SCIENTIFIC PAPER

Plodia interpunctella (Hübner) (LEPIDOPTERA: PYRALIDAE) LIFE CYCLE ON STORED WALNUTS UNDER CONTROLLED ENVIRONMENTAL CONDITIONS.

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Folia Entomológica Mexicana (*nueva serie*), *3*(2): 15–22, 2017.

Recibido: 24 de febrero 2017
Aceptado: 14 de julio 2017
Publicado en línea: 31 de agosto 2017

ISSN: 2448-4768

Plodia interpunctella (Hübner) (LEPIDOPTERA: PYRALIDAE) LIFE CYCLE ON STORED WALNUTS UNDER CONTROLLED ENVIRONMENTAL CONDITIONS

Ciclo de vida de *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) en nueces almacenadas bajo condiciones ambientales controladas

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ABSTRACT. *Plodia interpunctella*, known as Indian flour moth, is considered one of the most relevant pests of stored products worldwide. Infestation by *P. interpunctella* is one of the main causes of decrease in the production of walnuts in La Rioja, Argentina. Many investigations have shown that the biological cycle of this species depends on its diet and on environmental conditions. The study of the life cycle of *P. interpunctella* on stored walnuts was conducted under laboratory conditions at temperature values of 24 ± 1 ºC and average relative humidity of 56 ± 11 %. Eggs were collected daily and separated by batch. The presence of the head capsule exuvia was taken into account to determine the duration of the larval instars. Six larval instars were determined according to the exuvia found. The relationship between the length of larvae and the cephalic width was established by using the correlation coefficient, a key element in the instar characterization and for future tests for pest control. The duration of the complete life cycle of *P. interpunctella* fed on walnuts was of 71.56 days on average, from egg hatching until the death of the adult. The manipulation of larvae during the study did not affect significantly the duration of the cycle. Data obtained offer valuable information for pest management strategies for stored walnuts.

Key words: Larvae, instar, cephalic capsule, pest.

RESUMEN. *Plodia interpunctella*, conocida como polilla india de la harina, es considerada una de las plagas de productos almacenados de mayor relevancia a nivel mundial. La infestación por este lepidóptero es una de las principales causas que disminuyen la producción de nueces en La Rioja, Argentina. Muchas investigaciones han demostrado que el ciclo biológico de esta especie depende de su dieta y de las condiciones ambientales. El estudio del ciclo de *P. interpunctella* en nueces almacenadas se llevó a cabo en condiciones de laboratorio a valores de temperatura de 24 ± 1 ºC y humedad relativa ambiente de 56 ± 11 %. Se recogieron diariamente los huevos y se separaron por lotes. Se tuvo en cuenta la presencia de la exuvia de la cápsula cefálica para determinar la duración de los estadios larvales. Se determinaron seis estadios larvales de acuerdo con las exuvias encontradas. Se estableció la relación entre la longitud de la larva y el ancho cefálico utilizando el coeficiente de correlación, un elemento clave en la caracterización del estadio. La duración del ciclo biológico completo de *P. interpunctella* alimentada con nueces fue de 71.56 días en promedio, desde la eclosión de los huevos hasta la muerte del adulto. La manipulación de las larvas durante el estudio no afectó significativamente la duración del ciclo. Los datos obtenidos ofrecen información valiosa para las estrategias de control de plagas en el almacenaje de nueces.

Palabras clave: Larvas, estadio, cápsula cefálica, plaga.

INTRODUCTION

Plodia interpunctella (Hübner, 1813), also known as Indian flour moth, is considered as one of the most relevant pests of stored products worldwide. It was listed as a pest of different types of stored food in 1831 by Hamlin (Mohandass *et al*., 2007). A large variety of works on the developmental biology of *P. interpunctella* in different types of stored products have been published; products such as cereals (LeCato, 1976; Madrid and Sinha, 1983; Mbata, 1990), walnuts (Johnson *et al*., 1992), and garlic seeds (Perez-Mendoza and Aguilera-Peña, 2004); it has also been reported on dried fruits and vegetables (Cox and Bell, 1991; Na and Ryoo, 2000, respectively); peanuts (Mbata, 1987); sorghum and wheat (Allotey and Goswami, 1990) as well

as processed foods (Simmons and Nelson, 1975) among many others.

