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Interactive effect of diet and temperature on instar numbers in *Spodoptera litura*, with reference to head capsule width and weight

Hyoung-ho Mo [a], Keun Bok Jang [b], Jung-Joon Park [c], Sung-Eun Lee [d], Key-Il Shin [e], Joon-Ho Lee [f], Kijong Cho [a,*]

[a] Division of Environmental Science and Ecological Engineering, Korea University, Seoul 136-701, Republic of Korea
[b] Dongbang Agro Corporation, Seoul 151-801, Republic of Korea
[c] Department of Applied Biology, Institute of Agricultural and Life Science, Gyeongsang National University, Jinju 660-701, Republic of Korea
[d] School of Applied Biosciences, Kyungpook National University, Daegu 702-701, Republic of Korea
[e] Department of Statistics, Hankuk University of Foreign Studies, Yongin 449-791, Republic of Korea
[f] Entomology Program, Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Republic of Korea

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ABSTRACT

The effects of diet and temperature on instar numbers and head capsule width in *Spodoptera litura* F. were compared among individuals reared on an artificial diet, lettuce and perilla leaves at 25 and 30 °C. The number of instars that the insect completed varied as diet and temperature were changed. All the larvae developed through seven instars at 25 °C regardless of diet, but at 30 °C, the number of instars varied depending on the diet. All larvae fed on lettuce leaves had six instars, while larvae fed on the artificial diet passed through seven instars. On perilla, 52% of larval individuals had six instars, and the rest had seven instars. Head capsule width could be used effectively to determine the developmental stage of individual larvae. The frequency distribution of head capsule width showed six or seven distinct peaks, depending on diet and temperature conditions. The relationship between mean head capsule width and weight of larvae was described using an exponential model.

Introduction

The tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is polyphagous, damaging many economically important vegetables and ornamental plants throughout subtropical and temperate regions of Asia and Oceania (Feakin, 1973; Garad et al., 1984). The last instar larva is the most economically important because of its feeding damage to plants and its high resistance to insecticides (Kondo, 1987). Predicting the temporal occurrence and abundance of *S. litura* is essential for timing control tactics.

Determination of a population's instar distribution can provide important information for management of pest populations (McClellan and Logan, 1994). The ability to accurately assess instar distribution helps in the development of phenology models, and provides insight into and rationale for the timing of pesticide application. Variation in number of larval stages is common for many lepidopteran species (Esperk et al., 2007), and it has been reported that the number of instars may be related to factors such as temperature (Leonard, 1970) and nutrition (Igarashi, 1982). Morita and Tojo (1985) reported that instar numbers of *S. litura* were affected by food quality and rearing density during larval periods. However, there is little general understanding of the variability in instar number in *S. litura* populations, and there are few published data on either instar numbers, or head capsule width in relation to instars of *S. litura*.

In the present study, the effects of diet and temperature on the instar number and head capsule width were compared between insects reared on an artificial diet and those reared on host plants using laboratory populations of *S. litura*. The results presented here will provide basic information that can be used to develop accurate phenology models and monitoring tools for *S. litura* larval populations in the field.

Head capsule widths are often used to determine the larval age of various lepidopterans (Beaver and Sanderson, 1989; McClellan and Logan, 1994; de Groot, 1998). This method is based on the concept that there are discrete increases in head capsule width that are indicative of the larval instar (Dyar, 1890). Logan et al. (1998) provided a generalized computer program, H_{CAP}, for analysis of larval head capsule data; this program is a simple and rapid analytical tool for identifying instars and their distribution for various insect species.

Materials and methods

Insect rearing conditions

S. litura larvae used for this study were originally provided, in 1999, by Korea Research Institute of Chemical Technology, Daejon, Republic of Korea. This species has been reared on a bean-based artificial diet under conditions of 25 ± 1 °C and 70% RH with a photoperiod of 16:8 (L:D) h.

* Corresponding author. Tel.: +82 2 3290 3064; fax: +82 2 925 1970.
E-mail address: kjcho@korea.ac.kr (K. Cho).

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Composition (g) of the diet was as follows: agar (10), kidney bean (55), wheatgerm (30), yeast (25), sorbic acid (1.2), methyl p-hydroxybenzene (3), ascorbic acid (3), and salt mixture (3), mixed in 550 ml of distilled water. Each year, a wild population of *S. litura* was collected from various crop hosts and geographical areas, and added into the laboratory population to prevent inbreeding effects or genetic distortions.

After increasing the population size on the artificial diet, the *S. litura* population was divided into three subpopulations. The first subpopulation was maintained on the artificial diet to minimize any possible influence of prior experience of the host plant. The second and third subpopulations were reared on one of two natural plant hosts: lettuce (*Lactuca sativa* 'Sunny') and perilla (*Perilla frutescens*, var. *japonica* 'Daenongperilla'), respectively. These crop plants were selected because they are frequently grown together or adjacent to one another in Korea. All three subpopulations were maintained at conditions of 25 ± 2 °C, 65% ± 5% RH, and a photoperiod of 16:8 (L:D) h.

