Head Capsule Width is Useful for Determining Larval Instar in Heilipus lauri (Coleoptera: Curculionidae)

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Head capsule width is useful for determining larval instar in *Heilipus lauri* (Coleoptera: Curculionidae)

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The big avocado seed weevil, *Heilipus lauri* Boheman (Coleoptera: Curculionidae), is a curculionid pest that feeds on seeds of avocado, *Persea americana* Mill. (Laurales: Lauraceae). It was described by Boheman (1845) from specimens collected in Mexico, but it has also been reported in the Mexican states of Puebla, Morelos, Veracruz, Hidalgo, Guerrero, and Estado de Mexico (Peña 1998; Castañeda-Vildózola et al. 2013) and Colombia (Caicedo et al. 2010).

Heilipus lauri is considered an important quarantine pest. As a consequence, the Mexican government has restricted movement into *H. lauri*–free areas of any avocado fruits for human consumption and any material for propagation that comes from regions infested with this pest. This strategy has helped to ensure that the main avocado production areas of Michoacan remain free from this pest (SAGARPA 2002).

In order to establish rational pest management strategies, it is essential to first determine the basic life cycle of this pest, including the number of larval instars. Technical information on larval instars is essential for mortality–survivorship studies based on life tables and for population modeling, each of which inform the development of pest management strategies (Gold et al. 1999). One method for establishing the number of larval instars involves measuring the width of the larval head capsule throughout development and applying Dyar's rule (Panzavolta 2007; Cazado et al. 2014). This method is based on the assumption that the size of the head capsule is consistent throughout a given instar but undergoes a regular geometric increase as the larva passes from one instar to the next (Dyar 1890).

Many papers have reported the accuracy of this method for various weevil species (e.g., Reardon et al. 2002; Pantoja et al. 2006; Panzavolta 2007; Cazado et al. 2014), and this accuracy has been greatly increased with the development of particular software programs (Logan et al. 1998; Merville et al. 2014). One such program is the Hcap program (Logan et al. 1998), which allows an accurate separation between each larval instar to be defined based on the frequency of the sizes of the head capsules measured. In addition, this program estimates the mean and standard deviation of head capsule measurements for each larval instar, and the probability for overlap in sizes between instars.

The Hcap program has been used successfully to determine the number of instars in several curculionid species, such as *Pissodes castaneus* De Greer, *Tomicus destruens* Wollaston, and *Rhyssomatus subtilis* Fielder (Panzavolta 2007; Sabbatini-Peverieri & Faggi 2005; Cazado et al. 2014). The objective of this study was to define the number of larval instars in *H. lauri* from a larval population reared under laboratory conditions by measuring the width of the head capsules and using the Hcap program as well as by following Dyar's rule.

Heilipus lauri was reared from adults that were collected in Apr 2005 from avocado trees ('Colin V-33') cultivated in a non-commercial orchard from the municipality of Ixtapan de la Sal, Estado de México (18.8245°N, 99.6639°W; 1,685 m asl). The weevil adults collected were maintained under laboratory conditions (26 ± 2 °C, 60–70% RH, and a 12:12 h L:D photoperiod) and separated by sex using the methods described by Castañeda-Vildózola et al. (2013). Seventeen male and female weevil pairs were individually placed inside plastic boxes (14.0 × 10.5 cm), each containing an avocado fruit (Colin V-33) as food and for oviposition; the fruit was replaced every 3 d.

Avocado fruits were examined daily for the presence of eggs. Oviposition sites were cut for extraction of eggs using a camel hair brush. Eggs were placed in a Petri dish (9.0 × 1.0 cm) lined with wet filter paper. All Petri dishes of eggs were maintained at 26 ± 2 °C, 60% RH, and a 12:12 h L:D photoperiod and examined every 12 h to detect larval emergence. One thousand emerging neonate larvae were introduced into avocado fruits (Colin V-33) between Jun and Aug 2005 (n = 500 fruits; weight = 218.4 g; length = 9.4 cm; width = 6.8 cm). Specifically, a hole was made (1.0 × 1.0 mm) at the tip of each fruit, into which larvae were introduced using a camel hair brush (2 larvae per fruit), and each fruit was labeled with the date of larval introduction. Larvae were removed from decaying avocado fruits and transferred into fresh avocado fruits as necessary.

