STUDY OF COTTON BOLLWORM, *HELICOVERPA ARMIGERA* HÜBNER (LEPIDOPTERA: NOCTUIDAE) USING DYAR'S RULE

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ABSTRACT: Discrimination of different larval instars of insects is important in morphological, physiological and toxicological studies under laboratory conditions. The morphometric study of different parts of an insect's body is needed to obtain an index to distinguish different larval instars. In this study, Dyar's rule stating the ratio of size of each sclerotized body part in successive instars is in a constant range was studied on different larval instars of cotton bollworm, *Helicoverpa armigera*. The insects were reared at 26±2°C, 60±5% RH and a photoperiod of 16: 8 (L: D) h. for 10 generations. One hundred larvae of each instar were randomly selected and width of head capsule in widest region, as well as length and width of stigmata located on prothorax and first and last abdominal segments were measured by calibrated micrometer. A frequency distribution plot showed that due to overlap in stigmata dimension, size of the stigmata was not a suitable index for distinguishing different larval instars in this insect. The widths of head capsule for 1st to 6th larval instars were 0.257-0.314, 0.4-0.485, 0.6-0.743, 0.96-1.2, 1.5-1.8 and 2.55-2.8 mm, respectively. Dyar's ratios for 1st to 6th larval instar intervals were obtained 1.52, 1.512, 1.631, 1.612 and 1.552, respectively. There was no overlap in the data range; therefore, head capsule width can be used with high confidence as an index to distinguish larval instars. Linear regression analysis revealed significant effect of larval instars on head capsule (R^2 =0.999). Despite strong relationship between dimension of stigmata and instar numbers, some strong overlapping in stigmata dimensions in subsequent instars decreased the usefulness of stigmata dimensions for instar discrimination. Overlapping particularly in the first abdominal stigmata was more than others.

KEY WORDS: Larval instars, sclerotized, head capsule, stigmata.

The American bollworm, *Helicoverpa armigera* (Hübner) (Lep., Noctuidae), is one of the important pests of cotton, tomatoes, chickpea, tobacco, corn, sesame, hemp, sunflower, etc. in almost all of the agricultural areas of the world and causes both quantitative and qualitative damages (Fitt, 1989 and Matthews, 1999).

The effect of insecticides on the insects may vary based on developmental stages, and the proper timing of insecticides application generally depends on the predominance of a particular instar in field and laboratory experiments. Therefore, identification of instar is very important for both ecological studies and application of control measures. Information about life history of insects is important for understanding population dynamics, life table analysis, key factor analysis and other important ecological investigations, to determine their community structure.

During their immature development, larvae of insects and other arthropods moult regularly. This moulting process is under endocrine control, and follows the secretion of prothoracicotropic, ecdysone and juvenile hormones. As a result, the larval period of insects is divided into several discrete stages (Nation, 2002). The period between two successive moults is usually called an instar (Esperk et al., 2007).

Morphometric studies of different parts of an insect's body are needed to obtain an index to distinguish different larval instars. In different insects almost several instars are present at the same time and their size distribution overlap to some extent. Therefore, determination of appropriate instar for individual sampling is a major problem (Logan et al., 1998). Morphometric characters widely used by researchers to determine different developmental stages (Fink 1984, stark 1988, Holloway 1991, Guglielmino et al., 2006, Kayss et al., 2006).

Growth of insect larvae is discontinuous, with the most measurable change in size occurring following moults (Chapman, 1982). Heavily sclerotized structures, such as head capsules, remain approximately without change during an instar. Dyar was the first to suggest frequency distributions of head capsule width for instar determination more than 115 years ago (Dyar, 1890), and this has been successfully applied many times since (Logan, 1998).

Several studies have been undertaken to determine the number of larval instars in different insects. Danks and Corbet (1973), Savignac and Maire (1981) used head capsule to study insects Larval instar determination from genera *Aedes*, *Culex*, *Anopheles* and *Culiseta*. Hoddle (1990) determined three larval instars for *Apion ulicis* based on head capsule measurement ant Dyar's rule.

