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A technique for quick estimation of aphid numbers in field

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Investigations were made to develop a quick method for *in situ* estimation of aphid numbers using *Aphis punicae* Passerini (Aphididae: Homoptera) on pomegranate as case study. Regression analysis was made between aphid number and length of infestation on the sampling unit, viz. fresh terminal shoots of pomegranate in three arbitrary infestation classes – heavy, medium and low. The study indicated that for reliable estimation of aphid numbers under high-density infestation, power ($y = 202.74x^{0.5358}$; $R^2 = 0.8244$) and linear ($y = 56.57x + 187.35$; $R^2 = 0.7496$) models were adequate. Under medium density infestation, linear ($y = 11.517x + 55.634$; $R^2 = 0.7093$) and power ($y = 34.129x^{0.7049}$; $R^2 = 0.8268$) models and under low-density infestation linear model ($y = 9.75x–8.7548$; $R^2 = 0.7767$) were found adequate. The models worked out in the present study can be used for quick sampling of aphid numbers under field conditions by grading the infestation and measuring the length of the infestation, and substituting the same in the above models.

APHIDS are interesting subjects for studying 'population dynamics'. However, their high reproductive rates and colonizing habits lead to high unit density, rendering field enumeration difficult. Precision recording of the aphid numbers is a prerequisite to several ecological investigations and other studies related to their management.

The distribution of aphids on most plants is aggregated typical of many insects[1]. In general, aphids prefer to feed and reproduce on young growing parts compared to mature parts[2]. Further, the aphids that aggregate set different problems from those that disperse. Aggregates of aphids are usually easier to find than widely spaced species, but it is more difficult to estimate their numbers. Hence, the present study was carried out for developing a method for *in situ* estimation of aphid numbers in case of *Aphis punicae* Passerini (Aphididae: Homoptera) colonizing pomegranate (*Punica granatum* L.) trees. The population of aphids within the plant ranges from low to high density on tender shoots[3]. Thus mixed type of infestation does occur in the same plant, warranting grading of infestation as low, medium and high. The pomegranate aphid is a serious pest on pomegranate and in any management or field evaluation, investigators require quick estimation of large samples. Therefore, the present study was carried out to arrive at an estimation model for precision field-estimation of aphid numbers.

The experiment was carried out in the pomegranate (cv. *Ganesha*) field of Indian Institute of Horticultural Research, Bangalore (12°58′N; 77°35′E) during 1999–2000 for the pomegranate aphid, *A. punicae*. The aphid infestation was classified into three classes, viz. heavy, medium and low. Heavy (H) means aphids present in large numbers with dense infestation on leaves and stems of new terminal shoots. Medium (M) means aphids present in large numbers in recognizable colonies but diffused, and infesting a large proportion of the leaves and stems, and low (L) refers to aphids present in small numbers but not in recognizable colonies and diffused. The sampling unit constituted terminal tender shoots (approximately 15 cm length) on which aphids selectively colonize. However, aphids tend to congregate more towards the terminal end and rarely to the base of the shoot. Twenty-five sampling units (terminal shoots) of each class were randomly taken and the infestation length (linear spread of aphid colony along shoot axis) was measured in centimetres with a scale. Next, the sample shoots were carefully cut and brought to the laboratory in individual sealed polythene bags to prevent any escape of aphids, and the actual numbers of aphids on the shoot were counted under a stereo binocular microscope. The data were tabulated and subjected to linear and nonlinear analyses for all the three categories, with length (x) as independent factor and mean number of aphid per shoot (y) as dependent factor. These relationships take the following forms of equation: Linear – $y = a + bx$; Logarithmic – $y = C \ln x + b$; Exponential – $y = C e^{bx}$; Power – $y = C\ x^{b}$; Polynomial – $y = b + C_1x + C_2x^2 + C_3x_3 \ldots C_nx^n$ (where y is the predicted mean population, x is the factor, a is the intercept ($a = y – bx$) and b is the slope; thus C, $C_1 \ldots C_n$ and b are constants, ln is the natural logarithm and e is the base of the natural logarithm). The coefficient of determination (R^2) reflects the extent to which

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variability in estimates is accounted for by the sole factor x, which is the length of infestation. So, higher the R^2 value greater the reliability of the prediction model.