To obtain specimens of all possible instars for head capsule measurement, 35 larvae (approximately 16 fruits) were destructively

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sampled after 1, 3, 5, 10, 15, 20, 25, 30, 35, 40, and 45 d of incubation. Sampled larvae were killed in hot water (96 °C) and stored in glass vials in 70% ethanol prior to further processing. Digital photos of the head capsules of larvae (n = 385) were taken using a PAXCM 3 camera adapted to a Tessovar Carl Zeiss microscope. Each head capsule was measured from the digital image using the method developed by Hernández-Livera et al. (2005). Data on head capsule width were analyzed using the Hcap program (Logan et al. 1998). Dyar's constant was obtained for each larval instar by taking the mean head capsule width for that instar and dividing it by the mean head capsule width of the previous instar. The precision of Dyar's rule was tested using linear regression by fitting the number of larval instars obtained and the natural logarithm of the mean head capsule widths for each instar (Gaines & Campbell 1935). The significance of the slope of the regression line was tested with 1-way ANOVA using InfoStat software (2008).

The frequency distribution analysis of the head capsule widths of *H. lauri* larvae produced 4 peaks suggesting the existence of 4 larval instars (Fig. 1). The larval head capsule widths ranged from 512 to 2,088 µm (about 0.5–2.1 mm), with significant differences between each larval instar (Table 1). Allocation of all head capsule measurements to each of the 4 categories found was done with a maximum error probability of 1.8%.

The mean Dyar's constant for all instars was 1.42, where the relationship between the natural logarithm of the head capsule width and the number of larval instars provided a perfect geometric growth for each larval instar (Table 1; Fig. 2). Similar results have been obtained for other curculionids such as *Conotrachelus psidii* Marshall, *Cosmopolites sordidus* (German), *P. castaneus*, and *R. subtilis* (Bailez et al. 2003; Pantoja et al. 2006; Panzavolta 2007; Cazado et al. 2014).

The linear regression equation for the head capsule width versus larval instar was highly significant ($P \leq 0.0001$, $R = 0.992$, $R^2 = 0.988$) (Fig. 2). The excellent fit of the linear model suggests that there was nearly no overlap in the width of the head capsules among the different instars. The significant difference observed between the head capsule width for each larval instar (F = 3,900; df = 3,380; $P \leq 0.0001$) supports the results that there are only 4 larval instars for this species.

Results from the present study are different from those of García-Arellano (1962), who reported 5 larval instars for *H. lauri*. We believe this difference may be because a mathematical model to estimate larval instars more accurately was unavailable to García-Arellano (1962). Alternatively, the difference may be because the origin of the larvae used by García-Arellano (1962) was different to the origin of our larvae. In his study, García-Arellano (1962) collected larvae directly from the field, from different cultivars and orchards where they had been exposed to different temperatures, RH, and photoperiod. The quality and quantity of food resources are known to contribute to intraspecific variability in the number of larval instars in insects (Esperk et al. 2007), which may account for the difference between the 2 studies. Studies on other curculionids such as *Rhynchophorus ferrugineus* Olivier (Jaya et al. 2000), *C. sordidus* (Gold et al. 1999), and *Scyphophorus acupunctatus* Gyllenhal (Valdés-Estrada et al. 2010) have also reported intraspecific variability in the number of larval instars in response to variation in nutrition.

In contrast, our results are in agreement with reports on other species in the subfamily Molytinae that are closely related to *Heilipus* species and all have 4 larval instars (Kishi 1971; Bodenham et al. 1976; Raccete et al. 1992; Cerezke 1994; Leather et al. 1999; Bailez et al. 2003; Panzavolta 2007; Williams & Langor 2011; Cazado et al. 2014; Payán-Arzapalo et al. 2015). These include *Conotrachelus nenuphar* (Herbst), *Conotrachelus neomexicanus* Fall, *Conotrachelus perseae* Barber, *C. psidii*, *Hylobius abies* L., *Hylobius warreni* Wood, *P. castaneus*, *Pissodes nitidus* Roelofs, *Pissodes punctatus* Langor and Zhang, *Pissodes yunnanensis* Langor and Zhang, and *R. subtilis.* This commonality suggests that having 4 larval instars is a characteristic of the subfamily Molytinae.

