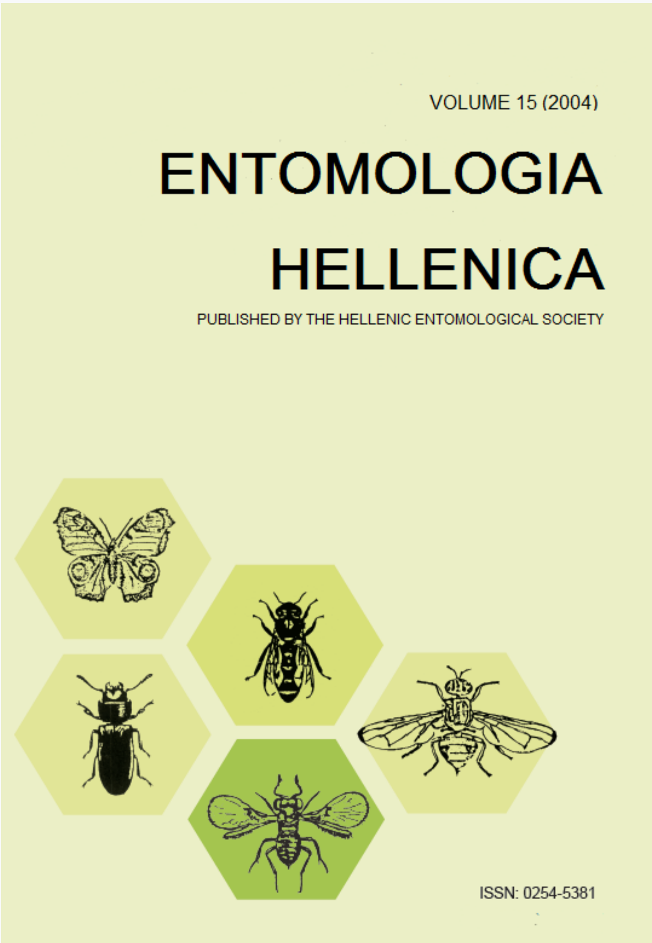
ENTOMOLOGIA HELLENICA

Vol 15 (2004)



Determination of Helicoverpa armigera (Lepidoptera: Noctuidae) larval instars and age based on head capsule width and larval weight

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doi: [10.12681/eh.14047](https://doi.org/10.12681/eh.14047)

To cite this article:

Stavridis, D. G., Ipsilandis, C. G., Katarachias, P. C., Milonas, P. G., Ifoulis, A. A., & Savopoulou-Soultani, M. (2004). Determination of Helicoverpa armigera (Lepidoptera: Noctuidae) larval instars and age based on head capsule width and larval weight. *ENTOMOLOGIA HELLENICA*, *15*, 53–61. https://doi.org/10.12681/eh.14047

ENTOMOLOGIA HELLENICA 15 (2003-2004)

Determination of *Helicoverpa armigera* (Lepidoptera: Noctuidae) Larval Instars and Age Based on Head Capsule Width and Larval Weight

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ABSTRACT

Larvae of *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae)were reared in laboratory conditions (26^{o}C, 16:8 L:D) and measurements of larval head capsule width, and body weight, were used in order to determine the boundaries of larval instars. Larvae of *H. armigera* completed development in 5 to 7 instars. Head capsule width could predict the larval instar only for L_1. The upper boundary of head width for L_1 was 0.4mm. Body weight could predict both L_1 and L_2 larval instars. Boundaries between L_1-L_2 instars were found to be 1 mg and for L_2-L_3 5,5 mg. Correlation and regression analysis suggest that a combination of head capsule width and body weight can predict both larval instars and chronological age under constant conditions in the laboratory.

