Characterization of *Hellula phidilealis* (Lepidoptera: Pyralidae) larval instars by application of the Dyar Rule

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The genus *Hellula* Guenée (Lepidoptera: Crambidae) spp. has acquired importance because of the damage it causes to crucifer crops. In India, for example, in the production of cauliflower, farmers apply insecticides 10 to 12 times per season to prevent damage from pests and diseases. Most of these applications are done to combat *Hellula undalis* (Fabricius) (Lepidoptera: Crambidae), which is prevalent especially during the wet season (Ahuja et al. 2012). According to Boopathi and Pathak (2012), *H. undalis* causes 5 to 10% of the damage in the vegetative and floret-forming stages of broccoli. Integrated Pest Management (IPM) requires knowledge of the pest's biology and ecology. Here we set out to determine the number of instars present in this species. The Dyar Rule establishes that growth in width of a larval head follows a regular geometric progression in its successive stages (Dyar 1890). In this way, the number of instars of lepidopterans can be estimated, as in the case of *Hellula phidilealis* (Walker) (Lepidoptera: Crambidae), by collecting all of the larval head capsules resulting from rearing, measuring them, and applying the Dyar Rule. The objective of this study was to determine the number of larval instars of *H. phidilealis* by measuring the width of the cephalic capsule, which is essential to determining the number of instars of this species, to design appropriate tactics for its control.

Hellula phidilealis larvae were collected in Atlixco, Puebla, Mexico (18.866647°N, 98.427983°W; 1,760 masl). The specimens were kept at 24 °C ± 2 °C, 55 ± 5% relative humidity, and a 12:12 h (L:D) photoperiod, and fed cabbage (*Brassica oleracea* var. *capitata* L. [Brassicaceae]). A total of 215 larvae of different instars were obtained from the third generation. A microscope (Carl Zeiss model SteREO Discovery V.20®, Jena, Germany) with a mounted Canon (model EOS 50D®, Ohta-ku, Tokyo, Japan) camera was used. Images in JPEG format were taken for later measurement with the Program Image J (v. 1.53e) (Rueden et al. 2017), and the external edges of the gena of each cephalic capsule was measured.

Measurements were then classified in simple frequency tables. To visualize an approximation of the groups of data of each instar, a frequency distribution graph was constructed with the measurements obtained. The data were subjected to a normality test by instar. In addition, a discriminant analysis was performed to separate the defined groups and reduce the likelihood of erroneous classifications. Each specimen analyzed was assigned a value of the weighted average of the values in the set of independent variables. After this value was determined, it was transformed to an a posteriori likelihood, which defines the probability that each of the specimens belongs to a certain group. The probabilities of overlapping were calculated to define the lower and upper limits of each group. The variables required for optimal separation of the groups were calculated with the assumption that distribution of cephalic capsule measurements for each instar is normal and that the distribution observed is the sum of individual normal distributions (Got 1988; Logan 1998). Finally, the Dyar rule was applied to obtain the average rate of increase and the expected values of the size of the cephalic capsule. The statistical analysis was carried out in R programming language, version 4.0.5 (R Core Team 2022).

As seen in Figure 1, when the different measurements of the studied specimens are graphed, 5 peaks are obtained, indicating a total of 5 instars, and that each has a normal distribution. By separating the sets, the class limits were obtained, and grouping the values in frequencies defined the class limits ($P = 0.05$). With a test of overlap (Logan 1998), the class limits in Table 1 were obtained. To verify the Dyar rule, the average rate of increase of the cephalic capsule size was calculated and, with that rate, the expected values for each instar. The change rate was 0.50, 0.58, 0.92, and 0.54 for instars L2, L3, L4, and L5, respectively, with an average of 0.63. Cadogan (1983), in an in vitro study, found that the larval period of *H. phidilealis* oscillated between 14 and 19 d (mean = 15.8), with 5 or 6 instars, coinciding with the findings of our study, although Cadogan did not indicate the technique used to determine each instar.

As Cadogan (1983) concludes, *H. phidilealis* possesses a reproductive potential that merits concern in the production of crucifers since, given favorable conditions, the species could become a serious pest for the species of this family. This study contributes to the knowledge of this species' ecology by stipulating the number of instars of this pest. This information can be useful in developing management tactics, because during later instars the caterpillars' feeding behavior changes from surface feeding to boring.