Walnut cultivation in Argentina encompasses around 16,834 ha from which a production of about 16,840 tons is obtained distributed in the Andean provinces of Catamarca, La Rioja, Mendoza, San Juan and Río Negro (Cólica, 2014). In La Rioja, the third province in production and cultivated area, it is one of the main sources of economical resources and the performance of the production depends, among many factors, on the phytosanitary situation. Infestation by *P. interpunctella* is one of the main causes of decrease in the production of walnuts. *P. interpunctella* larvae feed on the food surface and are responsible for the damage caused, and consequentially, the decrease in quality of the infested products. This takes place when most of the food is covered with silk produced by larvae on which feces, exuviae, head capsules and eggs accumulate. The Indian flour moth adults do not feed but are capable of producing a large number of descendants (Vukajlović and Pešić, 2012).

Many of the biological aspects of *P. interpunctella* such as life cycle, survival and fecundity are influenced by the type of food. The species shows differences concerning the development and number of larval instars in studies carried out on chickpea (Pérez *et al*., 2012), and stored garlic seeds (Pérez-Mendoza and Aguilera-Peña, 2004). Up to this moment, the larval instars of *P. interpunctella* using walnuts as host have not been determined.

Plodia interpunctella presents high fecundity values when the larvae feed on walnuts, almonds and wheat bran (Johnson *et al.*, 1992), in contrast to the values found in wheat and sorghum (Allotey and Goswami, 1990). The percentage of egg hatch is another biological parameter which varies with the type of diet used to feed larvae (Mohandass *et al*., 2007). With pistachios and almonds the egg hatch percentage was of 88% and 96% respectively (Johnson *et al*., 1992); whereas Allotey and Goswami (1990) proved that egg hatch in different stored products (peanut, corn, sorghum, wheat) could exceed 98.6%.

It has also been proven that temperature directly influences the duration of the biological cycle. It decreases significantly when temperature rises (Johnson *et al*., 1992; Na and Ryoo, 2000).

In summary, the duration of the complete life cycle of the species is directly conditioned by the type of diet during the larval stage (LeCato, 1976; Cline and Highland, 1985), by temperature (Johnson *et al*., 1992; Na and Ryoo, 2000) and other physical, chemical and biochemical factors (Na and Ryoo, 2000). Therefore, any model used to describe its complete development must take these aspects into consideration.

The aim of this paper was to study the life cycle of *P. interpunctella* raised in controlled laboratory conditions and fed on walnuts, as a base for the implementation of plague control strategies in this region.

MATERIALS AND METHOD

The study of *P. interpunctella* complete cycle was developed at the Universidad Nacional de Chilecito's High Complexity Laboratory, in La Rioja, Argentina. It was conducted from February to April 2016, under controlled laboratory conditions. In order to do so, a rearing chamber with temperature values of 24 ± 1 ºC and average relative humidity of 56 ± 11% was used. The walnuts (*Juglans regia*) infested by *P. interpunctella* used were provided by small and medium-sized producers of the Chilecito and Famatina departments, in La Rioja, Argentina.

Couples of adult *P. interpunctella* were separated and placed in test tubes with small quantities of walnuts to encourage copulation and oviposition (Mohandass *et al*., 2007); 24 hours later egg recollection began. Batches of 10-20 eggs were placed on Petri dishes (100 x 15 mm^2) and were controlled daily until their hatching. 168 eggs were obtained after 5 days. Following eclosion, each larva was separated and fed on walnuts. They were kept on rearing chambers and observed on a daily basis until the pupation time. The number of larval instars was established taking into consideration the emergence of the exuviae in the head capsule. After each molt, individuals were extracted for measurement; for that purpose, they were immobilized by means of a cold shock or by infusing them in alcohol 70%

for 5 minutes. The cephalic width and length of larvae were determined under stereoscopic microscope Leica mlz10F (microsystems) applying the software Suite V. 3.6.0 (Build: 488) and photographs were taken with a Leica DFC310 fx camera.

