Notas Científicas

Biology and morphometrics of the immature stages of *Epinotia aporema* on artificial diet

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Abstract – The objective of this work was to establish a life Table for the immature stages of *Epinotia aporema*, as part of a wider investigation on its biological control. Insects were reared on an artificial diet at 25±1ºC and a 16:8 (light:dark) hour photoperiod. For the identification of larval instars for the study of pathogen-insect interactions under laboratory conditions, head capsule widths (HCWs) were also determined. The egg incubation period was 4.13±0.30 days, larval stage took 11.64±0.49 days, and the development time of the pupal phase was sex-dependent with 8.51±0.69 days for the females and 9.41±0.65 days for the males. Five larval instars were identified.

Index terms: *Glycine max*, bean shoot borer, cephalic capsule, laboratory rearing, larvae,.

Biologia e morfometria dos estágios imaturos de *Epinotia aporema* em dieta artificial

Resumo – O objetivo deste trabalho foi estabelecer uma Tabela de vida para os estágios imaturos de *Epinotia aporema*, como parte de um estudo mais amplo para seu controle biológico. Os insetos foram criados em dieta artificial a 25±1ºC e 16:8 (luz:escuridão) horas de fotoperíodo. Para a identificação dos estágios larvais para estudos de interação inseto-patógeno em condições de laboratório, as larguras de cápsula cefálica também foram determinadas. O período de incubação dos ovos foi de 4.13±0.30 dias, o estágio larval foi de 11.64±0.49 dias, e o tempo de desenvolvimento das pupas dependeu do sexo, com 8.51±0.69 dias para as fêmeas e 9.41±0.65 dias para os machos. Foram identificados cinco estágios larvais.

Termos para indexação: *Glycine max*, broca-das-axilas, cápsula cefálica, cria em laboratório, larvas.

The bean shoot borer *Epinotia* (syn. *Crocidosema*) *aporema* Wals. (Lepidoptera: Tortricidae) is a major soybean pest in Argentina and other South-American countries (Morey, 1972; Corrêa & Smith, 1975; Pereyra et al. 1991; Liljesthröm et al. 2001). The available information on *E. aporema* life cycle is limited and mostly restricted to its occurrence and development on soybean crops under variable conditions (Morey, 1972; Iede & Foerster, 1982). Nevertheless, some basic aspects of the insect biology and morphometrics are still poorly documented.

Traditionally, various chemical insecticides have been used against this moth, but the interest in more environmentally friendly biopesticides such as *Bacillus thuringiensis* and baculoviruses has increased in the past few years (Ibarra et al. 1992; Sciocco-Cap et al. 2001; Sauka et al. 2007). A recently discovered granulovirus of *E. aporema* (EpapGV) (Sciocco-Cap et al. 2001; Goldberg et al. 2002) has been shown as a promising biological control agent when ingested by early larval instars. This led to the establishment of a laboratory-reared insect colony supplied with artificial diet, both to perform pathogen-host interaction studies and for virus multiplication. The infection process of EpapGV and other pathogens are age dependent.

The aim of this work was to construct a life Table of *E. aporema* larvae and to turn possible a reliable identification of its different instars. The present paper reports the duration of each *E. aporema* immature stage, the number of larval instars and the determination of their respective head capsule width (HCW) under controlled conditions of light, temperature and diet.

A virus-free colony of *E. aporema*, originated from field populations in Córdoba and Santa Fe provinces

(Argentina), and maintained in a rearing facility at 25±1ºC and at a photoperiod of 16:8 (L:D) hours provided the insects for the experiment. The sanitary status of the colony was periodically checked by PCR assays for EpapGV, according to Parola et al. (2002).

The artificial larval diet (modified from Greene et al, 1976) consisted of the following ingredients (for 1 kg diet): 800 mL water, 25 g soybean flour, 20 g casein, 50 g wheat germ, 62 g pinto beans (dry), 2 mL wheat germ oil, 3.5 g ascorbic acid, 16 g agar, 0.062 g oxofloxacine, 32 g brewers´ yeast, 1.5 g sorbic acid, 2.5 mL methyl-p-hydroxybenzoate, 0.25 g Vanderzant mix vitamins and 3 mL formaldehyde (40%). A solution containing 925 mL water, 60 g sugar, 5 mL beer and 10 g honey L^{-1} served as a food source for the adult moths. A group of newly emerged males (10) and females (5) was allowed to mate for two days and to lay eggs on paper for a 12-hour period. The resulting eggs were placed in Petri dishes and kept in a rearing chamber under the above-mentioned conditions of temperature and photoperiod until hatching. Egg hatchability and incubation period were recorded. Upon emergence, 50 larvae were placed individually in 50-mL acrylic vials containing artificial diet and kept there until pupation.

