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Quick estimation of egg number in compact ovisacs of pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green)

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ABSTRACT: Estimating the number of eggs in a mealybug ovisac (= egg sac) is not only tedious but time consuming. Here, we explain a simple method to estimate the number of eggs in a mealybug ovisac using the ovisac parameters *viz.*, length, width, weight. When subjected to correlation analysis these parameters revealed significant association ($P = 0.01$) between number of eggs per ovisac with ovisac length (r=0.81) and weight (r=0.69) but not with ovisac width. Further detailed step-wise regression analyses revealed that ovisac length (cm) and ovisac weight (mg) together can predict variability in the number of eggs to the tune of 72% ($-110.45 + 897.61\ x_1 + 28.72\ x_2$; F =46.63; edf =36; P <0.0001). Further, ovisac length alone could predict the egg number to the tune of 66% (F = 70.42; edf = 36; P<0.0001). This method can speed up the mealybug egg estimation *vis a vis* population in both field as well as laboratory conditions.

Keywords: Egg estimation, linear models, mealybugs, ovisac measurements

INTRODUCTION

Mealybugs are serious insect pests in horticultural crops including fruits, vegetables and ornamentals across tropical and subtropical regions. Of several species of mealybugs that attack horticultural crops, pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) is highly polyphagous and a very serious pest on several crops belonging to 76 plant families (Ben-Dov and German 2003). Adult female mealybugs are small (~3 mm), soft bodied, greyish pink in colour and sessile. Males are smaller than females and winged. On maturation, females secrete sticky, white wax filaments from her abdomen to cover their ovisac that holds her eggs. Upon mating, her whole pinkish-grey body gets filled with salmon-pink eggs that were bunched together in ovisac (= egg sac) covered by white, waxy material as mentioned earlier (http://www.cabi.org/isc/datasheet). The eggs are not visible, unless the ovisac is parted with a fine brush. This mealy wax protects them from pesticidal applications, thus making them 'hard to kill' pests. For this reason, often biocontrol methods, using predatory coccinellids are favourite options among scientific as well as farming communities. However, to make these predatory coccinellids sustainable in long run in depth studies on the field ecology and population estimation of both predator and prey are essential. While carrying out the prey population estimation or predator consumption studies, it is often becomes difficult to count the number of eggs in each ovisac to assess the predator feeding ability or future mealybug population.

Here in this study, we developed a ready reckoner using linear step-wise regression procedures to estimate the number of eggs in an ovisac of mealybug without attempting to count them by tedious manual procedures.

MATERIALS AND METHODS

Studies were carried out at the Division of Entomology and Nematology, Indian Institute of Horticultural Research, Hesseraghatta Lake PO, Bangalore 560089, India. The laboratory cultures of pink hibiscus mealybug, *M. hirsutus* were established in the laboratory on pumpkin (*Cucurbita moschata* L.) (Kairo *et al*., 1997). From an established mealybug colony, the freshly formed ovisacs (n = 40) were collected randomly and each sac was placed gently in a petriplate (4 cm dia) carefully without disturbing the sac for further measurements and data recording. The length and width (cm) of ovisacs were measured using vernier calipers, and the areas were calculated. The egg mass weight (mg) was measured using an analytical balance (Sartorius). To assess the number of eggs per sac, the ovisac was gently cut open with micro-forceps and the individual eggs were counted visually.

Data on ovisac length, width and weight were subjected to correlation and regression analyses with the number of eggs per sac as dependent variable. Significant correlation coefficient (r) values were taken as criteria to assess the most suitable factor (s) to develop linear models with the number of eggs per ovisac on the Y-axis. Detailed step-wise regression models were developed using ovisac measurements like length, width and weight

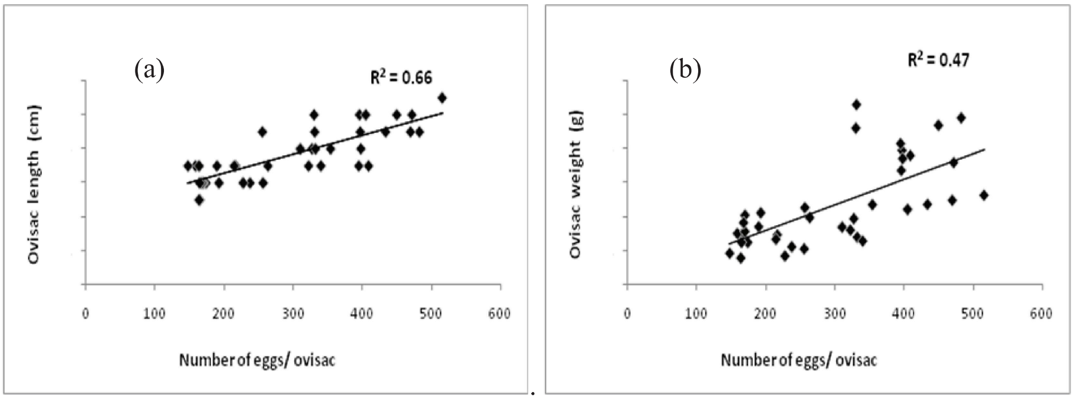

Fig.1. Relationship between number of eggs per ovisac length (a) and width (b).