The equations and R^2 values for the three categories, viz. high, medium and low are presented in Tables 1, 2 and 3 respectively.

In the case of high density among the models tested (Table 1), the best fit for mean aphid estimates was obtained with polynomial order (6) showing $R^2 = 0.8724$. However, the simple linear model ($y = 56.593\ x + 187.14$) was found to estimate the aphid numbers with 75% reliability ($R^2 = 0.7511$) and was comparable to polynomial order (2) ($R^2 = 0.7510$). The nonlinear models, log ($R^2 = 0.7257$) and exponential ($R^2 = 0.6514$) could not improve the reliability of aphid estimates over and above the linear models. The power model was also found to estimate the aphid numbers by explaining the total variability to the tune of 82% ($R^2 = 0.8252$). Among the polynomial models, the coefficient of determination increased to 85% for orders (3) and (4) and to 86% for order (5).

Here, the reliability of prediction for polynomial order was to the extent of 84–87%. It, however, did not differ much from the power model which can estimate to the extent of 82%. Hence considering the simplified calculation without compromising on reliability of estimation, the power ($y = 202.68x^{0.5359}$ with $R^2 = 0.8252$) and linear ($y = 56.593x + 187.14$; $R^2 = 0.7511$) models can be used to estimate aphid numbers under high density (Figure 1 *a*).

In the case of medium density of all models tried (Table 2), the power model could explain variability in aphid numbers to the extent of 82% ($R^2 = 0.8268$). The linear model explained the variability in numbers with a coefficient of determination (R^2) of 0.7093. The other models, viz. exponential ($R^2 = 0.6987$), log ($R^2 = 0.7341$), polynomial order (2) ($R^2 = 0.7431$), polynomial order (3) ($R^2 = 0.7476$), polynomial order (4) ($R^2 = 0.7479$), polynomial order (5) ($R^2 = 0.7562$) and polynomial order (6) ($R^2 = 0.7667$) could not explain variability in estimates beyond 76%. Therefore, for medium density, considering the convenience of calculation and sampling reliability the linear model ($y = 11.517\ x + 55.634$; $R^2 = 0.7093$) and the power model ($y = 34.129\ x^{0.7049}$ $R^2 = 0.8268$) can be used for estimating aphid numbers under field conditions (Figure 1 *b*).

In the case of low density among the models tried (Table 3) for estimation of aphid numbers, the linear model estimate accounted for the variability to an extent of 78%. The other models tried did not appreciably improve the coefficient of determination. However the best fit in increasing order was obtained with the polynomial models with $R^2 = 0.8149$ (for order (2)); 0.8763 (for order (3)); 0.8605 (for order (4)); 0.8763 (for order (5)) and 0.8881 (for order (6)) respectively. Considering the simplicity of calculation of the linear model ($y = 9.75\ x - 8.7548$, $R^2 = 0.7767$) it can be used to estimate aphid numbers under low density (Figure 1 *c*).

Further, to test the null hypothesis that actual numbers and estimated numbers do not differ significantly, the calculated population estimates using infestation length for high, medium and low densities along with actual counts were subjected to paired two sample *t*-test (Table 4). The analysis indicated that observed and calculated values for all the three categories, viz. high, medium and low did not differ significantly even at 1% probability level. Further, the coefficient of determination (R^2) for the estimated values was worked out. For high density, it was 1.00 and 0.9572 (for linear and power model respectively). For medium density, it was 1.00 and 0.9802 (for linear and power model respectively) and for low density it was 1.00 (for linear model) showing its high reliability in predicting the aphid population under respective categories of infestation. Both these tests clearly showed that the estimated counts and actual counts did not differ significantly. Thus, the models developed are reliable for field estimation of *A. punicae* in the field.

In the present study, infestation length of aphids and infestation grading which were used for generating the above models are easy to measure under field conditions.