Logan et al. (1998) used a computer program according to the head capsule for determining instars of Mountain pine Beetle. Hammack et al. (2003), Larocque, (2001), Rodriguez-Loeches and Barro (2008) used head capsule for instar determination. The body length and head capsule width was found to be the best criterion for the larval instar determination in *Neochetina eichhorniae* (Oke, 2009).

Several mathematical models have been used to describe linear measurements of sclerotized parts in successive instars. Linear progression, $y = a + bx$, and the geometric progression $y = a\ b^x$ which terms Dyar's rule were often used by entomologists to ascertain the actual number of instars (Klingenberg and Zimmermann, 1992). According to this law, the head capsule of caterpillars grows in a geometric progression, increasing in width at each moult by a constant ratio for a given species. This rule is also applied to many other parts of the body, such as body weight, larval length, length of posterior segment of prothorax, diameter of eye, etc. (Dyar, 1890). Another method is frequency, which is a simple and easy method for field population. Instars are indirectly determined through a plot of the number of individuals per size class, where each distinct peak in the plot infers one instar (Fink, 1984).

Hardwick found 30% larva matured in 5 instars, 69% in 6 instars and only 1% in 7 instars (Matthews, 1999). Rearing insects in controlled conditions that adjusts for the nearly best condition for insect's development and monitoring developmental stages for larval moulting is the best way for studying the life cycle of insects (Fink, 1984).

In this study some morphometric properties of all larval stages of cotton bollworm were studied and Dyar's rule was investigated. Larval head capsule width, and also prothorasic stigmata dimension in addition to first and last abdominal stigmata were studied.

MATERIAL AND METHODS

Insect: Insects used in this study were obtained from *H. armigeta* colony available in the department of plant protection, university of Tabriz. Insects reared in controlled condition of 26±2°C, 60±5% relative humidity and a photoperiod of 16: 8 (L: D) h. on artificial diet based on cowpea, for 10 generations. A strong homogenate population of insects allowed us to obtain sufficient number of larvae from each instars in the same generation.

Measurements: Daily monitoring of larvae carried out to record stage of development. The larvae of each instar were randomly selected and the width of the head capsule in the widest region as well as the length and the width of stigmata located on prothorax and first and last abdominal segments were measured by calibrated the micrometer located on a stereomicroscope.

Data analysis: Regression and correlation analysis were performed using SPSS (*ver.* 15.0) and MSTAT-C software. Analysis of variance was carried out based on one way ANOVA and the means were compared using Duncan's multiple-range test.

RESULTS AND DISCUSSION

Head Capsule Width: The head capsule width was relatively constant within the six instars. There was no overlapping in extremes of measurements between instars. The widths of head capsule in 1st to 6th larval instars were 0.257-0.314, 0.4-0.485, 0.6-0.743, 0.96-1.2, 1.5-1.8 and 2.55-2.8 mm, respectively (Table 1). Analysis of variance revealed significant differences ($p<0.05$) among different instars with respect to head capsule width. Mean comparison indicated significant differences among all the instars with maximum and minimum head capsule width in 1st and 6th instars, respectively.

Linear regression analysis showed significant relationship between larval instars and head capsule width (R^2 =0.999) (Fig. 2). Therefore head capsule width could be used for the estimation of larval instar in laboratory populations of cotton bollworm. The instars can be readily separated because of any overlapping in range of distinct instars. The frequency distribution of larval head capsule widths measured in this study is shown in Fig. 1. Dyar's ratio for subsequent instars was 1.52, 1.512, 1.631, 1.612 and 1.552 for first to last larval instars, respectively (Table 1).

Width and Length of Stigmata: There was significant difference among larval instars for stigmata dimension as revealed by analysis of variance ($p<0.05$). Mean comparison showed significant increase in the stigmata dimension from 1st to 6th instars.

Dyar's ratio measured for dimension of stigmata in different segments ranged from 1.667-1.911 and 1.454-1.937 for length and width of prothorax stigmata, 1.786-2.304 and 1.5-2.086 for length and width of first abdominal stigmata and 1.759-2.075 and 1.395-1.950 for length and width of last abdominal stigmata, respectively (Table 2).

