African Journal of Biotechnology Vol. 11(9), pp. 2365-2370, 31 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3338
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Discrimination of the *Palomena prasina* L. (Heteroptera: Pentatomidae) nymph stages and sex using some morphological parameters by the multiple regression analysis

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Accepted 9 December, 2011

Discrimination of different nymphal stages and sex (male and female) of insects is important in the morphological, physiological and toxicological studies under laboratory and field conditions. The morphometric study of different parts of an insect's body is needed to obtain an index to distinguish between different nymphal stages and sex. In the hazelnut production area of the Black Sea region, amongst the sucking type bugs the green shield bug (*Palomena prasina* L.) is the most important specie encountered due to its intensity and economical damage threshold. The study was aimed to develop modeling of the *P. prasina* nymph stages and sex (NSS) using body length and prothorax width. Eight regression equations were compared for accuracy and adaptability. The best model developed was as follows: NSS = 0.344 + 0.235W + 0.309L (R^2 = 0.9882), where NSS is nymph stages and sex, W is the prothorax width (cm) and L is the body length (cm). For validation of the model, estimated values for NSS showed strong agreement with the measured values. Therefore, it can be concluded that models presented herein may be useful for the estimation of the individual NSS with a high degree of accuracy.

Key words: Modeling, body length, prothorax width, *Palomena prasina,* nymph stages.

INTRODUCTION

Among the Pentatomidae (Heteroptera) species found in hazelnut growing areas of the Black Sea Region, green shield bug (*Palomena prasina* L.) is the most important species. Although, this pest is known as polyphagous and widespread, especially in fruit orchards of Turkey, it causes occurrence of the economic damage only in hazelnut orchards (Lodos, 1986; Tuncer et al., 2005; Saruhan and Tuncer, 2010). Its population level usually exceed the economic damage threshold in overall hazelnut orchards in Turkey. *P. prasina* feeds upon hazelnut fruits, causing dropping of premature nuts and kernel damage that creates important problems in export processing (Isık et al., 1987; Tuncer et al., 2005; Saruhan and Tuncer, 2005, 2010). Eggs were open within 15 to 21 days and the nymphs pass through the 5 instars. It complete all developmental stage in 5 to 6 weeks. The insect gives one gene-ration per year (Saruhan, 2004; Saruhan et al., 2010; Tuncer, 2011). Morphometric studies of different parts of an insect's body are needed to obtain an index to distinguish between different nymph stages. In different insects, almost several stages are present at the same time and their size distribution

Abbreviation: NSS, Nymph stages and sex.

overlap to some extent. Therefore, determination of the appropriate stages for individual samplings is a major problem.

Morphometric characters have been widely used by researchers to determine different developmental stages. Dyar's rule stating the ratio of size of each sclerotized body part in successive instars is in a constant range, and was studied on different larval instars of cotton bollworm, *Helicoverpa armigera* (Hubner) (Lep.: Noctuidae), (Davoud et al., 2010). Picaud and Petit (2008), are to test if there are relationships between the succession order of Caelifera and morphometric variables linked to displacement capacities of the different species. They focused on overall body size, wing to body ratios, and sexual size dimorphism of different characters. Develop-mental modeling are commonly explored that used computational or simulation techniques (Odabas et al., 2005, 2009).

The simulation software may be general-purpose, intended to capture a variety of developmental processes depending on the input files, or special-purpose, intended to capture a specific phenomenon. Input data range from a few parameters in models capturing a fundamental mechanism to thousands of measurements in calibrated descriptive models of specific plants or insects (species or individuals). Standard numerical outputs (that is, numbers or plots) may be complemented by computer-generated images and animations (Prusinkiewicz, 2004; Odabas et al., 2008; Caliskan et al., 2009; Odabas et al., 2010). Common measurements for prediction equations in some models carried out previously have included pro-thorax width, body length and different combination of these variables. The objective of this study was to deve-lop estimation of modeling for discriminations of NSS of *P. prasina* using body length, prothorax width and developing software for predicting nymph stages and sex.