The larvae which reached the last instar were placed in a 500 ml beaker. After pupation, each individual was separated, measured, sexed and placed in couples in test tubes with walnuts as food. They were observed daily until the emergence of adults. The sex of each individual was determined through the observation of the genital orifice (Butt and Cantu, 1962).

The number of eggs laid by female was quantified to measure fecundity. In order to do that, ten couples of adults were selected and located in 200 ml jars. Food, which was renewed every 24 hours, was placed in each container to stimulate oviposition. This was observed periodically in search for eggs; they were separated and quantified until the female died. The duration of the adult stage was registered.

The duration of the full handling-free cycle was determined by placing adult couples in test tubes in identical conditions to the above-described. Following oviposition, the dead adults were removed to prevent the larvae from eating them. Once they reached the pupal stage, the individuals were sexed, separated in couples and placed in test tubes with walnuts as food until the emergence of adults.

The program Infostat 2016 was used for the statistical study (Di Rienzo *et al*., 2016).

RESULTS AND DISCUSSION

The duration of the life cycle of *P. interpunctella* fed on walnuts was of 71.56 days on average, since eggs hatch until the death of the adult.

This study was carried out during the summer months due to the larval stage protraction observed during the low temperature seasons. This decrease in the metabolic activity coincides with the description of the diapause stage reported for this species and attributed by other researchers to the sudden temperature decrease and/or the shortening of the photoperiod (Tzanakakis, 1959; Mohandass *et al*., 2007).

The eggs of *P. interpunctella* are transparent at the moment of oviposition and turn white with time. They are oval and measure between 0.33-0.48 mm long and 0.27-0.30 mm wide. The incubation period was 4-5 days with an average of 4.20 days. This time is slightly superior to that found on chickpeas by Pérez *et al*. (2012) and on corn by Arbogast (2007) at 30 ºC and 75% of humidity. The hatch percentage was 88.60%, slightly inferior to the value obtained by Johnson *et al*. (1992) on walnuts at 25 ºC and 50 ± 5% HR. The differences found could be attributed to the type of diet and/or the environmental conditions.

The larval stage lasted 52 days, with six larval instars (Table I). However, other authors hold that the larval development undergoes five larval instars in chickpeas (Pérez *et al*., 2012) or in garlic seeds (Perez-Mendoza and Aguilera-Peña, 2004); or that they vary between five and seven instars (Vukajlović and Pešić, 2012).

Table 1: Mean larval length and cephalic width of six larval instars of *P. interpunctella* reared on walnuts.

	Cephalic width (mm)	Larval length (mm)
Instars	Mean ± S. D.	Mean ± S. D.
1	0.17 ± 0.01	1.02 ± 0.10
2	0.21 ± 0.02	1.75 ± 0.10
3	0.35 ± 0.02	2.68 ± 0.25
4	0.49 ± 0.05	3.74 ± 0.16
5	0.58 ± 0.02	5.44 ± 0.49
6	0.83 ± 0.02	9.05 ± 2.26*

*The standard deviation of the sixth instar shows the significant increase in size at the end of the larval stage.

First instar larvae measure around 1.02 mm and move energetically looking for food; behavior which coincides with the observations done by Sedlacek (1996). It was frequently observed that larvae resort to cannibalism of dead adults, especially in the absence of other food source. During this stage, larvae display a translucent white color, transparent setae, and a light brown head capsule which tends to become darker as time passes. This instar lasts, on average, 7.23 days (Fig. 1A).

It is observed that larvae continuously produce a silk wrapping inside, as well as on the surface of the product. If this is removed, larvae immediately begin the production of a new one. Such wrapping contains feces and exuviae of the head capsule, and apart from protecting, it would supply a suitable

microenvironment for the larval development. Approaching the end of the first instar, larvae exhibit a thickening in the last caudal segments together with a yellowish color which becomes more evident when reaching the second stage.

