

# Growth and Development of *Prodenia litura* FABRICIUS (Lepidoptera, Noctuidae) under the Conditions of Constant Temperature and Artificial Nutrition\*

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## INTRODUCTION

The artificial rearing of boring insects on a totally synthetic medium has been carried out by many workers. But few literatures on the artificial rearing of true leaf feeding insects, such as *Agrotis* and *Prodenia* are available. The present writers had frequently tried the artificial rearing of Tobacco-Cutworm, *Prodenia litura* FABRICIUS, using a totally synthetic medium as food. Although a number of larvae developed easily until normal pupae, it was difficult to obtain normal adults and their fertile eggs (Kiyoku 1964, Kiyoku and Tsukuda 1966). Recently, they found that the larvae reared on the synthetic basic media with peanut-oil or vitamin E developed normally, and the resulting adults laid eggs (Tsukuda and Kiyoku 1966). Furthermore, the artificial rearing of these insects was carried out repeatedly on the synthetic media which were supplemented with the peanut-oil or vitamin E, and the growth and development were observed in detail under such conditions as of an artificial nutrition and a constant temperature of 26°C.

## MATERIALS AND METHODS

Insect individuals used in these experiments are the offsprings of Tobacco-Cutworm, *Prodenia litura* FABRICIUS, which are maintained in our entomological laboratory on the basic semisynthetic medium using the *Kintoki*-beans (Kiyoku, 1965). The composition and their proportions in the synthetic media used are listed in Table I.

Of these ingredients, sodium propionate and sorbic acid are used to prevent the growth of noticeable microorganisms in the medium. At first, the sorbic acid is dissolved in a small quantity of ethyl ether and then the solution is mixed with the cellulose powder. The ascorbic acid and sodium propionate are dissolved in an amount of 10 cc out of the definite amount of water 55 cc for the whole amount of the ingredients. The agar is dissolved in the remaining water (45 cc) at 100°C and mixed thoroughly with other ingredients and the cellulose powder containing the sorbic acid.

\* We lectured already on this study at the 26th Annual Meeting of the Japanese Society of Entomology (Nagoya Univ. Oct. 1966)

Table 1. Composition of the totally synthetic medium for the tobacco-cutworm (g).

Ingredients	No. of rearing					
	I	II	III	IV	V	VI
Casein	1.0	1.0	1.0	1.0	1.0	1.2
Glucose	0.5	0.5	0.5	0.5	0.5	0.5
Sucrose	0.5	0.5	0.5	0.5	0.5	0.5
Cellulose powder	1.0	1.0	2.0	2.0	2.0	2.0
Cholesterol acetate	0.05	0.05	0.05	0.1	0.1	0.05
Choline chloride	0.15	0.15	0.15	0.15	0.15	0.15
Wesson's salt mixture	0.3	0.3	0.3	0.3	0.3	0.3
Yeast extract (Ebios)	1.5	1.5	1.5	1.5	1.5	1.5
L-ascorbic acid	0.2	0.3	0.2	0.4	0.4	0.4
Peanut-oil	—	0.2cc	0.08cc	0.15cc	—	0.1cc
Vitamin E	—	—	—	—	500mg	—
Bacto agar	0.4	0.4	0.4	0.4	0.4	0.4
Sodium carboxy methyl cellulose	0.5	0.5	0.5	0.5	0.5	0.5
Distilled water	55cc	55cc	55cc	55cc	55cc	55cc
Sorbic acid	0.08	0.08	0.08	0.08	0.08	0.08
Sodium propionate	0.08	0.08	0.08	0.08	0.08	0.08

The ingredients should be blent for at least 20 minutes after adding each ingredient. The mixture is cooled to less than 70°C and is mixed with the mixture of the ascorbic acid and sodium propionate previously mentioned. Careful preparation of the media seems to be important for healthy development of insects. These mixed diets are stored at 5°C.

Larvae were reared on a leaf of sweet-potato during the first instar after hatching. Thirty individuals of second instar larvae were placed in each rearing jar (9 cm in diameter, 8 cm in height) containing approximately three grams of the diet. Each jar was covered with a sheat of blotting paper. When the larvae reached the fourth instar, the larvae were removed to other petri-dishes (12 cm in diameter, 2.5 cm in height) with the lid of glass, on which a circular hole (3 cm in diameter) was cut. The hole was covered with the wire screen. A watch glass (4 cm in diameter) with the diet was placed in center of the petri-dish and a small quantity of sterilized sawdust was placed up to a depth of about 1 cm around the watch glass in the petri-dish. The full grown larvae dug into the sawdust and pupated therein. In this case, number of rearing larvae was seven or eight per petri-dish. The diets were

exchanged at two or three days intervals in the earlier period of rearing and every day in the later. All rearing was done at a constant temperature of 26°C and the photoperiod was left under the natural condition. Emerged three male and three female adults were confined in a glass bottle (10 cm in diameter, 13 cm in depth) and were fed on 8% sugar solution. The inner surfaces of the glass bottle and its lid were lined with paper, upon which most of eggs were laid.