A significant relationship was observed between instars and stigmata dimension as revealed by regression analysis (R^2= S1: 0.988 and 0.973, S8: 0.996 and 0.985, T: 0.996 and 0.984) (Fig. 3). In spite of the significant relationship between instar numbers and stigma dimension, overlapping was observed in stigmata dimensions in subsequent instars (Fig. 4), therefore, this may be resulted in decreased power of stigmata dimensions for instar estimation. Overlapping in the first abdominal stigmata was more than the others.

No significant correlation was observed between head capsule width and stigmata dimensions in larval instars of cotton bollworm except for prothorax length in 3rd instar and first abdominal stigmata width in 2nd instar larvae, however, the level of correlations was too low to be predictive. It suggested that head capsule width and stigmata dimensions grow independently through an insect's development.

Dyar's ratio ranges for stigmata were wider than head capsule dimension producing problems in diagnosis of two subsequent instars. Under constant food and other environmental factors, it is easer to study insects, since complexity of factors can variously affect biology of insects. Safranek and Williams (1984) showed that in normal feeding conditions, tobacco hornworm exhibits remarkable adherence to Dyar's rule. Kingsolver (2007) by studying laboratory and field's populations of *Manduca sexta* demonstrated that field population while feeding on a modified artificial diet showed substantial intraspecific variation in number of larval instars. Intra-specific variability in number of larval instars is widespread across insect taxa. Temperature, photoperiod, food quality and quantity, humidity, rearing density, physical condition, inheritance, and sex are the most common factors influencing number of instars (Esperk et al., 2007).

Hsia and Kao (1987) determined six larval instars for corn earworm larvae using the head capsule. Larval mean head capsule widths for insects reared in 28˚C were 0.28, 0.42, 0.71, 1.07, 1.73 and 2.91 mm for first to last larval instars, respectively. This in addition to Dyar's ratio confirmed our results with a little difference. Different research suggested Dyar's rule as a suitable criterion for instar determination and head capsule width as a reliable measurement for instar determination (Agrawal and Kumar Pati 2002, Francisco and Prado 2001, Stein 1981 and Donnell 1967). Head capsule growth is basically restricted to the period of ecdysis therefore the head sizes of successive larval instars tend to follow a regular progression.

Our observations indicated that the studied *H. armigera* colony in the measured individuals, had at least six larval instars and head capsule width in order with Dyar's rule was useful for distinguishing larval instars. Whereas overlapping in stigmata dimension reduced usefulness of this measure for larva instars determination. Therefore, it is not possible to use measurement of any sclerotized part of insect's body according to Dyar's rule for instar determination. Some researchers reported Dyar's rule for instar determination (Enrique 2006, Garcia-Barros 2006, Klingenberg and Zimmermann 1992, Fink 1984). However, Gaines and Campbell (1935) did not recommend Dyar's rule for instar determination because it may indicate false instars.

It should be mentioned that the growth of insects through time is not constant, since different ecological and physiological factors affect growth rate. Tateishi and Shimizu (1988) and Tateishi et al. (1989) studied hormonal bases of moulting and titer of PTTH before and after moulting with some evidence for unequal number of larval instars in common Armyworm populations. They reported that weight of larva in recent instar could determine the number of instars.

It is suggested that Dyar's ratio could be calculated for each insect and in distinct growing conditions. In spite of a linear relationship between larval instar and dimension of stigmata it is recommended not to use any sclerotized part of *H. armigera* larva for determination of larval instars. It is clear that the recent study has been carried out using laboratory homogenate population, thus extension of these results to field populations may cause some differences.

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Table 1. Mean, Standard Error (SE), Standard Deviation (SD), maximum, Minimum and Dyar's ratio in head capsule width of *H. armigera* different larval instars.