The second instar larvae have their body widened in the caudal region with an average length of 1.75 mm in length and 0.21 mm in cephalic width and exhibit transparent or white setae. In this period the thoracic shield can be seen for the first time, it has a slight light brown coloration which darkens and becomes more evident as time passes by (Fig. 1B).

The third instar larvae reach on average 2.68 mm in length and 0.35 mm in cephalic width. They show yellowish and elongated setae in the caudal end. They are white with a distinctive yellowish brown coloration from the last pair of prolegs to the caudal end. This coincides with a thickening of the body in the last pair of segments (Fig. 1C).

Fourth instar larvae present a prominent cephalic cap, wider than the body and colors vary from orange to light brown. During this period a slight pink color can be noticed in the intersegment spaces, especially on the sides, which becomes more evident as time passes by. Larvae on this instar measure on average 3.74 mm in length and 0.49 mm in cephalic width (Fig. 1D).

The fifth instar is characterized by a reddish color throughout the body. The cephalic capsule is oblong, and has a dark orange color which turns into light brown with time. Setae are brown in its base and lighter at the tips. Larvae measure 5.44 mm in length and 0.58 mm of cephalic width on average (Fig. 1E).

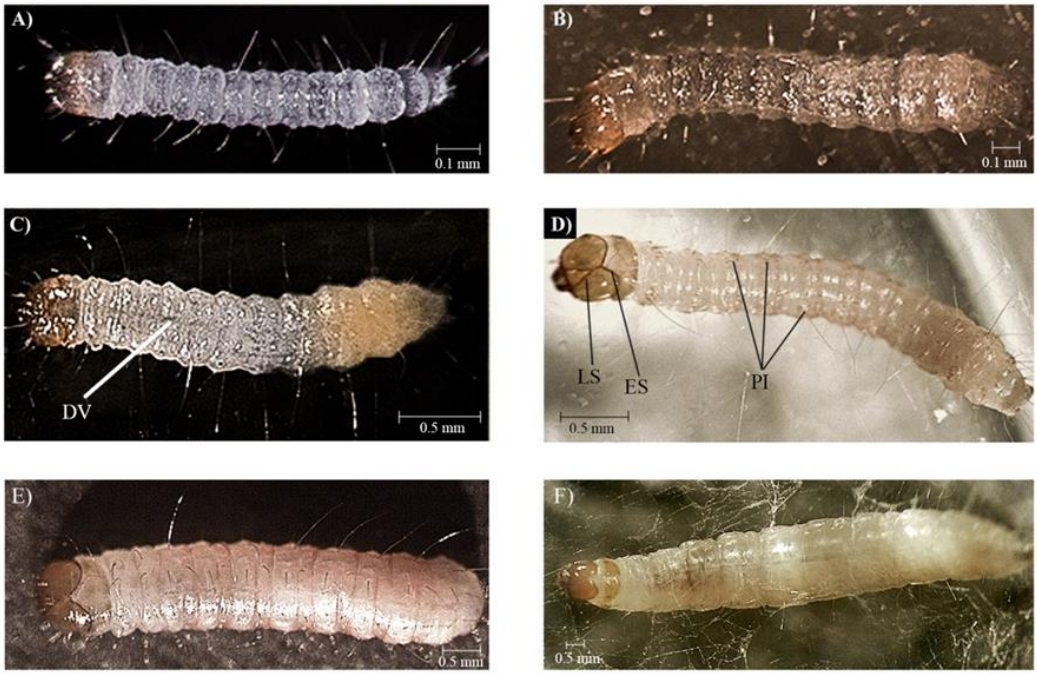

Figure 1: Larval instars of *P. interpunctella*: A) First instar. It shows the length of setae and the size and coloration of head capsule. B) Second instar. Internal content can be observed due to transparency of tegument. Thoracic shield starts to be seen. C) Third instar. The obvious variation of color of the last abdominal segments and the dorsal vessel (D. V.) can be seen through the tegument. D) Fourth instar. The epicranial suture (E. S.), lateral sutures (L. S.) and jaws are observed. The dark color of the pinnacles (P.I.) may be noticed. E) Fifth instar. The distinctive pink color of this instar is observed. F) Sixth instar. The presence of silk for pupation and an obvious development of the abdominal segments are observed.

