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Determination of Larval Instars of Black Cutworm *Agrotis ipsilon* (Hufnagel) (Lepidoptera, Noctuidae)

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Abstract

The numbers of larval instars of *Agrotis ipsilon* (Hufnagel) were determined using measurements of larval head-capsule width. The obtained results emphasized that the head capsule width recorded was 0.28 mm for the 1^{st} instar (newly hatched larvae) and 3.42 mm for the 6^{th} instar (taken to pupation). The overall results fit in with Dyar's rule.

Keywords: *Agrotis ipsilon*, head-capsule width, larval instars, Dyar’s Rule, number of instars.

1. Introduction

The larvae of *Agrotis ipsilon* (Hufnagel) (Lepidoptera, Noctuidae) is more commonly known as the black or greasy cutworm. It is a polyphagous serious pest of different economic plants found throughout the world (Rings and Johnson, 1975; El-Salamouny *et al*., 2003). Moreover, it is a serious pest of golf courses as well as vegetables and field corps (Hong and Williamson, 2004). The feeding habits vary across the six larval instars (Allan, 1975; Clement and McCartney, 1982).

There are several studies that apply Dyar's rule to other insect species. According to Dyar's rule, which is used for determining the instars and stages of many insect species, Moser *et al.* (1991) found that the width of the head capsules of *Ctenocephalides felis* (Bouche) of different instars had geometrically progressing growth. Goldson *et al.* (2001) found larval instar's distribution of the Argentina stem weevil by using head capsule width. Lauzon and Harper (1993) followed the growth of aquatic snipe fly, *Atherix lantha* Webb (Diptera, Athericidae), depending on measurements of second antennal segment of the larvae.

Rodriguez-Loeches and Barro (2008) found that the measurement of head capsule of *Phoenicoprocta capistrata* (Lepidoptera, Arctiidae) larvae followed a geometric pattern consistent with Dyar's rule. Moreover, Shashi and Singh (2009) determined the exact nymphal stages of *Chrotogonus trachypteras* (Blanchard).

In the present study, it was attempted to find out the number of larval instars depending on the measurement of larval head capsule width, and applying Dyar’s rule to the results.

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2. Material and Method

By using light traps, the moths were caught then placed in plastic boxes. They were fed with 10% honey water. The collection of eggs was facilitated by muslin cloth. The eggs were placed in an incubator (25°C) to hatch. The larvae were separated by plastic grids to form individual chambers and were kept at 25°C, 60-70% relative humidity. The larvae were reared on an artificial diet (Oxford recipe). The diet consisted of caseine, wheat germ, dry yeast, cholesterol, agar, wessons salt, vitamins and antibiotics mixture, fungicide and water (Hansen and Zethner, 1979). The experiment started with approximately 1000 newly hatched larvae which were reared as discussed above. Widths of larval head capsules were measured to determine the larval instars according to Dyar’s rule (Gullan and Cranston, 2005).

The width of the head capsule of each larva was measured using a calibrated ocular micrometer in a binocular dissecting microscope. For each instar, 100 larvae were randomly selected and their head capsules measured in interval periods. The rest were kept rearing on the diet until the last instar measurement of head capsule took place. Since it was difficult to measure a live larval head capsule, 70% alcohol was used to kill the larvae before measuring the head capsule. The larvae which were deemed to be in their final instar were not killed; instead, their head capsule was measured while they were alive. They were then kept until pupation to ensure that further molting did not occur.

3. Results and Discussion

The figures obtained from measuring the head capsule width were plotted on diagram paper against the number of larvae. In this way, a histogram (Figure 1) was produced, with six different groups of larvae.