MATERIALS AND METHODS

This study was conducted in the laboratory of the Plant Protection Department of Ondokuz Mayıs University, Faculty of Agriculture in 2010. Eggs were collected by beating sheet method from different hazelnut orchards grown in Samsun province, Turkey. The nymphs hatched were reared on fresh seeds of bean (*Phaseolus vulgaris* L.). Petri dishes, 9 cm in diameter were used in the experiments. Distilled water-saturated filter paper was put in the bottom of Petri dishes to regulate the humidity. Fresh bean fruits opened longitudinally and seeds were provided for insects to meet nutrition needs of nymphs in Petri dishes (Çetin and Karsavuran, 2000). Seeds of common bean in Petri dishes were changed once every two days. Petri dishes were checked every day until the nymphs reached adult stages. Moulting of nymphs was checked daily and digital compass were used to measure the body length and prothorax width of each stages. A total of 50 individuals from each biological stage were exposed to this process.

Model construction

Multiple regression analysis of the data was performed to develop nymph stages and sex. The general purpose of multiple regressions is to learn more about the relationship between several independent or predictor variables and a dependent or criterion variable. Given a data set $\{y_i, x_{i1}, \ldots, x_{ip}\}_{i=1}^{n}$ of *n* statistical units, a linear regression model assumes that the relationship between the dependent variable y_i and the *p*-vector of regressor's x_i is linear. Thus, the model takes form

$$y_i = \beta_1 x_{i1} + \cdots + \beta_p x_{ip} + \varepsilon_i = x_i'\beta + \varepsilon_i, \quad i = 1, \ldots, n,$$

Where ′ denotes the transpose, so that $x_i'\beta$ is the inner product between vectors x_i and β. often these *n* equations are stacked together and written in vector form as $y = X\beta + \varepsilon$, where

$$y = \begin{pmatrix} y_1 \\ y_2 \\ \vdots \\ y_n \end{pmatrix}, x = \begin{pmatrix} x_1' \\ x_2' \\ \vdots \\ x_n' \end{pmatrix} = \begin{pmatrix} x_{11} & \cdots & x_{1p} \\ x_{21} & \cdots & x_{2p} \\ \vdots & \ddots & \vdots \\ x_{n1} & \cdots & x_{np} \end{pmatrix}, \beta = \begin{pmatrix} \beta_1 \\ \vdots \\ \beta_p \end{pmatrix}, \varepsilon = \begin{pmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \vdots \\ \varepsilon_n \end{pmatrix}$$

Some remarks on terminology and general use

y_i is called the *dependent variable*. The decision as to which variable in a data set is modelled as the dependent variable and which are modelled as the independent variables may be based on a presumption that the value of one of the variables is caused by, or directly influenced by the other variables. x_i are called independent variables. Usually a constant is included as one of the regressors. For example, we can take $x_{i1} = 1$ for $i = 1, \ldots, n$. The corresponding element of *β* is called the intercept. The regressors x_i may be viewed either as random variables, which we simply observe, or they can be considered as predetermined fixed values which we can be choose. Both interpretations may be appropriate in different cases, and they generally lead to the same estimation procedures; however different approaches to asymptotic analysis are used in the two situations. β is a *p*-dimensional parameter

Table 1. Regression models for the estimation of nymph stages and sex of *Palomena prasina*.

Regression model	Equation	R^2
NSS = 0.344 + 0.235 W + 0.309 L	1	0.9882*
NSS = 0.477 + 0.554 W + 0.008 L	2	0.9852*
NSS = 0.548 + 0.425 L + 0.003 W^2	3	0.9859*
NSS = 1.670 + 0.044 LW	4	0.9540*
NSS = 2.231 + 0.003 WL^2	5	0.8805*
NSS = 2.119 + 0.005 W^2L	6	0.9032*
NSS = 1.520 + 0.003 L^2 + 0.067 W^2	7	0.9618*
NSS = 2.460 + 0.0003 L^2W^2	8	0.8298*

All variables in the models above are significant at P = 0.05. NSS is growth stages and sex; L is length; W is width. Asteriks denote that P<0.0001.

vector.