Table 1. Models to estimate field aphid numbers using infested shoot length under high infestation

Model	Equation
Linear	$y = 56.593x + 187.14$ ($R^2 = 0.7511$)
Log	$y = 182.34 \ln(x) + 194.51$ ($R^2 = 0.7267$)
Power	$y = 202.68x^{0.5359}$ ($R^2 = 0.8252$)
Exponential	$y = 215.33\ e^{0.1454x}$ ($R^2 = 0.6523$)
Polynomial order (2)	$y = -0.6455x^2 + 63.096x + 174.71$ ($R^2 = 0.7524$)
Polynomial order (3)	$y = 2.405x^3 - 41.644x^2 + 244.76x - 15.918$ ($R^2 = 0.8479$)

Table 2. Models to estimate field aphid numbers using infested shoot length under medium infestation

Model	Equation
Linear	$y = 11.517x + 55.634$ ($R^2 = 0.7093$)
Log	$y = 100.6 \ln(x) - 43.518$ ($R^2 = 0.7341$)
Power	$y = 34.129x^{0.7049}$ ($R^2 = 0.8268$)
Exponential	$y = 72.131\ e^{0.0755x}$ ($R^2 = 0.6987$)
Polynomial order (2)	$y = -0.4302x^2 + 21.219x + 13.762$ ($R^2 = 0.7431$)
Polynomial order (3)	$y = 0.0263x^3 - 1.3883x^2 + 31.098x - 12.464$ ($R^2 = 0.7476$)

Table 3. Models to estimate field aphid numbers using infested shoot length under low infestation

Model	Equation
Linear	$y = 9.75x - 8.7548$ ($R^2 = 0.7767$)
Log	$y = 50.678 \ln(x) - 28.585$ ($R^2 = 0.5943$)
Power	$y = 5.8422x^{1.0979}$ ($R^2 = 0.6787$)
Exponential	$y = 10.992\ e^{0.1752x}$ ($R^2 = 0.6103$)
Polynomial order (2)	$y = 0.4443x^2 + 2.0313x + 12.624$ ($R^2 = 0.8149$)
Polynomial order (3)	$y = 0.1085x^3 - 2.3244x^2 + 20.02x - 15.402$ ($R^2 = 0.8372$)