The number and duration of larval instars, as determined by the successive molts, were assessed. For each larval stage, 31 to 36 shed head capsules were photographed at 40x magnification with an Olympus SZX9 stereomicroscope (Olympus Optical Co. LTD, Tokio, Japan) and measured using the image analysis program Image-Pro Plus (Media Cybernetics Inc., Bethesda, Maryland, USA). The increase ratio in HCW between successive instars was also calculated. Pupae originating from the surviving larvae were sexed and controlled daily until adult emergence to record the duration of the pupal phase (differences in duration between sexes were analyzed by ANOVA). All biological parameters were expressed by means and standard errors (SE).

Egg hatchability was 95% (573 out of 604 eggs laid during the 12-hour oviposition period), with an incubation period of 4.13±0.30 days (n=573). This study identified five larval instars of *E. aporema*, except for a single individual, which underwent only four instars (not included in mean calculations). Previous authors working under different conditions also reported the occurrence of five larval instars (Morey, 1972; Iede & Foerster, 1982; Pereyra & Sánchez, 1998), although they found longer embryonic, larval and pupal development times, possibly due to lower environmental temperatures and different diets. A characteristic feature of the larvae was that the first to fourth instars had black heads, while the fifth instar had a light-brown head. In the case of the exceptional specimen that went through four instars, its head was black from the first to the third instar and turned to light brown in the last instar. Thus, head color appeared as a distinctive trait to identify last instar larvae in *E. aporema*, regardless of the number of molts.

The total duration of the larval stage was 11.64±0.49 days (n=43). This value is considerably lower than the developmental time (from hatch to pupation) of 15.13–17.20 days reported by Pereyra & Sánchez (1998) for *E. aporema* larvae fed on a variety of legume species, under similar conditions of temperature and photoperiod. The duration of the pupal stage varied significantly ($p<0.01$) with sex, with females emerging about one day earlier than males. The mean duration for each of five instars and pupal stage is summarized in Table 1. The range of HCWs did not overlap between the different instars and had an average growth rate (mean increase rate: 1.47) that followed Dyar's rule (Dyar, 1890), which predicts a constant ratio of increasing width at each molt, usually of the order of 1.4. This result showed the accuracy of HCW measurements for determining instars in *E. aporema* larvae, at least when reared under controlled conditions. The only specimen with four larval instars (not included in mean calculations) presented similar HCW values, except for the third instar (in this case HCW was 0.53 mm, which is between the normal values of a third and fourth instar larvae). HCWs and increase rates are presented in Table 2.

This article represents a contribution to the knowledge of the *E. aporema* life cycle and provides useful

Table 1. Duration (days) of the larval instars and pupal stage of *Epinotia aporema* reared at 25±1ºC and 16:8 (L:D) hour photoperiod on artificial diet.

Instar	Number of individuals	Mean±standard error
I	46	2.06±0.11
II	46	1.60±0.12
III	44	1.41±0.14
IV	44	1.88±0.23
V	43	4.71±0.28
Pupae (female)	25	8.51±0.69
Pupae (male)	19	9.41±0.65

Table 2. Head capsule width (mm) of the larval instars of *Epinotia aporema* reared at 25±1ºC and 16:8 (L:D) hour photoperiod on artificial diet.

Instar	Number of individuals	Mean±standard error	Range	Increase rate
I	32	0.21±0.01	0.20–0.22	-
II	36	0.31±0.01	0.29–0.33	1.48
III	31	0.45±0.02	0.40–0.49	1.45
IV	35	0.67± 0.02	0.64–0.72	1.49
V	31	0.97±0.08	0.79–1.14	1.45

practical information for investigating pathogen-host relationships and other insect age-dependent issues.

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