Its elements are also called effects, or regression coefficients. This variable captures all other factors which influence the dependent variable y_i other than the regressors x_i. The relationship between the error term and the regressors, for example whether they are correlated as a crucial step in formulating a linear regression model, will determine the method to use for estimation (Erper et al., 2011).The most common regression equations used to develop NSS models were evaluated for accuracy and adaptability. All equations were composed of various subsets of independent variables, such as body length (L) and prothorax width (W). Eight models were determined and selected as the most suitable for estimating NSS of *P. prasina*. All variables in the models below were significant at *P = 0.05* level.

$$NSS = a + bW + cL \quad (1)$$

$$NSS = a + bW + cL^2 \quad (2)$$

$$NSS = a + bL + cW^2 \quad (3)$$

$$NSS = a + bLW \quad (4)$$

$$NSS = a + bWL^2 \quad (5)$$

$$NSS = a + b\,W^2L \quad (6)$$

$$NSS = a + bL^2 + cW^2 \quad (7)$$

$$NSS = a + bL^2W^2 \quad (8)$$

Where, NSS is the nymph stages and sex; L is the body length (cm); W is the prothoax width (cm) and a, b, and c are the co-efficiencies. All data was analyzed using the R-program. Slopes, intercepts and regression coefficients of the models were compared using the R-program. Correlation coefficients were calculated between measured and estimated data (Cho et al., 2007; Caliskan et al., 2010a, b; Celik and Odabas, 2009). SlideWrite program was used for 3-D graphic.

RESULTS AND DISCUSSION

Of the all models, body length (L) and prothorax width (W) were selected for estimation of the NSS of *P. prasina* (Table 1). Equation 1 had a higher R^2 value than other equations tested. Table 1 shows that the R^2 values are ranging between values 0.9882 to 0.8298. Equation 1 (NSS = 0.344 + 0.235W + 0.309L) was found to have the highest R^2 value (R^2= 0.9882) and the lowest R^2 value (R^2= 0.8298) was found Equation 8 (NSS = 2.460 + $0.0003L^2W^2$). The other models can also be used to predicted stages and sex of *P. prasina.* That is why the researchers can prefer the model whichever they want. Figure 1 shows that both body length and prothorax width were highly related to NSS of *P. prasina*. Equations with *P* > 0.05 and lower R^2 values were eliminated at the beginning of this study. To estimate the NSS of *P. prasina*, 8 models using L and W were selected (Table 1). Of the eight models, Equation 1 showed the highest relationship. According to obtained results, body length and prothorax width contribute to accurately discrimi-nation of NSS by the developed software herein. The using of software is very simple. When the researcher entered the data, the program shows the NSS as a number. The numbers are from 1 to 7. The numbers show us the NSS of *P. prasina.* Number 1 is first instar, number 2 is second instar, number 3 is third instar, number 4 is fourth instar, number 5 is fifth instar, number 6 is male and number 7 is female (Figure 1).

As a result of multi-regression analysis of NSS of *P. prasina,* the effects of body length and prothorax width were found to be significant. When the prothorax width and body length are increased from 1 to 7 cm, the NSS increase also (Figure 2). Increasing prothorax width and body length effected positively. Prothorax width is more effective than body length for determining NSS (Figure 2). The estimation models that aim to predict the NSS of *P. prasina* can provide more accurate data to researches in biological studies on heteropteran insect species. Moreover, these kinds of models enable researchers to