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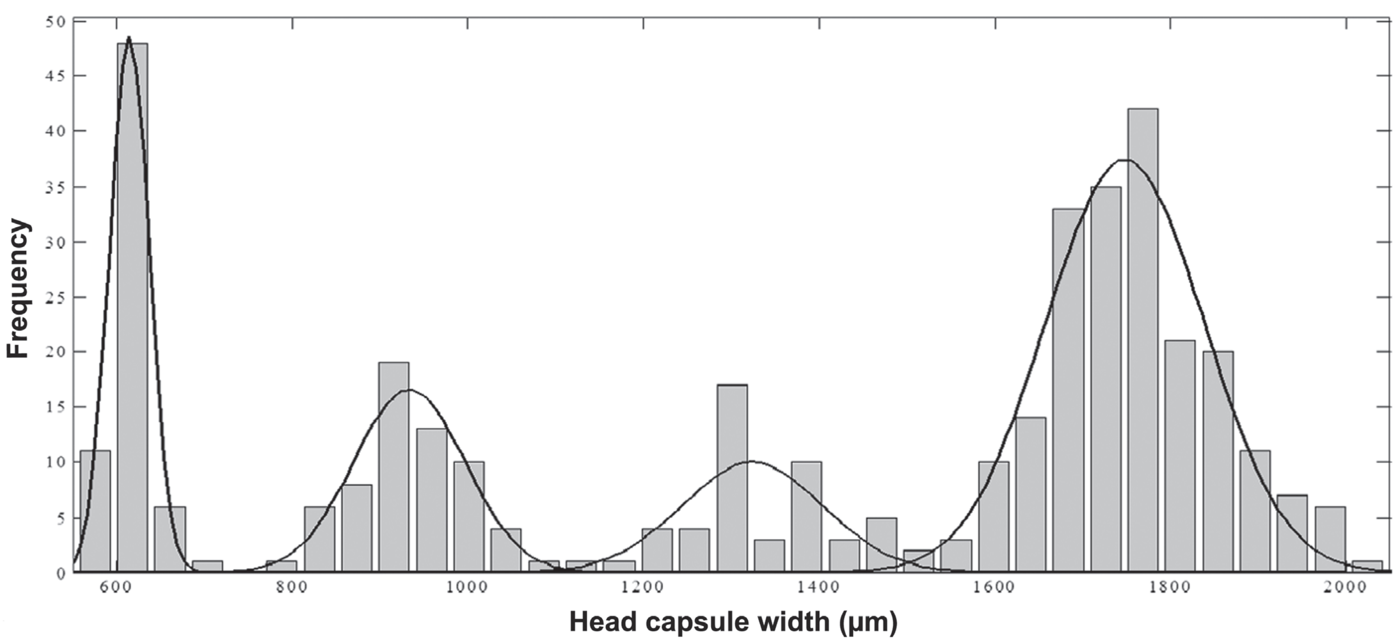

Fig. 1. Frequency distribution of the head capsule widths (µm) of *Heilipus lauri* larvae. Graph generated by the Hcap program.

Table 1. Means, range, and probability of misclassification for the measurements (width) of head capsules of the larval instars of *Heilipus lauri* calculated using the Hcap program.