Organically grown leaves of lettuce and perilla were provided to the larvae as a food source. Two or three leaves of each plant were placed with one-day-old larvae in a plastic box (25 × 15 × 8 cm) lined with a paper towel. Larvae were allowed to complete their larval periods on the leaves of each host plant. The fresh leaves were replenished daily until pupation. Resulting adults were transferred to a wax-paper cage with a 20% sucrose solution as a food source. Egg masses were collected daily by cutting the wax paper using a surgical blade, and the collected egg masses were placed in the separate plastic boxes with different food sources.

Effect of diet and temperature on instar numbers of S. litura

This experiment was started from eggs obtained from the three subpopulations described above. First instar larvae (age < 1-day-old) were selected from several egg masses. Individual larvae were placed randomly on each diet (n = 150 larvae per diet) with a camel's-hair brush. Larvae reared on the artificial diet, lettuce, or perilla leaves were placed individually in petri dishes (diameter, 9 cm) and provided with the artificial diet, lettuce, or perilla leaves, respectively. Perilla and lettuce are economically important crops and preferred hosts of *S. litura*. Also their leaves are easy to control as food of *S. litura*. For lettuce and perilla populations, two leaf disks (diameter, 5 cm) were provided daily. Each petri dish was sealed with plastic wrap to maintain humidity conditions. Old food was removed and fresh diet or leaves were added when needed. Egg periods were determined by daily observation prior to transferring the larvae to each dietary treatment.

Observations of individual larvae were started 24 h after initial transfer and continued at 24-h intervals until the larvae died or pupated. However, individual larvae maintained at 30 °C were examined at 12-h intervals because of fast larval growth. Duration of each larval period was determined by recording the existence of casting skin, and prepupal stage was included in the last larval period. The pupae were recovered from the petri dishes with soft-touch forceps on the 1st day of pupation. Sex was not considered in this study, because it is known that the duration of larval development, larval head capsule width, and pupal weight of *S. litura* are not significantly different between sexes (Morita and Tojo, 1985).

Within 24 h of molting into the next instars, larvae were weighed to the nearest 0.01 mg using a chemical balance (AUX 200, Shimadzu, Kyoto, Japan). The 1st instar could not be weighed because this instar was too light to obtain an accurate measurement.

Measurement and analysis of head capsule widths

The head capsule widths of larvae from the three different diets at 25 and 30 °C were measured with an ocular micrometer (Kyowa Optical, Japan) to the nearest 0.01 mm. Rearing conditions were the same as described in the development study. Head capsule widths were measured within 24 h after molting.

The H_{CAP} program was used to analyze the data on larval head capsule width (Logan et al., 1998). The program determined the following: optimum instar classification rules; estimated mean and standard deviation of head capsule width for each instar; estimated numbers of each instar; and probability of misclassification. Logan et al. (1998) explained the statistical assumptions and utility of this program in detail.

Statistical analysis

Data were subjected to a two-way analysis of variance (ANOVA) in which two tests for additive effects (diet and temperature) and one test for interaction (diet × temperature) were performed on the developmental rate (SAS Institute, 1999). All tests were two tailed with significance assessed at the $p < 0.05$ level. Growth ratio (head capsule width at instar x + 1/head capsule width at instar x) was calculated for each larval group reared under the different conditions. Finally, regression analysis was used to establish relationships between head capsule width and larval weight, using a PROC NLIN procedure in SAS (SAS Institute, 1999).

Results

Variation in number of instars according to different diet and rearing temperature

More than 90% of eggs, which were obtained from the populations reared on the three different diets, hatched at 25 and 30 °C. No *S. litura* larvae required more than seven instars to complete their larval periods and enter the pupal stage. The instar number varied, depending on diet and temperature (Fig. 1 and Table 1). All larvae developed through seven instars at 25 °C irrespective of diet, but at 30 °C the instar number differed according to diet. All larvae fed on lettuce leaves passed through six instars, while larvae fed on the artificial diet passed through seven instars. On perilla, 52% of individuals completed their larval stage with six instars, and the remainder completed the larval stage with seven instars. Since instar numbers varied on the perilla diet, the larval development periods for this diet were calculated separately as 6- and 7-instar types.

Larval head capsule width

Head capsule widths ranged from 0.20 to 3.20 mm. The frequency distribution produced by H_{CAP} showed six or seven peaks, depending on the diet and temperature (Fig. 1). Peak numbers observed in the head capsule distributions matched the instar numbers found in the larval developmental studies, indicating that the distribution of head capsule width represented the instar distribution in larval stages of *S. litura*. The larvae fed on lettuce at 30 °C showed six distinctive peaks, but all the others had seven peaks.

The mean head capsule widths, boundary points, and probability of misclassification are listed in Tables 2–4. The estimated probability of misclassification was always <5%, except for the 4th and 5th instars of larvae fed on perilla at 30 °C. Relatively high misclassification probabilities was observed in the 4th (5.3%) and 5th (7.2%) instars of the group fed on perilla at 30 °C, which might have resulted from the variation of instar numbers observed in this treatment (Table 1). The mean head capsule width was not significantly different in the first four instars in all treatments ($P < 0.05$), but in the last three instars mean head capsule width differed between the 6- and 7-instar types. Because of the variation in number of instars in larvae fed on perilla at 30 °C, the data from this condition were excluded from the comparison. Larvae with 7 instars fed on the artificial diet, lettuce, or perilla at 25 °C had head capsules of a not significantly different mean width. Larvae that