## RESULTS

### *The results of rearing experiment.*

Data concerning the life-history of Tobacco-Cutworm reared on these synthetic media are summarized in Table 2.

Table 2. Life-history data of the tobacco-cutworm reared on the totally synthetic media.

No. of rearing	RI	RII	RIII	RIV	RV	RVI	Control*
Number of larvae tested	60	60	30	30	30	30	60
Percentage of pupation	60	60	30	86.6	66.6	30	78.3
Percentage of emergence	100	66.7	♂ 66.6 ♀ 66.6	83.3 78.6	75.0 50.0	100 100	91.5
Percentage of yield of adults	60	40	20	70	43.3	30	71.7
Duration of larval period (days)	28.0	24.7	♂ 15.8 ♀ 16.8	17.9 17.1	19.3 20.7	18.0 20.0	16.5 16.6
Duration of pupal period (days)	9.7	15.5	♂ 10.2 ♀ 9.2	8.9 9.0	9.1 8.8	13.0 12.0	10.6 8.9
Pupal weight (mg.)	206.0 211.0	317.6	♂ 351.9 ♀ 312.2	340.4 340.8	342.6 387.9	305.5 336.5	332.9 328.2
Number of eggs per female	0	138	786	804	268	1016	1045
Hatchability (%)	—	0	0	67.5	30.1	50.0	95.5

\* Data obtained from the cultures on the basic semisynthetic media using the *Kintoki*-deans (Kiyoku, 1965).

According to Table 2, the best growth and development were obtained in the rearing experiment RIV, that is, percentage of pupation was 86.6%. Adult moths successfully emerged from 83.3% of the male pupae and 78.6% of the female ones. At the constant temperature of 26°C, an average of 17.9 and 17.1 days were required to complete the larval stage for the male and female, respectively. The duration of pupal period was 8.9 days for male and 9.0 days for female. The mean weight of

the resulting pupae was 340.4 mg for male and 340.8 mg for female. The emerged adults laid 804 eggs per female and these eggs were hatchable. Thus, many healthy larvae of the second generation have been obtained from the present rearing experiment.

Such results as described above are never inferior or rather superior to those of the control shown in the last column of Table 2. As shown in the first column of Table 2, however, larvae reared on the media containing no peanut-oil or vitamin E developed normally until emergence, but the resultant adults did not lay eggs. Moreover, it was noticeable that although females reared on the media containing peanut-oil or vitamin E laid eggs, the percentage of eggs that hatched was low when amount of these substances supplemented to the basic media was not sufficient (RII, RIII). These results indicate that the adding of peanut-oil or vitamin E and sufficient amount of cellulose to the basic media is important to maintain a high percentage of normal adult emergence and egg-hatch.

The progenies from the adults obtained from the artificial rearing have been reared on the media similar to those used in artificial rearing of the parent generation (RIV). These rearing results seem to be as satisfactory as that of the parent generation except the hatchability. These are summarized as follows. The percentages of pupation and emergence for the male and the female are 86.6%, 93.7% and 90.0%, respectively. The percentage of yield of adults becomes 80.0%. The durations of larval period are 16.6 (♂) and 16.4 days (♀), those of pupal period 10.2 (♂) and 8.6 days (♀). The mean weight for male pupae is 350.7 mg and for female pupae 334.5 mg. The number of eggs per female is 626, and their hatchability 28%. From the experimental results mentioned above, it has become apparent that consecutive generations of the tobacco-cutworm are successfully reared on this medium.

#### *Observations on the growth and development.*

In another experiment, detailed observations were made on the growth and development of these insects reared individually on these synthetic media, using a glass cylinder measuring about 3 cm in diameter and 12 cm in length. Number of the instars passed, durations of the time required to complete each stage and pupal weight are given in Table 3.

Data obtained were varied considerably with the rearing plots. For example, durations of each instar for individuals growing normally were 2, 3, 3, 4, 4 and 6 days (from second to seventh instar), whereas for individuals developing slowly were 4, 3, 5, 11, 6 and 7 days, respectively. Thus, the total developmental time (in days) for all larval stage averaged 22 days, ranging from 20 to 36 days. However, almost all tobacco-cutworms reared on the artificial diet moulted six times and passed through seven instars during the larval period. Comparing with the growth and development of insects reared on the leaves of sweet-potato, it was found that the numbers of moulting and instar were larger by one and the duration of growing period tended

Table 3. Durations of the time required to complete larval and pupal stage, and pupal weight of insects reared individually on the totally synthetic media.