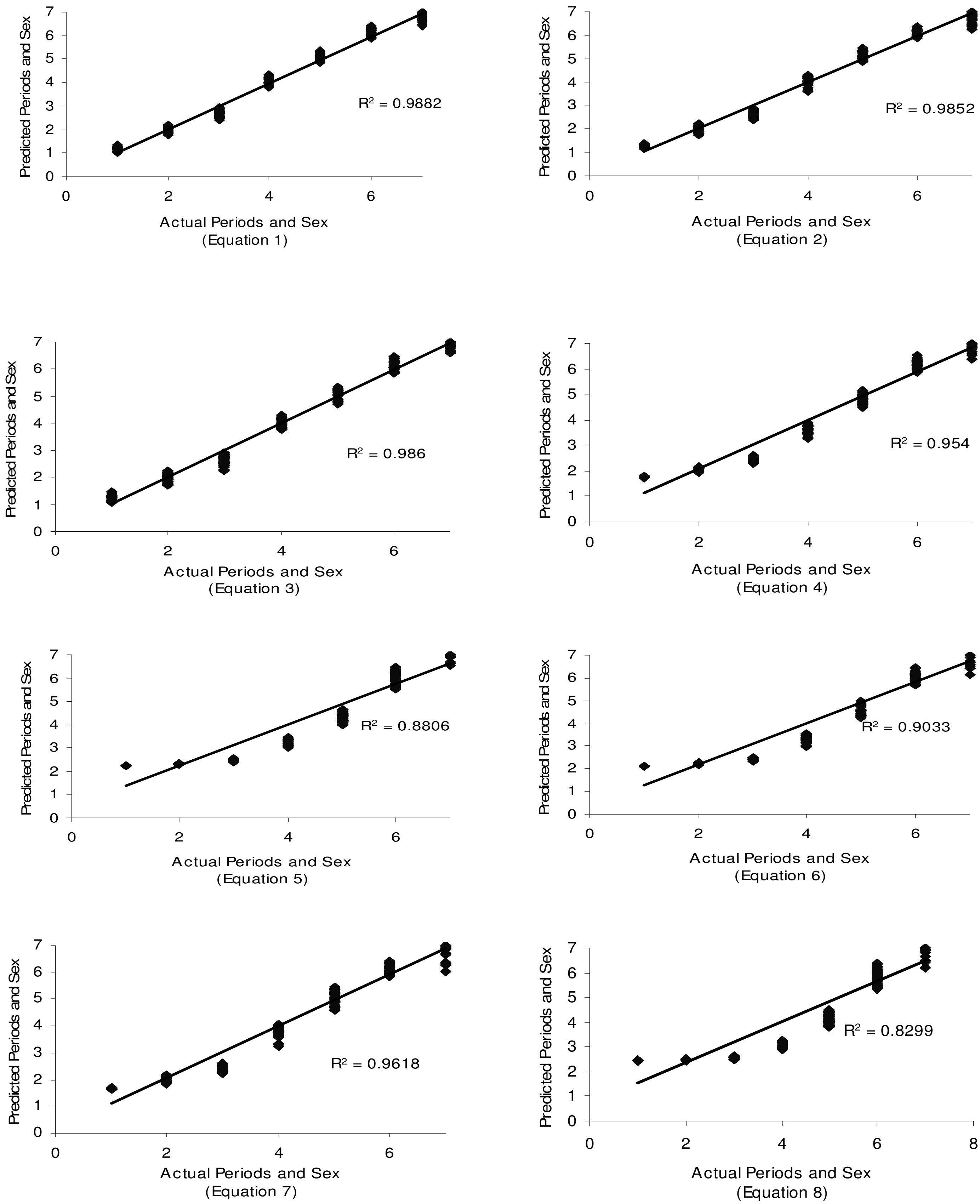

Figure 1. Relationship between actual and predicted the NSS of *Palomena prasina*.