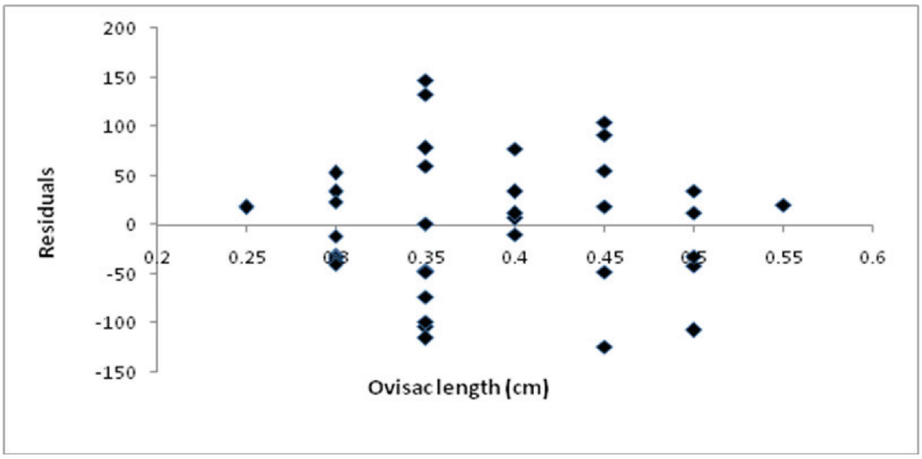

Fig.2. Plot of residulas against the ovisac length when used as independent variable

individually as well as in various combinations to realize maximum coefficient of determination for estimating egg number in the ovisac of pink mealybug (Little and Hills, 1978). Additionally, as a measure of goodness-of-fit, the extent of variability in the egg number per ovisac in the developed models was assessed based on the coefficient of determination (R^2) (Draper and Smith, 1981). The Variance Inflation Factor (VIF) that assess the extent of multi- collinearity was estimated to measure the correlated increase in the variance of the estimated regression coefficient because of collinearity among the variables (Kamala Jayanthi and Verghese, 2011).

RESULTS AND DISCUSSION

Correlation of number of mealybug eggs with ovisac size parameters *viz.*, length, width, weight revealed that egg number is positively and significantly (P=0.01) correlated with ovisac length (r = 0.81) and weight (r = 0.69) but not with ovisac width.

Step-wise regression analysis of dependent variables with egg number could explain variability to the tune of 66% with ovisac length (y= -147.206+ 1168.787x, R^2= 0.66, F=70.43, edf=37, P<0.001; VIF=2.90) and 47% with ovisac weight (y= 153.84+ 61.87x, R^2= 47, F=32.77, *edf*=37, P<0.001; VIF=1.89). The multiple regression analysis with both ovisac length and weight could explain variability to the tune of 72% (y= -110.45+ 897.61x + 28.72x, R^2=0.72, F=46.63, edf=37, P<0.001; VIF=3.59) (Table 1, Fig. 1). Addition of ovisac width as third independent variable could not improve the R^2 value over and above 72%. The VIF values for these models were found to be within the acceptable limits (<10). Plotting the residuals of observed and estimated number of eggs per ovisac using the length of ovisac as independent variable showed a random dispersal of points across x-axis explaining the good fit of linear model indicating the usefulness of ovisac length as potent independent variable to predict egg numbers (Fig.2)

Table 1. Regression analysis

Dependent variables	Regression equation	R^2	VIF	*P*
All variables [Ovisac length cm) (x_1); weight (mg) (x_2); mealysac width (cm) (x_3)]	$-116.53 + 886.63x_1 + 29.14\ x_2 + 32.05x_3$	0.72	6.65	$F = 30.25$; *edf* = 36; $P < 0.0001$
Only statistically significant variables [ovisac length cm) (x_1); weight (mg) (x_2)]	$-110.45 + 897.61\ x_1 + 28.72\ x_2$	0.72	3.59	$F = 46.63$; *edf* =36; $P < 0.0001$
Ovisac length (cm) (x_1)	$-147.21 + 1168.79\ x_1$	0.66	2.90	$F = 70.42$; *edf* = 36; $P < 0.0001$
Ovisac weight (mg) (x_2)	$153.84 + 61.87\ x_2$	0.47	1.89	$F = 32.77$; *edf* = 36; $P < 0.0001$
Ovisac width (cm) (x_3)	$164.02 + 456.50\ x_3$	0.05	1.05	$F = 1.75$; *edf* = 36; $P = 0.19$

Assessment of number of eggs in a mealybug ovisac by counting oviposited eggs is not only tedious but time-consuming method particularly in the case of *M. hirsutus*, where eggs are laid in closed ovisacs and are not easily observed. We devised a non-destructive, fast and easy method to estimate the number of eggs in a mealybug ovisac. Earlier studies with casuarina moth, *Lymantria xylina* Swinhoe, a defoliator of hard wood and fruit trees revealed positive correlation between number of eggs per mass and egg mass size parameters like length, width, area and width (Tse-Chi Shen *et al.* 2003). Here, they found ~98% of the variation in the number of eggs per egg mass just by weight alone and they recommended use of weight of egg mass and other egg mass density parameters as reliable variables to estimate casuarina moth population size. These egg mass parameters during overwintering period proved to be very handy for forest and orchard managers to predict casuarina moth population. Similarly, in the present study also either the ovisac length alone or in combination with ovisac width were found to be good indicators (Table 1, Figs 1-2) to estimate egg number in ovisac of pink hibiscus mealybug.

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