During the sixth instar, the larvae present a coloration ranging from pink to yellow and even a green hue. The cephalic end is smaller than the rest of the body. Larvae are less active and reduce feeding; they leave the silk coverage and begin to move away from the food.

Finally, the cephalic capsule opens through its cephalic fissures during the molting process

preventing its measurement, thus coinciding with what was described for last instar of chickpea-fed larvae (Pérez *et al*., 2012). Taking into account that the width of the head does not change growth during this stage (Dyar and Rhinebeck, 1890), the measures of cephalic width were taken before molting.

Prior to pupation, larvae produce a new cocoon and enter a period of pre-pupae which can last from a few hours to one day in length. In this phase, the larvae present a tapered shape and do not feed; they have a slight greenish coloration and its head bents forward (Figs. 2A-B)

The sixth instar represents the period of major growth in the larval development of *P. interpunctella.* Larvae can measure from 6 mm at the start of this phase and reach a length of about 12 mm before pupation (Figs. 2C-D).

Figure 2: Prepupae (left). A) Side view. It shows orthognathous position of the head, characteristic of the prepupae stage. B) Dorsal view. Larvae (right). Fifth (C) and sixth (D) larval instars. Note the difference in size between two consecutive larval instars.

The pupal stage lasts on average 10.30 days. The pupae are about 7 mm long and light brown (Figs. 3A-C). Before the emergence of the adults, the typical coloration of the wings can be seen through the chrysalis.

The adult stage is reached between 65 and 67 days after eclosion. The average length of adults is 10.80 mm and females generally measure between 0.50 and 1 mm more than males. The individuals exhibit all the morphological features of the adult stage described by Hamlin (1831) for the species. They have a wingspan of 16.30 mm on average. This stage ends with the death of the adult, after 5 to 10 days in the case of females, and 7 to 10 days for males (Figs. 4 A-B).

Copulation takes place between 24 and 48 hours after adult emergence. Based on the observations, each female copulates only once with each male, but could do it with more than one in presence of several males. Each female can lay 40 to 180 eggs during its whole adult life. Few eggs are laid during the first days of oviposition (2-10), and in general, during the 3rd and 5th day, oviposition is at its peak. In the subsequent days, oviposition decreases until the death of adults (Fig. 5). These data support the observations of Mohandass *et al*. (2007), in the sense that as the adults grow old, oviposition becomes erratic and has no defined peaks.

Taking into consideration that the head capsules undergo an exponential growth in successive larval stages (Dyar and Rhinebeck, 1890), the relation between the length of larvae and the cephalic width has been determined. A high correlation between

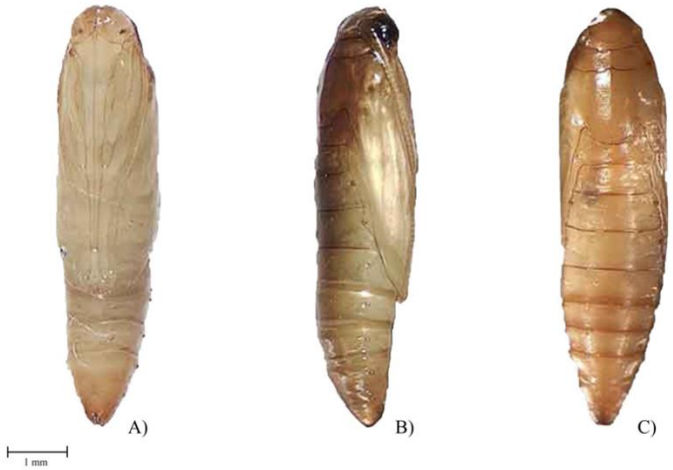

Figure 3. Pictures of pupal stage of *P. interpunctella.* A) Ventral view (female). Two-day-old pupae. B) Side view of pupae 3-4 days old. C) Dorsal view. Pupae 6-7 days old with final coloring.

