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ARTIFICIAL REARING OF THE DIAMONDBACK MOTH, *PLUTELLA XYLOSTELLA* (L.), ON A SEMI-SYNTHETIC DIET

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Jean-Hwa Hsiao and Roger F. Hou (1978) Artificial rearing of the diamondback moth, *Plutella xylostella* (L.), on a semi-synthetic diet. *Bull. Inst. Zool., Academia Sinica* 17(2): 97-102. An improved semi-synthetic diet was developed for rearing the diamondback moth, *Plutella xylostella* (L.). The modifications were made by increasing the amounts of dry leaf powder with cabbage leaves instead of rape leaves, and by adding cholesterol, linseed oil and *i*-inositol. The diamondback moths were reared on this diet filled in a glass tube (3×7 cm) for at least 3 consecutive generations without any deterioration. In continuous rearing at 24-26°C, 70% R.H., developmental period from the larval to the adult stage was 18.5 days; adult longevity being about 9.5 days, and the diet-fed females laid ca. 126-151 eggs in their life. Adult emergence was 45.7%, 51.7%, and 58.2% for the 1st, 2nd, and, 3rd generation, respectively.

A successful artificial diet for insects is considered to be one which is satisfied the chemical, physical and nutritional requirements of these organisms[9,10]. With the development of standardized artificial diets, continuous maintenance of laboratory colonies of certain insect species has become greatly facilitated for more than two decades, and several phytophagous insects have been reared successfully on agar-base diets which differ in physical composition from the natural foods[2,3,11,18].

Artificial rearing of lepidopterous insects on media has been much more successful than that of other insects. Laboratory-reared populations can supply physiologically uniform animals for research on insect pathogens, insect pheromones, insect-resistant factors in plants, effects of insecticides and radiation inducing sterility as well as for the study of life cycles of some little-understood insects. Therefore, entomologists have drawn much attention to studies on these particular problems.

An artificial diet for the diamondback moth, *Plutella xylostella* (L.), was first formulated by Biever and Boldt[4]. Agui *et al.*[1] also developed a method for rearing this insect on a soybean meal diet for 3 generations in Japan. However, their feeding diets and rearing techniques are impracticable and unsatisfied for growth and development of the local populations here. The present work is to re-establish a better method and to develop an optimal diet for rearing the diamondback moth more efficiently in Taiwan.

MATERIALS AND METHODS

The stock culture of diamondback moths was established from field-collected larvae. Several generations were reared on cabbage heads in the laboratory before testing.

The composition of four different diets is listed in Table 1. The basic diet was adopted from that reported by Biever and Boldt[4] for the diamondback moth. We modified the diet by adding linseed oil, *i*-inositol and cholesterol. The cabbage leaf powder or juice was added to replace the rape leaf powder.

To prepare the diet, the desired amounts of the following ingredients: vitamin-free casein,

TABLE 1
Composition of semi-synthetic diets for feeding the diamondback moth, *P. xylostella*

Constituent	Amounts in 100 g diet			
	I*	II	III	IV
Vitamin-free casein	3.50 g	3.50 g	3.50 g	3.50 g
Alphacel	0.50	0.50	0.50	0.50
Wesson's salt	1.00	1.00	1.00	1.00
Sucrose	3.50	3.50	3.50	3.50
Wheat germ	3.00	3.00	3.00	3.00
Methyl-*p*-hydroxybenzoate	0.15	0.15	0.15	0.15
Choline chloride	0.10	0.10	0.10	0.10
Ascorbic acid	0.40	0.40	0.40	0.40
Aureomycin	0.015	0.015	0.015	0.015
Cabbage powder	1.25	—	3.00	—
Cholesterol	—	—	0.25	0.25
i-inositol	—	—	0.018	0.018
Agar	2.25	2.25	2.25	2.25
Distilled water	84.00 ml	62.00 ml	84.00 ml	84.00 ml
KOH (4M)	0.50	0.50	0.50	0.50
Formaldehyde (37%)	0.05	0.05	0.05	0.05
B-vitamin sol'n***	1.00	1.00	1.00	1.00
Linseed oil	—	—	0.65	0.65
Leaf juice**	—	22.00	—	—

* After Biever and Boldt (1971).
** 60 g fresh leaves/100 ml distilled water.
*** Niacin 100 mg; Calcium pantothenate 100 mg; Riboflavin 50 mg; Thiamine HCl 25 mg; Pyridoxine HCl 25 mg; Folic acid 25 mg; Biotin 2 mg and Vitamin B_{12} 0.2 mg in 100 ml H_2O sol'n.

Alphacel, Wesson's salt mixture, sucrose, wheat germ, methyl-p-hydroxybenzoate, potassium hydroxide, formaldehyde, cholesterol, linseed oil and leaf powder were mixed thoroughly in a 100-ml beaker and then poured into a waring blender. The agar was melted with 62 ml of distilled water in a water bath and then cooled down to 75°C before poured to the mixture of other components in the blender. The resultant solution was blended for at least 30 sec. Aureomycin was next added and blended for 15 sec more. Finally, the vitamin mixture consisting of ascorbic acid, choline chloride, *i*-inositol and B-vitamin solution was added into the blender and blended for 1–2 min. The completed diet was dispensed immediately into glass tubes (3×7 cm). Each tube was filled with ca. 5 ml of warm diet and then stopped with a cotton plug. The diet was stored at 4°C before use.