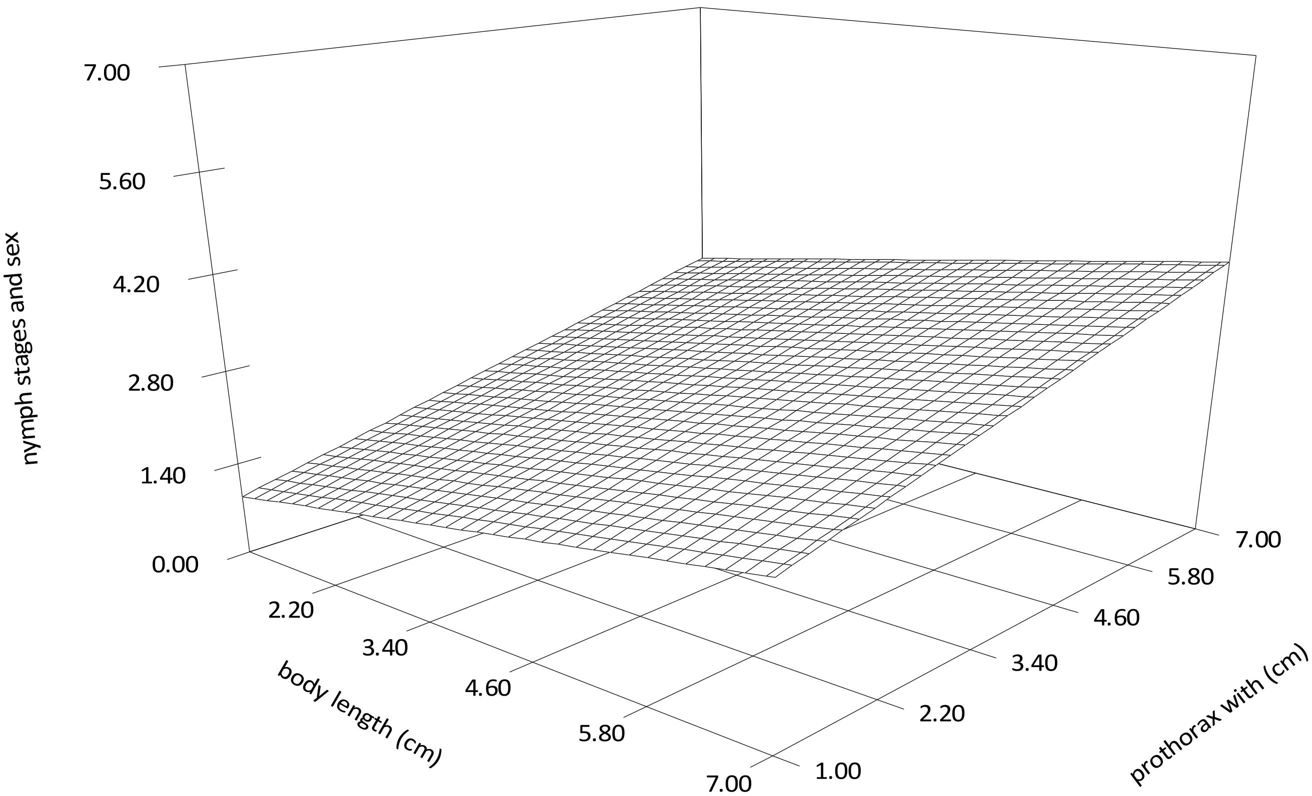

Figure 2. Changes NSS of *Palomena prasina* with body length (cm) and prothorax with (cm).

carry out NSS on the same metamorphoses studies. *P. prasina* of 1 to 2 and 3, 4, 5 nymphal stages are very difficult to distinguish morphologically. However, their stages can be understood by following the developments in the laboratory. Furthermore, estimation of NSS saves times. There are no published reports related to NSS prediction model for *P. prasina*. In this study, NSS are well correlated with body length and prothorax width, with high R^2 values (Tables 1). The body size of *P. prasina* was significant factor in the estimation of NSS. This method was rapid and was relatively accurate. According to the results of the current study, NSS of *P. prasina* may be estimated by nonlinear regression models including body length and prothorax width.

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