Introduction

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) is a major pest of a wide range of host plants, including field crop plants. Several studies have been conducted on the development of *H. armigera* on plants and artificial diets. Larvae required 5, 6 or 7 instars to complete their development, which resulted in a variation in duration of larval development. Such variation made the determination of the timing of control application difficult, as all instars are not equally susceptible to the control means. Timing the application of various control methods is important to achieve sufficient control of the pest. For example insect growth regulators might be more efficient against late instars of the pest (Gurr et al. 1999). It is essential to identify the various larval instars and to know the larval performance during any individual instar for better understanding of cultured and field insects' behavior. Instar has been widely determined for lepidopterous larvae on the basis of head capsule width (Dyar 1890, Gaines and Campbell 1935, Fox et al. 1972, Muggli and Miller 1980, Frick and Wilson 1981, Smith et al. 1986, Godin et al. 2002). Although measurement of head capsule width has currently been used to determine instars of various insects, it cannot be used to discriminate between instars unequivocally in cases where head capsule widths of successive instars overlap (Got 1988). Examination of the larva itself was therefore necessary, but because of the variability in number of instars found among the larvae, the question arose as to whether determination of instar and nearness to pupation was possible by measurement, not only of the width of head capsule, but of the larval weight as well. Under optimal conditions, most lepidopterous larvae develop through a minimal number of instars, growing at a nearly maximal growth rate. However, environmental conditions are seldom stable enough to support this maximum growth rate throughout the duration of larval development. Supernumerary molts may be hereditary or may be caused by inadequate nutrition or when environmental conditions depart from the optimum (Hoskins and Craig 1935, Allegret 1964, Wigglesworth 1965, Leonard 1970, Schmidt and Lauer 1977, Schmidt et al. 1977, Scriber 1977).

We document and support our interpretation that accurate assignment of instar number of *H. armigera* with variable number of instars is possible when combined with parameters such as larval weight and larval head capsule width.

Materials and Methods

Insects. Our laboratory colony was started in 1995 with larvae collected in Thessaloniki (Macedonia, Northern Greece) and reared on an artificial diet in laboratory conditions (26°C, 16:8 L:D, 70% RH). The components of the diet were: water (4 l), agar (106g), maize meal (760g), brewer's yeast (200g), wheat germ (184g), ascorbic acid (28g), benzoic acid (12g), methyl p-hydroxybenzoate (9.2g), vanderzant vitamin mixture (0.2g) and Wesson's salt mixture (30g). Eggs were procured by placing 6 pairs of moths in a cylindrical, 1l cardboard carton lined with wax paper. The top of each carton was removed and replaced with a circular piece of wire screen, and the bottom was left open. The cartons were set on petri dishes containing moist sand. The moths were transferred to fresh cartons daily until they ceased laying eggs.

Larvae were reared in insect rearing trays divided in cells (3 x 4cm). A cube (2x2x2cm) of artificial diet was placed in each cell together with a neonate, randomly selected from the colony, and sealed with a plastic tray sealer. The cells were examined daily and the head capsules, shed in each ecdysis, were collected and preserved individually. Voucher specimens of the head capsules collected have been deposited in the collection of the Laboratory of Applied Zoology and Parasitology, Faculty of Agriculture of the Aristotle University of Thessaloniki.

Upon pupation, the sex was determined and marked. Larvae were maintained at 26±1°C, and a photoperiod of 16:8 (L:D)h. They were weighed 3d after each ecdysis.

Statistics. Measurements of larval head capsule width and body weight, and the pupal weight and length, were analysed using STATISTICA (StatSoft Inc 2001). The descriptive statistics, for larval head capsule width (in mm) and larval weight (in mg), were computed throughout all larval instars (L_1-L_6), for the mean value, the standard error of mean (SEM), the standard deviation, the minimum/maximum values. The distributions of these variables for all larval instars were computed, and the boundaries of adjacent instar curves were found, using minimum and maximum values or the combination of coupled values for head capsule width and body weight. Head capsule width and body weight numerical data were transformed into logarithmic scale for achieving normality. An analysis of variance (one-way ANOVA) carried out, with factor the instars and dependent variables the transformed data of head capsule width and body weight, using SPSS statistical package (SPSS Inc. 2003). Regression analysis was made using Minitab (Minitab Inc 2000), in order to find the dependence of the number of instars on the variables head capsule width and body weight. The same analysis was carried out between duration as the dependent variable and head capsule width and body weight as independent variables.

Results and Discussion

Our data show the existence of 5 or 6 instars on the artificial diet (Table 1). Analysis of variance showed significant differences between all instars for both variables head capsule width and body weight (Table 1). In laboratory, about 75% of *H. armigera* larvae completed their development in 5 instars, 24% in 6 instars and 1 % in 7 instars. The L_2/L_1 inter-instar ratio was slightly lower than the L_3/L_2 and L_5/L_4 and even lower than that of L_4/L_3. The L_4/L_3 was the highest ratio, when 5 instars were completed, while L_6/L_5 and L_5/L_4 inter instar ratios were lower than L_3/L_2 and still lower than that of L_2/L_1, when 6 instars were completed. The L_4/L_3 ratio was also the highest one. The average ratios of 1.78 (L_5) or 1.59 (L_6), are higher than those commonly found (1.40) in other insects (Chapman 1969).

To determine whether a larva falls within a certain instar, both head capsule width and body weight measurements from all larvae (whether they completed their development in 5 or 6 instars) were used and comparisons were made in accordance to the distributions found (Figs.1 and 2).