The leaf powder was prepared from the fresh cabbage leaves which were dried at 60°C for 24 hr and then ground up with a waring blender.

To ensure the cholesterol being mixed uniformly with other dietary components, we dissolved 250 mg of it in 2.5 ml of hot 98% ethanol and emulsified the solution in 22 ml distilled water with 1 ml of 0.5% Tween 80.

Chemical incompatibilities may result from mixing vitamins with strong oxidants, e. g., formaldehyde and potassium hydroxide, causing a loss of some vitamins in the diet. Therefore, these oxidants should be mixed well before the vitamin mixture is added. Ascorbic acid, for example, is heat labile above 60°C[15], thus the diet temperature should be properly managed during preparation.

Newly-hatched larvae were transferred into the diet-containing tubes for continuous rearing, but the larvae of 24–36 hr after hatching on their host plants were used for selecting an appropriate diet.

Approximately 30 larvae were kept in each tube for various tests and three replicates were made in each series of tests. The feeding diet was changed regularly every 2–3 days during the testing period in order to keep it fresh.

Evaluation of the feeding diets was based on larval period, larval and pupal body weight, and adult emergence compared to those obtained from insects fed on cabbage.

The wheat germ, Alphacel, vitamin-free casein and Tween 80 were purchased from the ICN Nutritional Biochemicals Corp., Cleveland, Ohio, U. S. A. Choline chloride, *i*-inositol, and B-vitamins were purchased from the Sigma Chemical Company, St. Louis, Missouri, U. S. A. L-ascorbic acid, cholesterol and linseed oil were obtained from the Wako Pure Chemical Industrial Corp., Osaka, Japan.

RESULTS

Data for development of the diamondback moth reared on cabbage leaves and on different diets under similar environmental conditions are summarized in Table 2. It is indicated that the larval growth and development was poor on the diet IV without cabbage leaves and that no adult emerged from the few larvae which pupated. Diet I containing 1.25 g of dry leaf powder promoted the larval growth, although 32.47% abnormal adults was present. No adult emergence occurred when the dry leaf powder in the diet I was replaced by leaf juice. Insects fed on diet III which contains more leaf powder showed the best survival and pupation among four different diets. In comparison with those insects on cabbage leaves, the diet III is able to support the insect growth and development optimally.

As shown in Table 3, it appears to be no significant difference in body weight of 3rd instar larvae fed on the diet I and III and those on plants. However, the larval weight is different significantly between insects fed on diet I and III at the 4th instar. Larvae fed on diet III required only 10.44 days for growing from 3rd to 4th instar; the pupal duration was found to be 6.43 days and the adult longevity was up to 9.71 days. These performances are similar to the insects fed on cabbage leaves and are significantly distinct from those on other diets (Table 4). From our feeding experiments, it is likely that diet III is adequate for rearing the diamondback moth compared to using the host plant as food as evaluated by survival, growth and development. The diet-fed females deposited as many eggs as those from natural food on cabbage leaves. Therefore, we were able to rear this insect continuously with the diet III through generations. In Table 5 is given insect development and reproduction reared for 3 generations. The adult emergence from diet-fed larvae appears

TABLE 2
Larval survival, pupation and adult emergence of diamondback moths reared on different diets in the laboratory

Diet	Larval survival (%)	Pupation (%)	Adult emergence (%)	
			Normal	Abnormal*
I	61.86	58.83	26.36	32.47
II	48.53	46.93	0	0
III	83.40	83.40	83.40	0
IV	8.31	8.31	0	0
Cabbage leaves	84.80	84.80	84.80	0

* Showing wing deformity.

TABLE 3
Mean body weight of diamondback moths fed on different diets and on the cabbage

Diet	Mean body weight (mg)		
	3rd instar	4th instar	pupae
I	0.34[a]	4.77[b]	4.10[b]
II	0.24[b]	4.13[c]	3.97[bc]
III	0.36[a]	5.79[a]	5.20[a]
IV	0.23[b]	4.04[c]	3.91[c]
Cabbage leaves	0.37[a]	5.71[a]	5.29[a]

Means followed by the same letter are not significantly different at the 5% level.

TABLE 4

Growth period, pupal duration and adult longevity of diamondback moths reared on different diets

Diet	Growth period* (days)	Pupal duration (days)	Adult longevity (days)
I	13.89^{b}	8.90^{b}	6.73^{b}
II	16.80^{c}	—**	
III	10.44^{a}	6.43^{a}	9.71^{a}
IV	18.25^{d}	—**	
Cabbage leaves	10.23^{a}	6.09^{a}	9.97^{a}

* From 3rd to 4th instar.
** No adult emerged.
Means followed by the same letter are not significantly different at the 5% level.