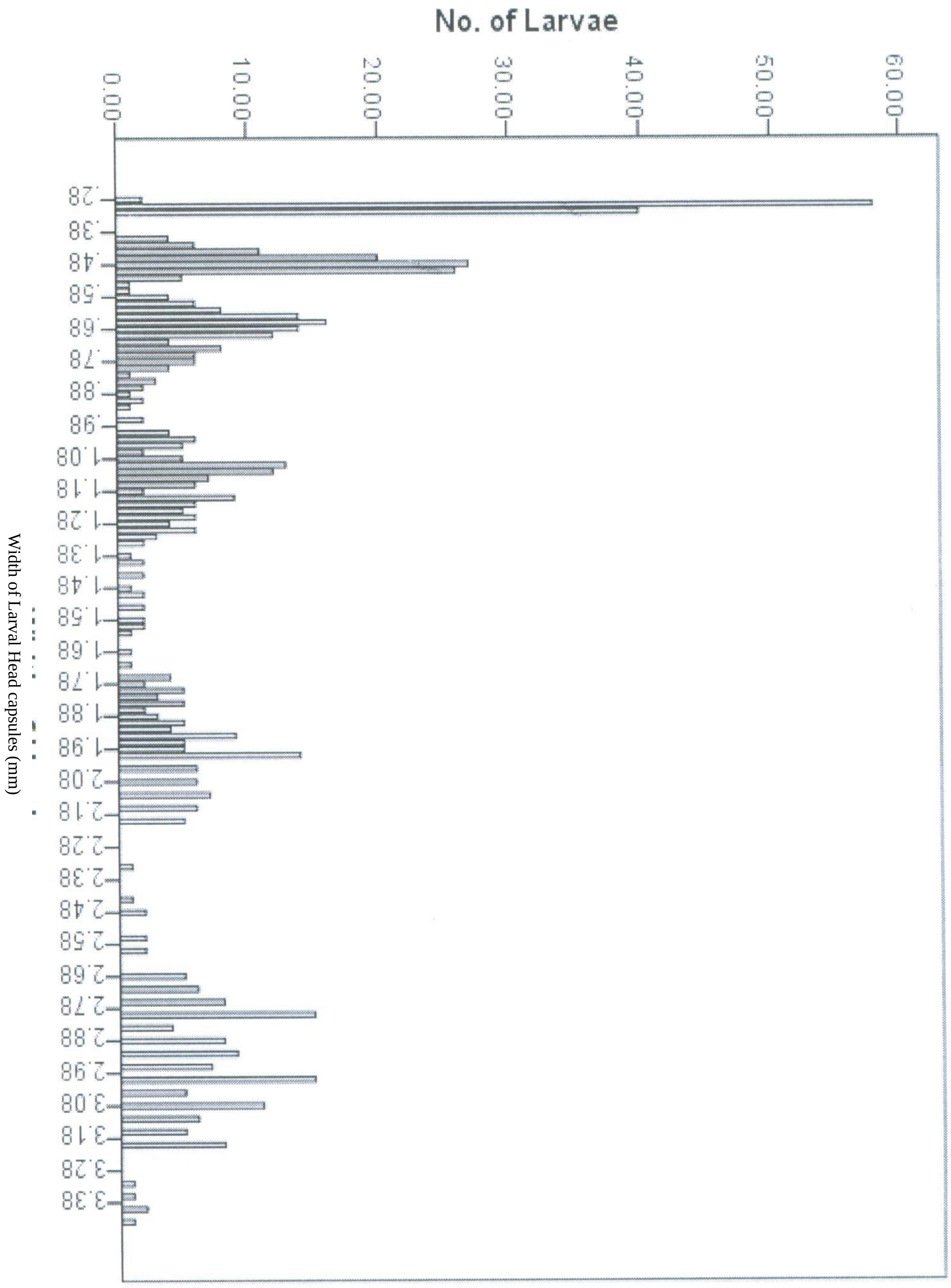

Figure 1: The measurement of larval head capsule width plotted against the number of larvae producing 6 different groups

The 1st group included only newly hatched larvae which were consequently representative of the 1st stage instar. On the other hand, the 6th group included the larvae which were pupated without further molting, so it is regarded as the final instars.

For many population studies, it is important to know the number of larval or nymphet instars within a species in order to be able to recognize the instars to which any immature individual belongs. More frequently, the only obvious difference between successive larval or nymphal instars is the increase in size that occurs after each molt. Thus, it should be possible to determine the actual number of instars in the life history of a species from a frequency histogram of the measurements of a sclerotized body part such as the width of the head capsule or the length of the mandible. Dyar's rule developed from his observation of the measurement of the larval head capsule width of some Lepidoptera species (Gullan and Cranston, 2005). Dyar's rule states that if the logarithms of the measurements of some sclerotized body part in different instars is plotted against the instars number, a straight line should result; any deviation from a straight line indicates a missing instars. In figure 2, one can notice that a straight line is produced, which indicates that there is no missing instars, fitting in with Dyar's rule. In figures 1 and 2 along with Table 1, we can see that there are six larval instars of *A. ipsilon.* These results agree with Zaz (1999), and Alnaji and Ghafoor (1988) who found that the larvae have six instars. On the other hand, Luckman *et al.* (1976) reported that larvae have seven instars. But Allan (1975) stated that the black cutworm has five instars only.