Several authors (Hardwick, 1965; Kirkpatrick, 1961; Twine, 1978) have reported considerable differences in head capsule width of cotton bollworm larvae. This variation might be because of differences in the ecological conditions such as temperature, diet, humidity, and crowding under which the larvae developed. Muggli and Miller (1980) demonstrated that instar determination on the basis of head capsule measurement might differ with different population densities. We found (Table 1) that the larvae of the first two instars have a lower individual variation of capsule width than those of any other instar. These instar larvae are more homogenous because they are exposed to external ecological conditions for a shorter period of time mainly due to their short developmental time. The first instar larvae reported 13 times increase in their body weight, which was followed by the 2nd, 3rd, 4th and 6th instar. No significant differences were found between males and females.

From the correlation analysis (Petridis 2000) it was found that the number of instars is highly correlated with the head capsule width (R^2=0.954, P=0.001) or with body weight (R^2=0.932, P=0.001) or considering both (R^2=0.969, P=0.001). The respective regression equations were found to be:

$Y = 3{,}29478 + 4{,}25855*X_1$

$Y = 1{,}40874 + 1{,}53322*X_2$

$Y = 2{,}55 + 2{,}67*X_1 + 0{,}603*X_2$

Where Y is the instar, X_1 is the head capsule width (in mm) and X_2 the body weight (in mg)

Another important larval feature, the larval age in days, was highly correlated with the head capsule width (R^2=0.695, P=0.001) or with body weight (R^2=0.729, P=0.001) or considering both (R^2=0.739, P=0.001) (Fig. 3). The respective regression equations were found to be:

$Y = 9{,}91 + 11{,}8*X_1$

$Y = 4{,}53 + 4{,}42*X_2$

$Y = 6{,}33 + 4{,}21*X_1 + 2{,}96*X_2$

Where Y is the larval age in days, X_1 is the head capsule width (in mm) and X_2 the body weight (in mg).

With the above equations larval age, could be predicted when both head capsule width and body weight are known.

The applicability of Dyar's (1890) rule, stating that the width of head capsule of lepidopterous larva is more or less constant for any instar of a given species, was tested. Also, the successive larval instars of a given species show more or less regular geometrical progression in the growth of head capsule. However, Dyar's hypothesis applies in some cases (Fox et al. 1972), but not in others (Jobin et al. 1992). Dyar's hypothesis that proposes a constant growth ratio

TABLE 1. Head capsule width (in mm) of *H. armigera* instars and body weight (in mg).

Instar	n	Mean ± SEM	Range	Interinstar Ratio (Dyar's constant)	Instar	Mean ± SEM	Range	Interinstar Ratio (Dyar's constant)
		Head capsule (mm)				Head capsule (mm)		
L1	187	0.298 ± 0.004a	0.2 - 0.4		L1	0.301 ± 0.002a	0.2 - 0.4	
L2	187	0.495 ± 0.007b	0.4 - 0.7	1.66	L2	0.511 ± 0.004b	0.4 - 0.7	1.70
L3	187	0.818 ± 0.015c	0.6 - 1.2	1.65	L3	0.888 ± 0.012c	0.5 - 1.3	1.74
L4	180	1.424 ± 0.055d	0.9 - 3.3	1.74	L4	1.719 ± 0.032d	1.1 - 3.1	1.94
L5	189	2.071 ± 0.062e	1.4 - 3.5	1.45	L5	2.954 ± 0.033e	1.6 - 4.0	1.72
L6	56	2.992 ± 0.044f	1.8 - 4.5	1.45				
		Larval weight (mg)				Larval weight (mg)		
L1	183	0.721 ± 0.049a	0.3 - 1.1		L1	0.737 ± 0.048a	0.3 - 1.1	
L2	183	1.871 ± 0.111b	0.5 - 4.5	2.60	L2	2.864 ± 0.088b	0.5 - 5.5	3.89
L3	185	9.650 ± 0.569c	2 - 27	5.16	L3	16.347 ± 0.613c	4 - 34	5.71
L4	186	32.001 ± 2.135d	4 - 68	3.32	L4	68.896 ± 3.493d	10 - 163	4.22
L5	185	131.492 ± 5.837e	20 - 216	4.11	L5	356.224 ± 9.097f	127 - 562	5.17
L6	49	377.312 ± 17.097f	103 - 665	2.87				

Means in each column followed by the same letter are not significantly different at $p = 0.05$ according to Student-Newman-Keuls test after ANOVA.