				Probability of misclassification			
Instar	*n*	Mean ± SD (µm)	Range (µm)	*i* as *i* – 1	*i* as *i* + 1	Total	Dyar's ratio
1	66	615 ± 22.6	512–703	0.000000	0.000046	0.000046	—
2	62	934 ± 63.5	703–1,111	0.000145	0.002544	0.002689	1.52
3	48	1,324 ± 81.2	1,111–1,502	0.004473	0.014206	0.018679	1.42
4	205	1,748 ± 90.1	1,502–2,088	0.003169	0.000000	0.003169	1.32

Summary

The purpose of this study was to determine the number of larval instars for *Heilipus lauri* Boheman (Coleoptera: Curculionidae). We reared 385 larvae under laboratory conditions and measured the width of the larval head capsules from digital images. Data were analyzed using the Hcap program and Dyar's rule. Analysis of the frequency distribution showed the existence of 4 distinct peaks. The mean Dyar's constant was 1.42, where the relationship between the natural logarithm of the head capsule widths and the number of larval instars resulted in a near-perfect geometric growth for each instar. The excellent fit of the data to a linear model suggests that there was nearly no overlap among the 4 instars in the head capsule measurements, making it possible to distinguish the 4 instars of *H. lauri* accurately.

Key Words: weevil; avocado pest; Molytinae; Dyar's rule

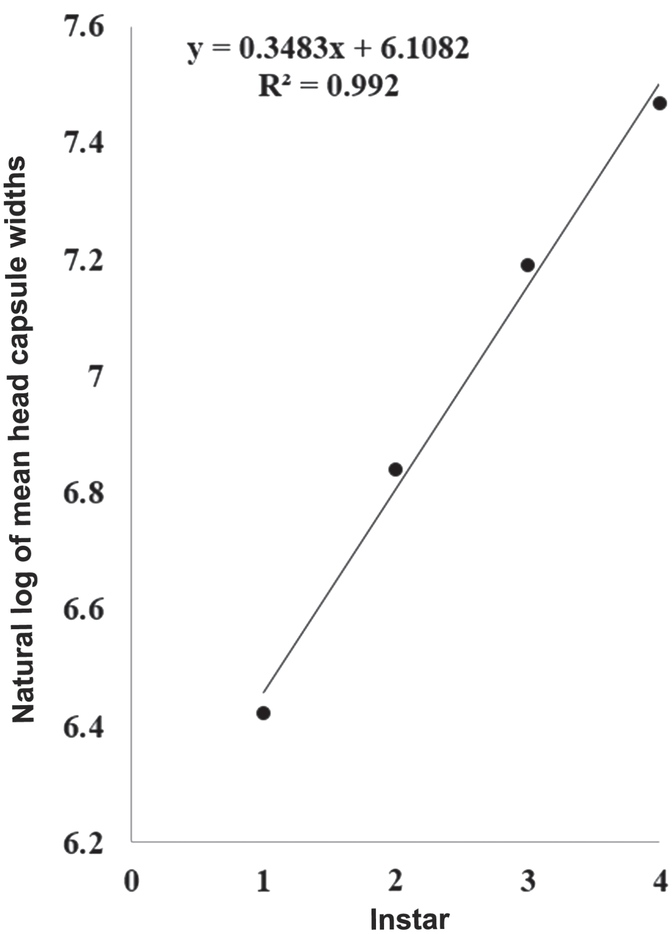

Fig. 2. Linear regression relationship between the natural logarithm of the mean larval head capsule width and the instars of *Heilipus lauri* larvae.

Sumario

El objetivo de este estudio fue determinar el número de estadios larvarios de *Heilipus lauri* Boheman (Coleoptera: Curculionidae). Un total de 385 larvas fueron criadas en condiciones de laboratorio. El ancho de la capsula cefálica de cada larva fue medido por medio de un sistema computarizado de análisis de imágenes. Los datos fueron analizados con el programa HCap y la regla de Dyar. El gráfico de distribución de frecuencias mostró cuatro picos de distribución del ancho de la cápsula cefálica. El promedio de la constante de Dyar fue de 1.42 donde la relación entre el logaritmo natural del ancho de la cápsula cefálica y el número de estadio larvario resultó en un perfecto crecimiento geométrico de cada estadio larvario. El excelente ajuste lineal de las datos sugiere que no existen instares traslapados y es posible distinguir cuatro estadios larvarios en *H. lauri.*

Palabras Clave: picudos; plagas del aguacate; Molytinae; regla de Dyar

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