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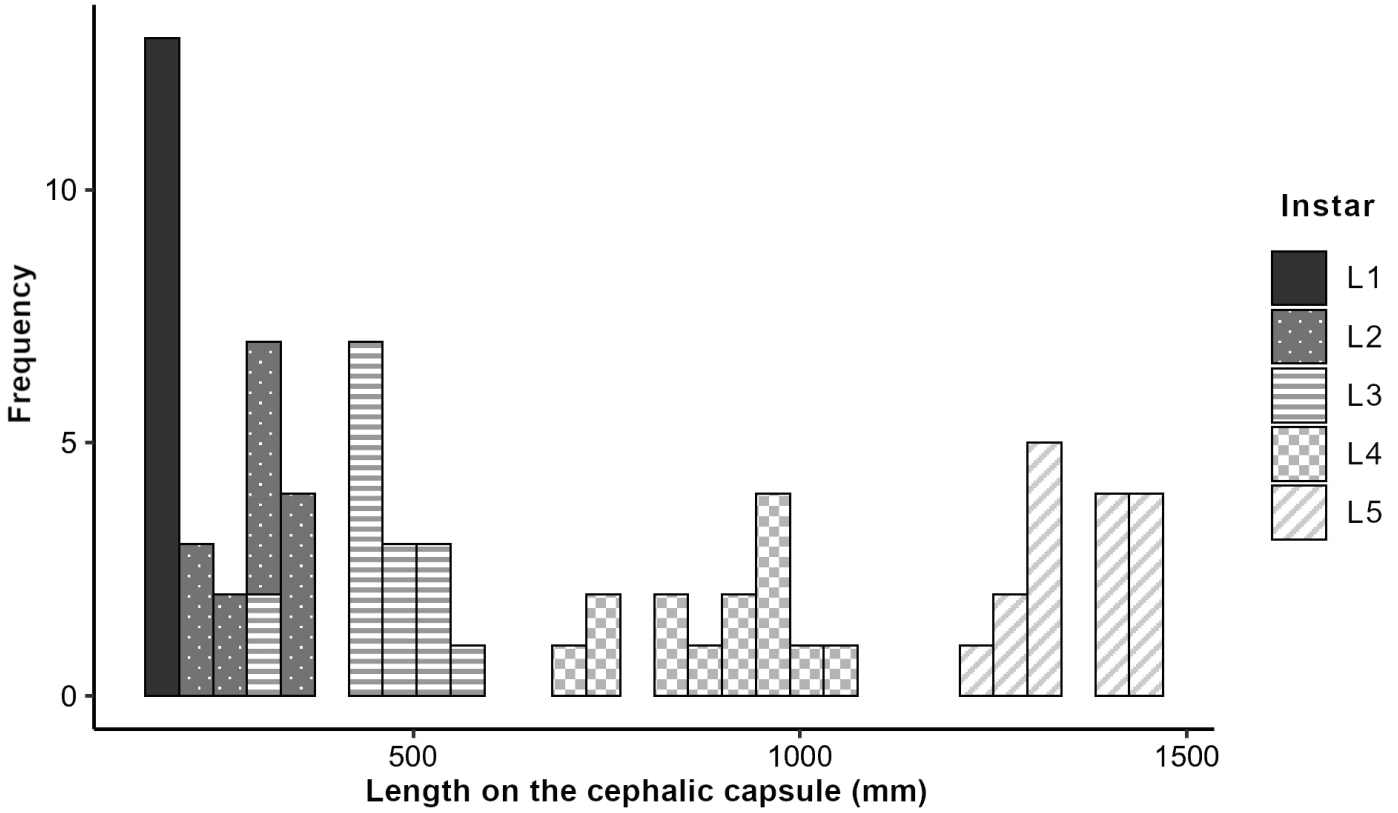


Fig. 1. Normal distribution of the cephalic capsule measurements of *Hellula phidilealis* larval instars.

Summary

Hellula phidilealis (Walker) (Lepidoptera: Pyralidae) is a species associated with cultivation of crucifers. The damage it produces depends on the phenological stage of the plants, its population density, and the instar in which it is found. For this reason, it is important to identify the instars of the species to contribute to defining management tactics focused on its control in hosts of economic importance. The Dyar rule establishes that the width of the cephalic capsule of the larvae increases at a regular geometric progression. The objective of this study was to determine the rates of increase in the size of the cephalic capsules to determine the number of larval instars of *H. phidilealis*. Because it is a species that has recently acquired importance in Mexico, it is necessary to conduct basic research to determine its biology to enable implementation of appropriate management tactics for each instar. To obtain the images, a Carl Zeiss® (SteREO Discovery V.20) microscope equipped with a Canon® (EOS 50D) camera was used, and JPEG images were captured for later measurement with the program Image J (v. 1.53e). The larval instars of *H. phidilealis* were adjusted to the Dyar rule (R^2 = 0.9639) with a change rate of 0.50, 0.58, 0.92, and 0.54 for instars L2, L3, L4, and L5, respectively; the average of the rates was 0.63.

Key Words: change rate, cephalic capsule, management in crucifers

Sumario

Hellula phidilealis (Walker) (Lepidoptera: Pyralidae) es una especie asociada con el cultivo de crucíferas, el daño que produce depende de la etapa fenológica de las plantas, la densidad poblacional y del ínstar en el que se encuentre. Por lo anterior, es de importancia identificar los ínstares de esta especie, lo que coadyuvará a definir las tácticas de manejo enfocadas para su control en hospedantes de importancia económica. La Ley de Dyar establece que la anchura de las cápsulas cefálica de las larvas, se incrementa de acuerdo con una progresión geométrica regular. El objetivo de este trabajo fue determinar las tasas de incremento en el tamaño de las cápsulas cefálicas para determinar el número de ínstares larvales de *H. phidilealis*. Al ser una especie que recientemente ha adquirido importancia en México, es necesario desarrollar investigación básica que permita conocer la biología de la plaga para implementar las tácticas de manejo pertinentes para cada ínstar. Para la obtención de imágenes, se utilizó un microscopio Carl Zeiss ® (SteREO Discovery V.20) acondicionado con una cámara Canon® (EOS 50D); se capturaron imágenes JPEG de cada espécimen para su poste-

Table 1. Values observed (mean of each interval) and expected (calculated with the Dyar Rule) for the width (µm) of *Hellula phidilealis* cephalic capsules.

Instar	# Specimens	Lower limit	Upper limit	Observed values	Standard deviation	Expected values
1	22	150	241	192	6.04	178
2	37	242	369	288	42.16	295
3	49	370	693	455	71.39	489
4	34	694	1,146	874	100	810
5	40	1,147	1,478	1,343	67.51	1,344

rior medición con el programa Image J (v. 1.53e). Los ínstares larvales de *H. phidilealis* se ajustaron a la Regla de Dyar (R^2 = 0.9639) con una tasa de cambio de 0.50, 0.58, 0.92, y 0.54 para los ínstares L2, L3, L4, y L5, respectivamente, el promedio de las tasas fue de 0.63.

Palabras Clave: tasa de cambio, capsula cefálica, manejo en crucíferas.

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