Instar and stage	No. of rearing											
	1	2	3	4	5	7	8	9	10	11	12	
Duration of 2nd inster period (days)	3	4	3	2	2	3	2	2	2	4	4	
3rd	3	3	4	3	3	2	3	3	4	3	2	
4th	4	4	5	3	3	3	3	4	3	5	3	
5th	9	4	4	4	4	4	4	3	4	11	2	
6th	4	4	4	4	5	5	5	5	5	6	3	
7th	7	6	6	6	5	5	7	7	10	7	6	
Total	30	25	26	22	22	22	24	24	28	36	20	
Duration of prepupal period (days)	1	2	2	2	3	1	1	2	3	2	2	
Duration of pupal period (days)	11	—	13	—	11	12	—	11	—	—	—	
Pupal weight (mg)	399	454	356	327	301	313	287	337	352	308	360	
Sex	♀	♀	♂	♀	♀	♀	♀	♀	♂	♀	♂	

to become somewhat longer than those of individuals reared on the leaves of sweet-potato. On the other hand, there were no great differences in the durations of prepupal and pupal period, and in pupal weight between two cultures. The appearance and structure of the insect reared on the synthetic media differed more or less from those of individuals fed on sweet-potato leaves. Main differences are briefly summarized in Table 4.

Table 4. Differences in the appearance and morphological characters of individuals between three cultures.

Kind of food	Characters	Number of moulting and Instar	Width of larval head (mm)	Color of larvae	Wing pattern and color of adults	Abnormality in wings
Leaves of sweet-potato		5:6	5th instar 1.75—1.85 6th instar 2.87—3.15	No yellowish brown ring	Normal	No
Basic semi-synthetic media using the Kintoki-beans		6:7	5th instar 1.22—1.40 6th instar 1.92—2.10 7th instar 2.80—3.15	Yellowish brown ring, on the surface of abdominal legs	Indistinct and pale	Rarely
Totally synthetic media		6:7	5th instar 1.16—1.40 6th instar 1.92—2.10 7th instar 2.80—2.97	"	Indistinct and pale, body hair less dense	Frequently

As described above, these larvae moulted six times and passed seven instars during larval satge. There was no significant difference depending on food with regards to the width of head capsule of the larvae until the fourth instar, but it appeared considerably smaller in both fifth and sixth instars for the individuals reared on the synthetic and semisynthetic media than for those reared on the sweet-potato leaves. The width of head capsule of the last instar (7th instar) of the former, however, became almost the same as that of the latter (6th instar). Yellowish brown ring was obviously seen on the surface of abdominal legs of larvae reared on both synthetic media. It was often found that the wing pattern and body colour of resulting adults tended to be indistinct and pale, respectively. Moreover, their body-hairs were usually less dense and their wings were frequently abnormal in size and shape.

### SUMMARY

Thus for experiments on the artificial rearing of true leaf feeding insects, such as *Agrotis* and *Prodenia*, on a totally synthetic media were not so numerous as those of insects boring into leaves and stems. In the present experiments two consecutive generations of the tobacco-cutworm, *Prodenia litura* FABRICIUS, have been reared succesfully on the totally synthetic media which are composed of casein, glucose, cellulose, cholesterol, choline chloride, ascorbic acid, yeast extract, peanut-oil, Wesson's salts mixture, sodium carboxy methyl cellulose, sorbic acid, sodium propionate, agar and water. Generally speaking, the life historical characters of insects obtained from these rearing experiments are never inferior or rather superior to those of the control reared on the sweet-potato leaves. Consequently, the writers believe that it is essential for obtaining normal adults and their hatchable eggs to add sufficient amounts of the cellulose and either the peanut-oil or vitamin E to the basic synthetic media.

The wings of the emerged adults, however, showed more frequently abnormality in size and shape and eggs laid by these individuals were somewhat smaller in number, and a certain percentage being not hatchable. Therefore, the fecundity and hatchability of eggs in these experiments considerably decreased as compared with those of the control. We shall be able to raise still more both the percentage of emerged normal adults and the hatchability of their eggs in further experiments.

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定温・人工栄養条件下におけるハスモンヨトウ

*Prodenia litura* FABRICIUS の発育

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摘 要

*Prodenia* や *Agrotis* などのような食葉害虫の全合成飼料による完全飼育の例は比較的少ない。筆者らは表1に示すような全合成飼料によってハスモンヨトウを連続して2世代間飼育することができた。一般にその飼育成績は良好で成虫の翅が畸型を示すものの割合が多いこと、卵の孵化率がやや低いことを除けば対照区と大差がなかった。正常な成虫を羽化させそれらに孵化力のある卵を産ませるためには、基礎飼料に Peanut-oil または Vitamin E を添加し更に飼料成分中のセルローズの量を多めにすることが必要であるように思われた。厳密にいうとこの全合成飼料による昆虫の発育は対照に比べて幼虫の脱皮回数および令数が1多く、発育期間がやや長い。成虫の翅の畸型を示すものがなおしばしば見られる。産卵数もやや少なく、その孵化率もやや劣る。しかしこれらの点は次の実験で改良されると思う。