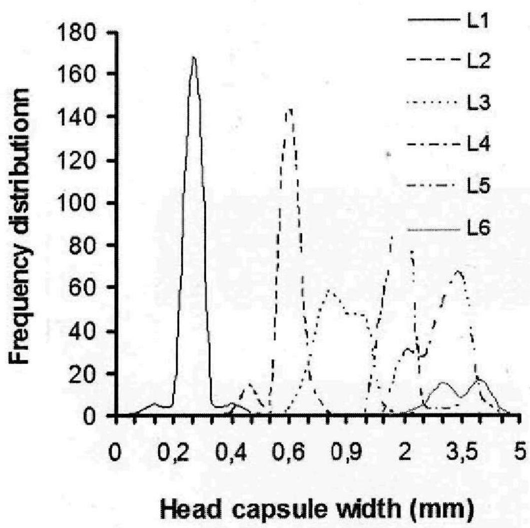

FIG. 1. Larval head capsule width in mm, for L_1-L_6 larval instars.

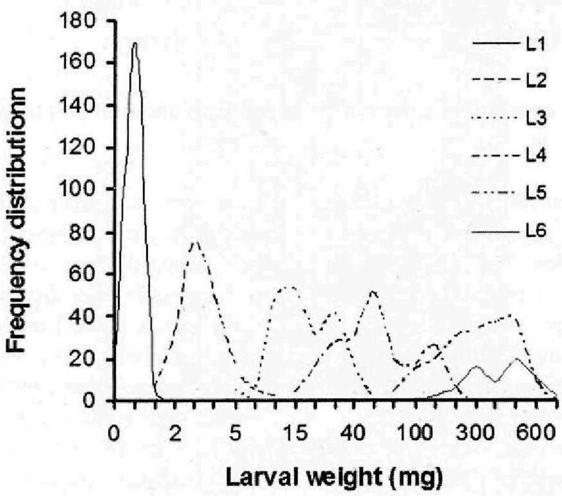

FIG. 2. Larval body weight in mg, for L_1-L_6 larval instars.

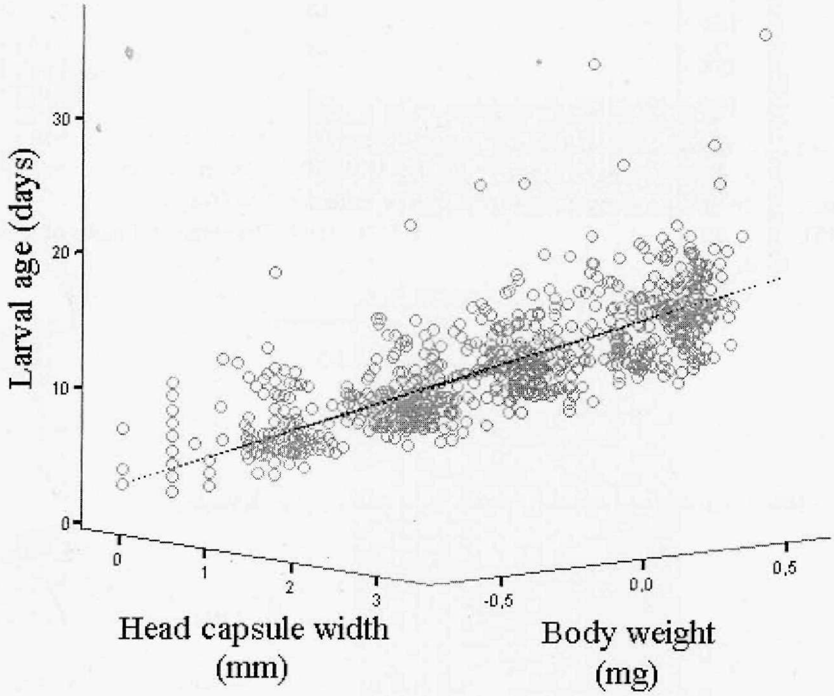

FIG. 3. Relationship of *H. armigera* larval age with the head capsule width and body weight

throughout larval development in Lepidoptera disagrees with our results for last instar both for those completed 5 or 6 instars. A decrease in growth ratio observed in the last instar might indicate a physiological stress in the population. The average ratio in the present study is beyond the range reported by Dyar (1890) for Lepidoptera when 5 instars are taken into consideration. When the sixth instar included in the analysis, the ratio falls within the range reported for Lepidoptera in relevant studies (Dyar 1890, Daly 1985). Geographic variation, alternating temperatures and food type may influence the morphometrics of larvae of the same instar. Laboratory data may differ from that in the field. A departure from Dyars' rule is common for populations suffering greater mortality than usual under field conditions.

