instar larvae which are less tolerant than either the eggs or the second-instar larvae to low humidity (Gordon, 1984).

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