TABLE 5

Continuous rearing of *P. xylostella* on the diet III at 24–26°C, 70% R. H.

Generation	Developmental period* (days)	Adult emergence (%)	Adult longevity (days)	Reproduction**
P	18.56	45.73	9.58	151
F1	18.34	51.70	9.74	126
F2	18.58	58.30	9.67	138
Check***	17.34	78.82	9.72	147

* From larval to the adult stage.
** Eggs/♀/life.
*** Insects reared on cabbage leaves.

to be poorer than that from host plants.

It is conceivable that our best diet is still inferior to the natural food of the insect in adult emergence. Nevertheless the diet we developed can be applied to rear the diamondback moth through generations to some extent, and our feeding method is practical for artificial rearing of this species in the laboratory.

DISCUSSION

In the present study, we obtained 45.73, 51.70 and 58.30% of survival for 1st, 2nd and 3rd generation, respectively, fed on diet III. This is better than that reported by Agui *et al.*[1] in which they only obtained 35.00 or 39.13% from Japanese populations. Previous workers have indicated the low survival in rearing some lepidopterans, e. g., *Carpocapsa pomonella* L., *Rhyacionia frustrana* Comstock on artificial diets[5,6]. However, Biever and Boldt[4] were able to get 70% survival in rearing the diamondback moth on an artificial diet containing rape leaf powder in the United States. Since our dietray composition is mostly similar to Biever and Boldt's diet except addition of cabbage leaf powder instead of rape leaf powder, we assume that the natural components from different plants could stimulate feeding and support insect growth at various degree.

Growth period from the larval to the pupal stage was 18–19 days on our diet, this is slightly longer than 16 days reported by Biever and Boldt[4]. However, Agui *et al.*[1] showed that the growth period was 21.00 and 24.89 days for the 1st and 3rd generation, respectively. For those insect populations grown on host plants, the life cycle was reported to be 30 days in Canada, but only ca. 18 days at 23°C in Taiwan[14,19].

Agui *et al.*[1] observed that most of the diet-fed larvae died at the 1st and 2nd instar. The reason for a high mortality during these stages was found to be that young larvae did not initiate feeding on the diet. Similarly, our diets were poor in incitement of larval feeding, especially the first instar larvae, causing most of mortality at this stage. Therefore we postulate that there could be some particular chemicals missing the diets, which are supposed to be phagostimulatory to the diamondback moth.

Nayar and Thorsteinson[17] demonstrated that some mustard oil glucosides in plants, e. g., sinigrin, sinalbin, etc., acted as phagostimulants for the larvae of diamondback moths. Some lipids and related compounds have also been implicated in insect phagostimulation; wheat germ oil and soybean oil enhance the feeding activity of locusts and silkworms. In addition, fatty acids were described as feeding stimulants for certain insect species[6]. Since some of these compounds are not commercially available here, we thus added linseed oil into our diet to serve as a phagostimulant.

In order to compare dietary effects of leaf juice and leaf powder on the insect growth, we added either one to the diets. As indicated in our results, we were unable to obtain any adult from feeding the diamondback moths on a diet containing cabbage leaf juice. It is presumed that the juice we added is insufficient to provide substances which are required for supporting insect growth, or that some active compounds may be lost when making the plant tissues into a juicy form. Many larvae fed on the diet I and II without cholesterol died in the process of molting. It seems that the sterol contents in the leaf powder or other dietary components are not enough for larval growth and development. Since cholesterol has been considered to be a precursor for ecdysone(12), we added it to promote the molting.

It was indicated that *i*-inositol was unnecessary for some insects fed on artificial diets(8), but Forgash(7) first determined the nutritive value of *i*-inositol for the American cockroach, *Periplaneta americana*. Hamamura *et al.*(13) reported that *i*-inositol seemed to be able to stimulate the acceptance of food by *Bombyx mori*. In developing our basic diet, we added *i*-inositol to strengthen its acceptability.

Although the survival, growth and development of diet-fed larvae was inferior to those fed on their natural hosts, the diet III presents feasibility for rearing the diamondback moth through generations in the laboratory. Improvement in dietary composition as well as feeding techniques toward a better rearing remains to be undertaken.

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小菜蛾人工飼料飼育之研究

蕭金華　侯豐男

小菜蛾已成功地在一種半合成人工飼料上連續飼育三代以上。本飼料乃沿用以前的配方，以甘藍葉粉取代油菜粉並增高其用量，另加入膽固醇 (Cholesterol)、亞蔴仁油 (Linseed oil) 及肌醇 (*i*-inositol)，經改進配成。

在溫度 24～26°C，相對濕度 70% 的條件下，連續飼育的昆蟲，由幼蟲至成蟲的發育期約爲 18.5 天，成蟲壽命約 9.4 天，每雌蟲一生產卵 126～151 個，連續飼育三代的羽化率分別爲 45.7%、58.3%、51.7%。此與對照組以甘藍葉飼育者頗相似，惟羽化率較低。