Larval instar	Mean	SE	SD	Min	Max	Dyar's ratio
I	0.280	0.001	0.012	0.257	0.314	
						1.520
II	0.426	0.002	0.021	0.400	0.486	
						1.512
III	0.644	0.004	0.038	0.600	0.743	
						1.631
VI	1.050	0.007	0.066	0.960	1.200	
						1.612
V	1.693	0.007	0.075	1.500	1.800	
						1.552
VI	2.628	0.006	0.060	2.550	2.800	

Table 2. Mean, Standard Error (SE), Standard Deviation (SD), maximum, Minimum and Dyar's ratio in stigmata of *H. armigera* larval instars (T: prothorax, S1and S8: first and last abdominal stigmata)

Instar		T						S1						S8					
		Mean	SE	SD	Min	Max	Dyar's Ratio	Mean	SE	SD	Min	Max	Dyar's Ratio	Mean	SE	SD	Min	Max	Dyar's Ratio
I	Length	0.024	0.002	0.007	0.014	0.029	1.911	0.016	0.001	0.005	0.014	0.029	2.304	0.029	0.000	0.000	0.029	0.029	2.075
II	Length	0.046	0.002	0.010	0.029	0.057	1.723	0.038	0.002	0.007	0.029	0.043	1.773	0.059	0.002	0.007	0.043	0.071	1.662
III	Length	0.080	0.002	0.011	0.071	0.100	1.712	0.067	0.002	0.010	0.057	0.086	1.655	0.099	0.002	0.010	0.086	0.114	1.763
IV	Length	0.137	0.005	0.021	0.117	0.167	1.557	0.111	0.004	0.019	0.083	0.167	1.544	0.174	0.005	0.023	0.150	0.233	1.591
V	Length	0.213	0.004	0.020	0.167	0.233	1.677	0.172	0.004	0.016	0.133	0.200	1.786	0.277	0.006	0.029	0.233	0.333	1.759
VI	Length	0.357	0.007	0.031	0.300	0.433		0.307	0.004	0.017	0.267	0.333		0.487	0.010	0.044	0.367	0.567	
I	Width	0.023	0.002	0.007	0.014	0.029	1.937	0.016	0.001	0.005	0.014	0.029	2.086	0.029	0.000	0.000	0.029	0.029	1.95
II	Width	0.044	0.002	0.009	0.029	0.057	1.725	0.034	0.002	0.007	0.029	0.043	1.770	0.056	0.001	0.006	0.043	0.071	1.589
III	Width	0.076	0.002	0.011	0.057	0.100	1.461	0.061	0.001	0.006	0.057	0.071	1.712	0.089	0.002	0.007	0.071	0.100	1.627
IV	Width	0.112	0.004	0.017	0.080	0.133	1.477	0.104	0.003	0.015	0.080	0.133	1.282	0.144	0.004	0.016	0.117	0.167	1.433
V	Width	0.165	0.004	0.017	0.133	0.200	1.454	0.133	0.003	0.015	0.100	0.167	1.5	0.207	0.004	0.017	0.167	0.233	1.395
VI	Width	0.240	0.004	0.017	0.200	0.267		0.200	0.002	0.011	0.167	0.233		0.288	0.006	0.027	0.233	0.333	

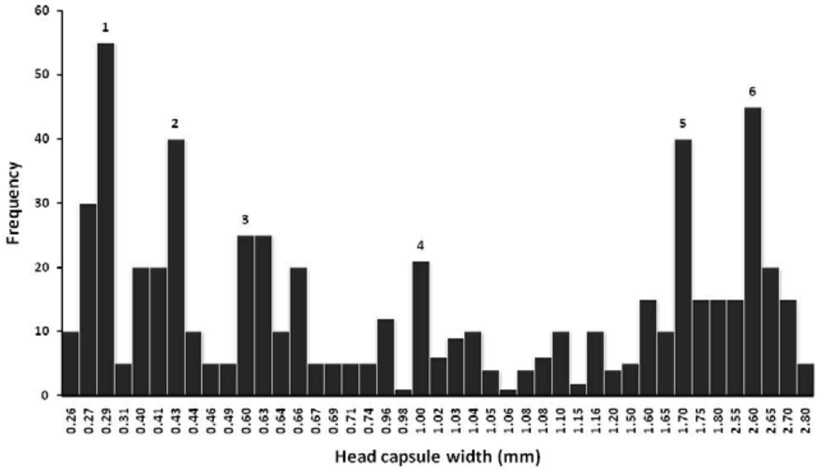

Figure 1. Head capsule width frequency histogram for *H. armigera* larvae.