Hardwick (1965) reared larvae of *H. armigera* on shelled peas in the laboratory at a constant temperature of 25 °C and found that 30% of the larvae completed their development in 5 instars, 69% in 6, and 1% in 7 instars. Hardwick showed that under the same conditions, the number of larval instars in other species of *Heliothis* also varied from 5 to 7, but the percentages of larvae completing development with 5, 6 or 7 instars varied for each species. Poitout and Cayrol (1969) found that the number of larval instars varied, although there were generally 5 or 6 instars, occasionally there were 7 to 9. They attributed this variation to differences in the vigor of the culture used in each study. Crowding in early larval instars was found to cause supernumerary instars in *Leucania separata*, and it was induced possibly to compensate for reduced size due to food deficiency (Iwao 1962). Furthermore, it was shown in *Spodoptera litura* (F.), that overcrowding in the parental larval stages acts to induce extra moults in the progeny (Yamanaka et al. 1975).

Nijhout (1975) first demonstrated that in *Manduca sexta* (L.), in which the individuals with head capsules wider than a defined threshold size proceed to pupate at the following moult whereas those with smaller capsules undergo further larval moult. It seems that *H. armigera* larva has a control mechanism that can 'measure' its own absolute weight and/or head capsule width so as individuals with weight lower than 105 mg and head capsule narrower than 1.7 mm undergo further larval growth instead to pupate. These critical values were defined on the basis of laboratory observation data of individuals (summarized in tables 1 and 2), meaning that over these values L_5 larvae always pupate and in opposite, under these values, L_6 larvae are formed instead to pupate. Morita and Tojo (1984) elucidated that in *Spodoptera* larvae the penultimate instar can be recognized by their head capsules being wider than 1.65 mm. This may suggest that strains exist having a different tendency for supernumerary ecdysis.

In summary, our results suggest that instar determination of *H. armigera* is possible combining measurements on head capsule width and larval weight. However, as this species is capable of responding to environmental conditions by adjusting its number of molts, a detailed field study in different geographic locations is needed to further understand this insect's biology.

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KEY WORDS: *Helicoverpa armigera*, head capsule width, body weight, larval instars

Προσδιορισμός των προνυμφικών σταδίων και της ηλικίας προνυμφών του *Helicoverpa armigera* (Lepidoptera: Noctuidae) με βάση το πλάτος της κεφαλικής κάψας και το βάρος τους

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ΠΕΡΙΛΗΨΗ

Προνύμφες του *Helicoverpa armigera* (Lepidoptera: Noctuidae) εκτράφηκαν σε χώρο με 26°C, φωτόφαση 16 ωρών και σχετική υγρασία 60-75%. Μετρήθηκαν, ανά προνυμφικό στάδιο, το πλάτος της κεφαλικής κάψας και το βάρος κάθε προνύμφης 2-3 ημέρες μετά την έκδυση, με σκοπό τον προσδιορισμό του προνυμφικού σταδίου και της ηλικίας. Χρησιμοποιήθηκε επί πλέον το βάρος της προνύμφης γιατί δεν είναι ασφαλής ο προσδιορισμός των προνυμφικών σταδίων μόνο από το πλάτος της κεφαλικής κάψας, καθόσον υπάρχει αλληλεπικάλυψη μεταξύ πλάτους ενός σταδίου και του προηγούμενου και επόμενού του, και ακόμη παρατηρείται διαφορετικός αριθμός σταδίων μεταξύ των προνυμφών. Η ανάπτυξη συμπληρώνεται σε 5 προνυμφικά στάδια στο 75% των προνυμφών, σε 6 στο 24% και σε 7 στο1%. Το πλάτος της κεφαλικής κάψας ήταν αρκετό για τη διάκριση των προνυμφών της 1ου σταδίου μόνο και το μέγιστο πλάτος ήταν 0,4mm. Το βάρος της προνύμφης ήταν ικανό μόνο του να προσδιορίσει προνύμφες 1ης και 2ης ηλικίας. Η διαφορά μεταξύ 1ου και 2ου σταδίου ήταν 1mg και μεταξύ 2ου και 3ου σταδίου 5,5mg. Συσχέτιση και ανάλυση παραλλακτικότητας των δύο παραμέτρων μας δίνει τη δυνατότητα προσδιορισμού όλων των προνυμφικών σταδίων μιας προνύμφης με πιθανότητα 96,9%,.καθώς και την ηλικία της σε ημέρες από την εκκόλαψη.