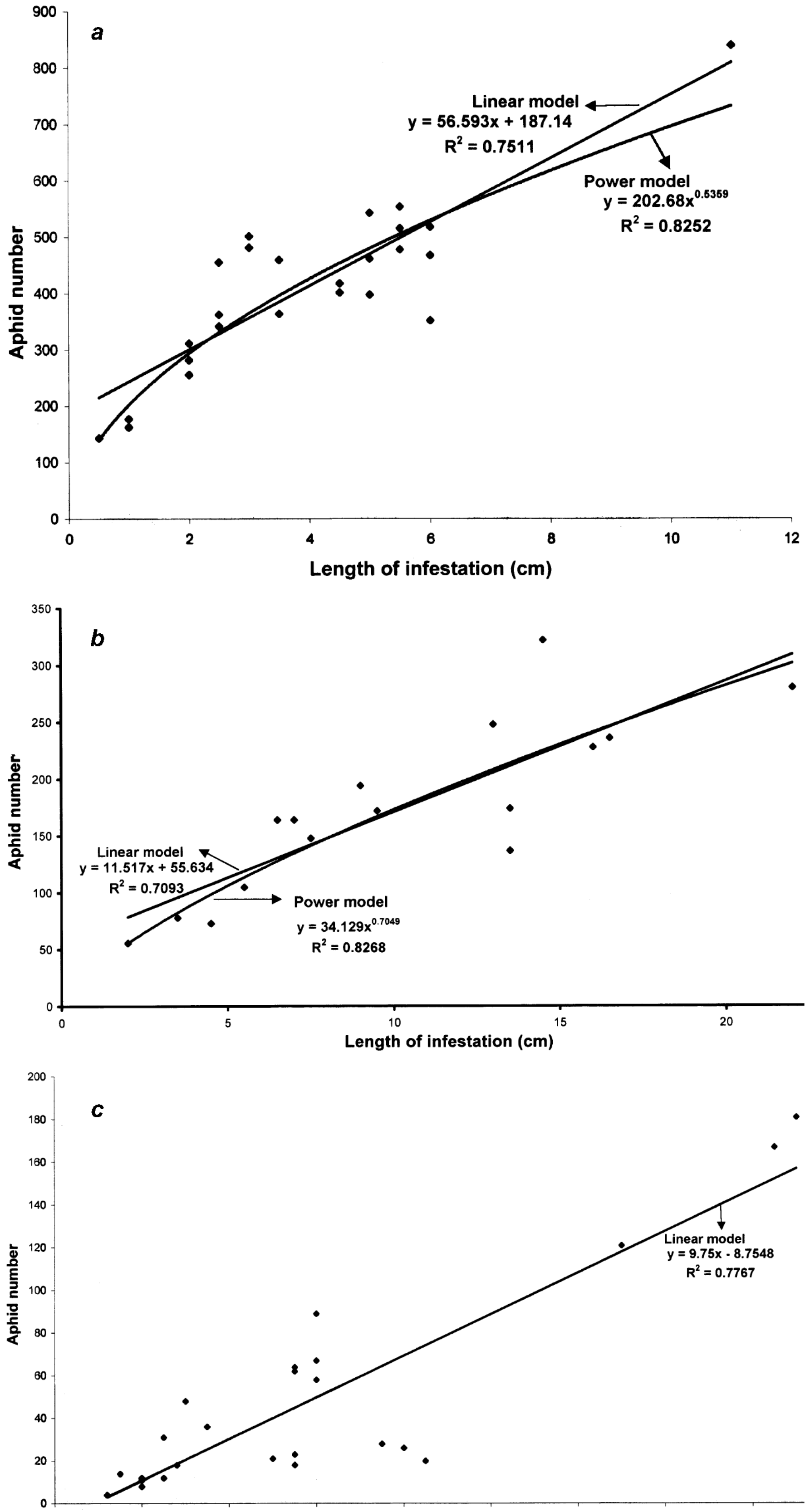

Figure 1. Relationship between length of infestation and aphid number: ***a***, high density; ***b***, medium density; ***c***, low density.

Table 4. Paired two-sample *t*-test for means of predicted number of aphids (through linear model) vs observed number of aphids

Infestation density	Sample size	Observed sample mean (no. of aphids)	Predicted sample mean (no. of aphids)	Variance of observed sample	Variance of predicted sample	Pearson correlation
High	25	410.12	418.03 (NS)	21794.11	17550.4	0.87
Medium	25	173.69	173.76 (NS)	5741.696	4070.112	0.84
Low	25	45.54	46.12 (NS)	2379.65	1871.99	0.88

NS, Not significant.

It was found that the estimated aphid number did not differ significantly with actual counts, making the above sampling models self-weighting. Aphids are small insects, densely-colonizing plant parts. Visual *in situ* estimation of numbers is difficult and fraught with inaccuracy, time-consuming and a strain on the eyes. But in ecological studies, precision estimates with a tolerance of 25% margin of error is acceptable[1]. Towards this, the present study has helped in arriving at models for three grades of infestation or density. By measuring the length of the colony on a longitudinal axis of the shoot, and substituting it in the equations, aphid estimates can be obtained. This further ensures quick estimation, especially in case of insects like aphids whose numbers are highly variable on a temporal scale. Using the models the estimation of population total (y) can be simply made by substituting that particular infested shoot length in the place of (x). This quick method of calculation of aphid estimates may be done after grading new shoots for high, medium or low infestation and measuring the length of the spread of the infestation. As ecological studies and integrated pest management decisions require reliable estimates of aphid numbers under field conditions, the models worked out in the present study can be used for quick sampling of aphid numbers under field conditions. The study also showed that the more complicated nonlinear models can be dispensed with, or may be used when further accuracy is desired.

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