Figure 4: Adult of *P. interpunctella.* A) Dorsal view. Picture of an adult with the distinctive pattern of color of the species. B) Lateral view. Schematic figure of an adult.

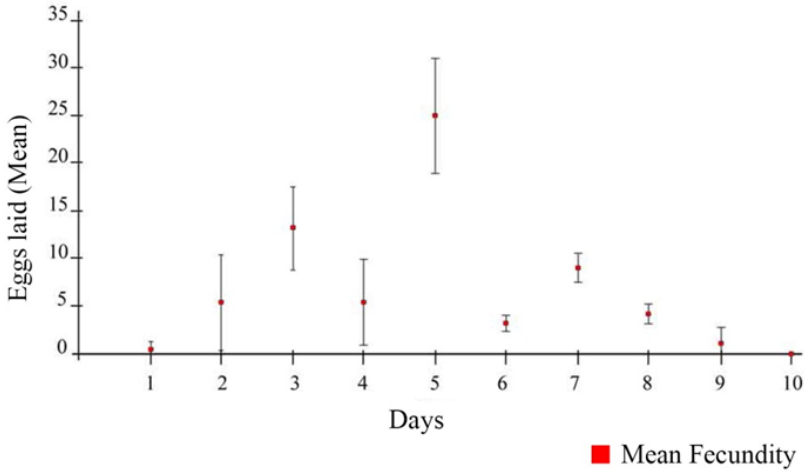

Figure 5: Percentage of eggs laid per day of *P. interpunctella* reared on walnuts. The values in the graph represent mean and standard deviation of oviposition.

both dimensions (R = 0.98) was found, which allows to infer the larval instar by knowing the cephalic width (Fig. 6). A similar value was established by Pérez *et al*. (2012) for larvae fed on chickpeas.

Finally, the life cycle of *P. interpunctella* controlled in equal experimental conditions but without disturbance lasted 68 days on average. Although other authors consider that the disturbance of the larvae to measure the head capsules could prolong the development time and increase mortality (Mohandass *et al*., 2007), in this study the manipulation of larvae did not interfere significantly in the biological cycle.

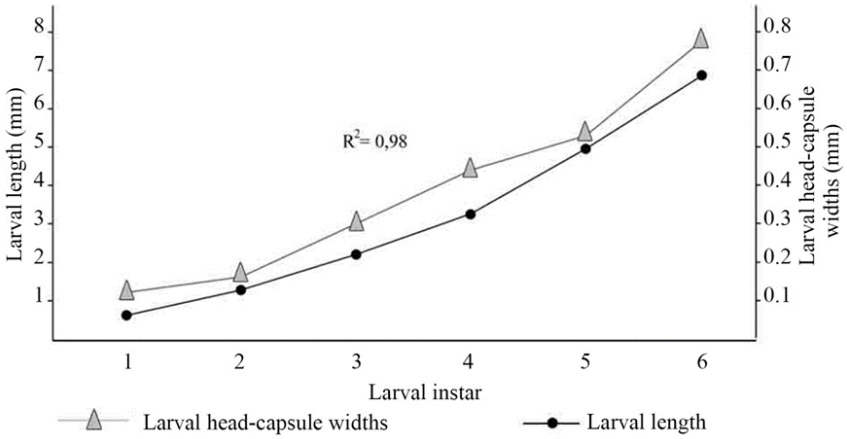

Figure 6. Relationship between larval length and head capsule width of *P. interpunctella* reared on walnuts.

CONCLUSIONS

This paper is the first description of the complete life cycle of *P. interpunctella* using walnuts as host. Six instars are defined and morphologically described in the larval stage.

The high correlation between cephalic width and larval length not only allows us to determine the instar by measuring the head capsule, but it could also be helpful for future population studies and laboratory assays destined to species control.

In-depth knowledge of the developmental stages of *P. interpunctella* in rearing conditions which imitates the ones existing in small and medium-size producers' storage facilities of the region is useful for the subsequent design of pest control strategies.

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