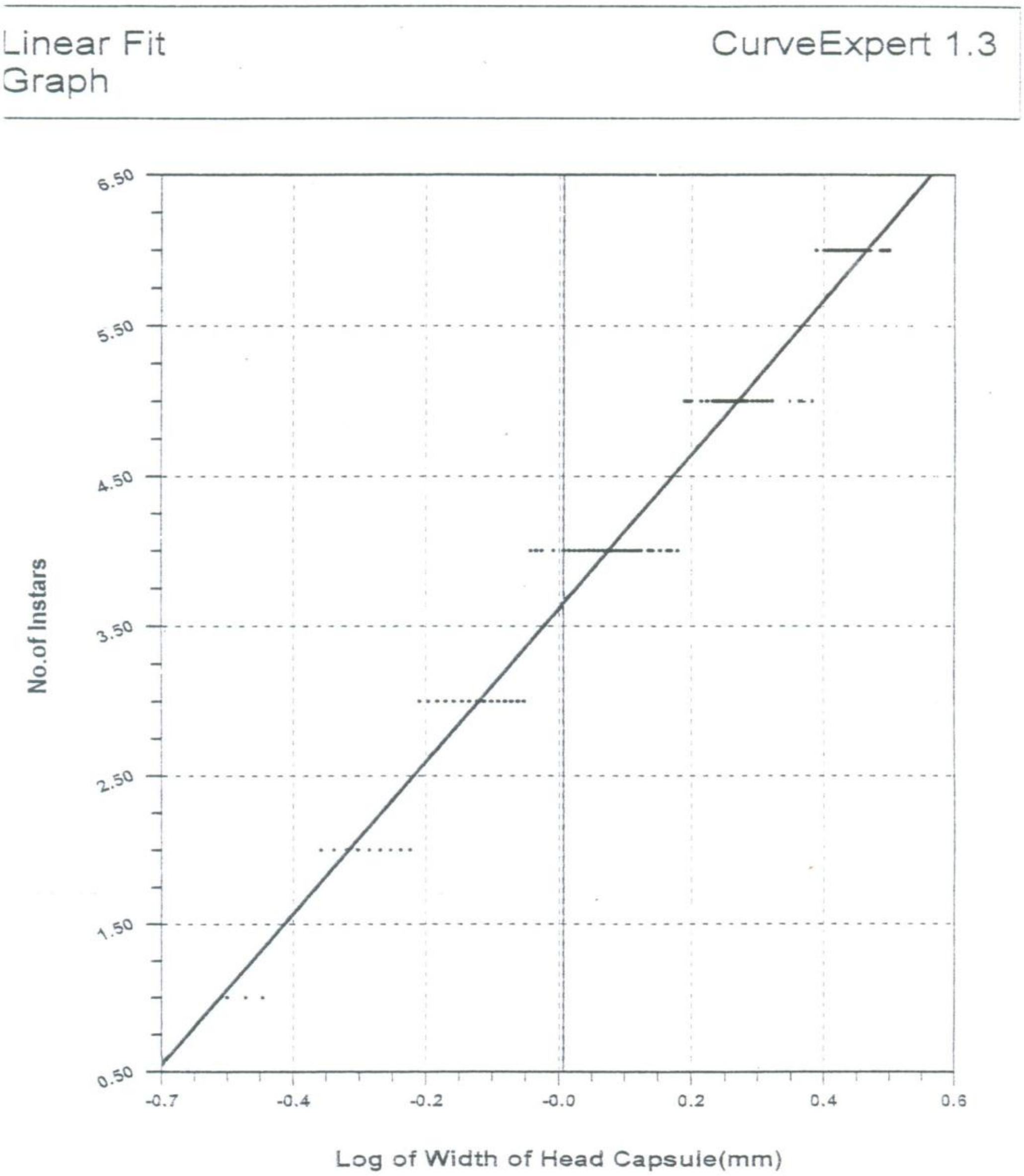

Figure 2. A linear regression shows a straight line which fitted with Dyar's rule

Table 1. Mean measurements width (mm) of larval head capsules of the *Agrotis ipsilon*

Larval instars	No. of larvae	Average of head capsule width (mm)± S.E
1st	100	0.307±0.0015
2nd	100	0.4726±0.0030
3rd	102	0.681±0.056
4th	108	1.16129±0.009
5th	98	1.96816±0.012
6th	117	2.95982±0.012

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