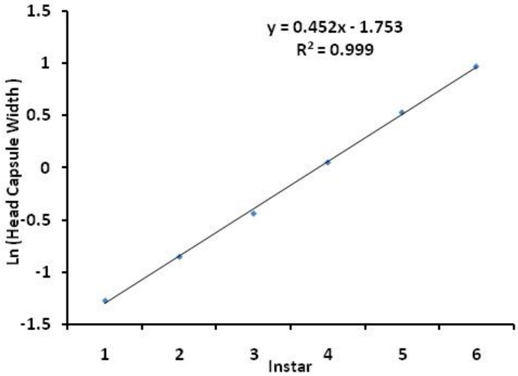

Figure 2. Relationship between head capsule width and instar of *H. armigera*.

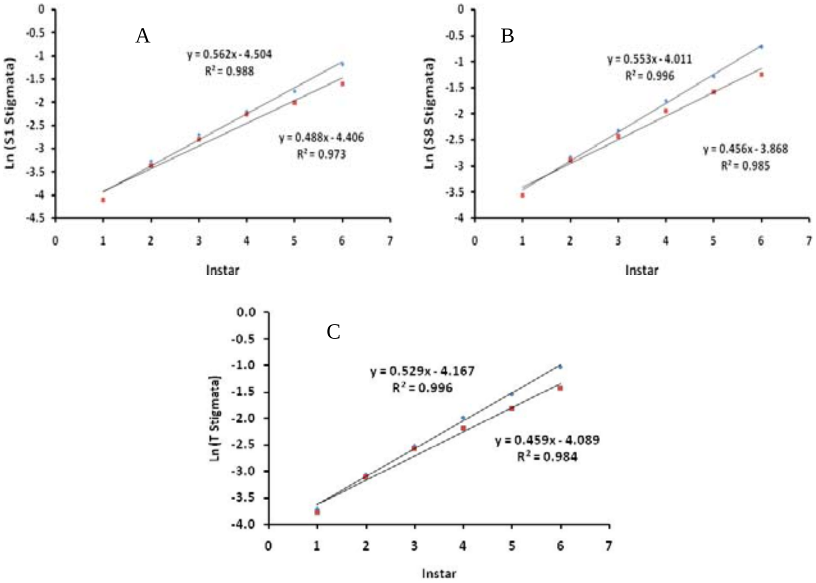

Figure 3. Relationship between different stigmata dimension and instar of *H. armigera*
A: First abdominal stigmata (S1), B: Last abdominal stigmata (S8), C: Prothorax stigmata (T)
■Width of stigmata, ◆Length of stigmata

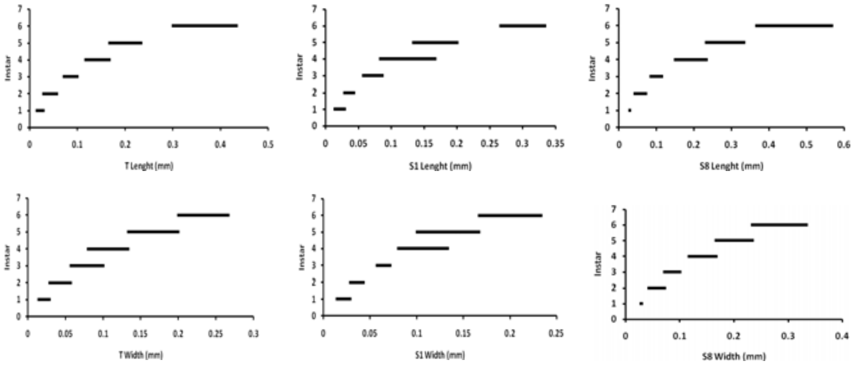

Figure 4. Relationship between stigmata dimension and instars of *H. armigera*, shows some overlapping in width and length range in subsequent instars. T: Protorax stigmata, S1: First abdominal stigmata, S8: Last abdominal stigmata.