

Mass Rearing of Diamondback Moth

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Abstract

Mass rearing methods on artificial diets and cruciferous seedlings for the diamondback moth, *Plutella xylostella* L, are reviewed and described briefly.

Introduction

Research on the diamondback moth (DBM), *Plutella xylostella* L (Lepidoptera: Yponomeutidae), has recently become increasingly important after findings that the insect has developed resistance to various insecticides and that it produces sex pheromone which has potential in controlling the pest. For obtaining reliable and reproducible information from the physiological, toxicological, and pathological experiments of any insect, it is desirable to have uninterrupted supply of physiologically homogeneous individuals. Therefore, development of laboratory rearing techniques is necessary. According to Singh (1977), the goal of mass rearing is to produce the maximum number of insects in the shortest possible time and under the most economical conditions such as minimum labor and space. To meet these practical requirements, both rearing materials and equipment should be kept as simple and inexpensive as possible but they must be nutritionally and behaviorally optimal for the insect.

Only limited published information exists on the rearing of DBM on semi-synthetic diets or cruciferous seedlings. The present paper attempts to review the existing literature and to describe the techniques for laboratory and mass rearing of DBM.

Rearing on Semi-Synthetic Diets

Dietary formulation

Most of the diets developed for lepidopterans are formulated as the semi-synthetic containing natural food either fresh or dried. Biever and Boldt (1971) first adopted a semi-synthetic diet (Table 1), which was previously formulated for *Heliothis zea* and *H. virescens* by Berger (1963), for rearing DBM. Later, Hsiao and Hou (1978) modified this diet by adding linseed oil, i-inositol and cholesterol (Table 1). These diets can be regarded as wheat germ-based diets. However, Hou and Hsiao (1979) suggested deletion of formaldehyde from the diet in the presence of antibiotics. Agui et al (1975) formulated two diets for the cabbage armyworm, *Mamestra brassicae*, that could be used for rearing DBM as well. The composition of their diet is shown in Table 2. Both diets contain a large amount of finely chopped cabbage leaves instead of dried leaf powder. The addition of fresh materials may make the dietary effect inconsistent due to variation in water content of fresh leaves in each preparation. Besides, preparation of the diet is impossible when fresh leaves are not available. In comparison, dried leaf powder can be stored for months if kept cool and is thus convenient for dietary preparations.

Table 1. Composition of semi-synthetic diets for rearing of DBM

Ingredient	Amounts in 100 g diet	
	Biever and Boldt	Hsiao and Hou
Casein, vitamin-free	3.500 g	3.500 g
Alphacel	0.500	0.500
Wesson's salt mixt.	1.000	1.000
Sucrose	3.500	3.500
Wheat germ	3.000	3.000
Methyl-p-hydroxybenzoate	0.150	0.150
Choline chloride	0.100	0.100
Ascorbic acid	0.400	0.400
Aureomycin	0.015	0.015
Cabbage leaf powder	1.250	3.000
Cholesterol	—	0.250
i-inositol	—	0.018
Agar	2.250	2.250
Distilled water	84.00 ml	84.00 ml
KOH (4M)	0.50	0.50
Formaldehyde	0.05	0.05
B-vitamin solution	1.00	1.00
Linseed oil	—	0.65

Table 2. Composition of semi-synthetic diets for *Mamestra brassicae* and DBM^a

Ingredient	Diet I	Diet II
Water	100.00 ml	100.00 ml
Agar	7.50 g	5.00 g
Potato starch	5.00	5.00
Sucrose	5.00	5.00
Soybean flour	20.00	20.00
Wesson's salt mixture	1.75	1.75
K ₃ PO ₄	0.50	0.50
Citric acid	0.25	0.25
Dried brewers' yeast	7.50	7.50
Cholesterol	0.25	0.25
Soybean oil	1.50	1.50
Powdered filter paper	11.25	—
Wheat germ	—	20.00
L-ascorbic acid	2.00	2.00
Propionic acid	0.35 ml	0.35 ml
Finely chopped cabbage leaves	65.00	75.00

^a Source: Agui et al 1975.

Preparation of diet

The proper preparation procedure is extremely important in determining whether the diet is acceptable by various stages of insects and is nutritionally optimal for their growth and development. When making a suitable diet, at least two technical factors should be considered: dietary ingredients should be well mixed and should retain all nutrients as far as possible for the final diet. Hsiao and Hou (1978) prepared a diet by mixing solid dietary components, liquids, and anti-mold compounds with a melted

agar solution, and then added vitamins and antibiotics. However, an improved preparation method, involving heating wheat germ before adding to other components and changing the mixing order, resulted in a better diet (Hsiao and Hou 1982). The improved procedure is as follows: Alphacel, cabbage leaf powder, and the heated wheat germ are mixed thoroughly and then cholesterol and linseed oil dissolved in ether are added to this mixture, stirring well to evaporate the ether. Sucrose, casein, methyl-p-hydroxybenzoate, and Wesson's salt mixture are added to the melted agar solution, and the resulting solution is poured together with the solid mixture into a blender. Immediately add aureomycin, ascorbic acid, i-inositol, choline chloride, potassium hydroxide and vitamin B. The whole mixture is ground thoroughly in the blender for 1-2 min and is then ready to be dispensed (Hsiao, M. L. and R. F. Hou unpublished data). The diet can be dispensed in vials, dishes or other appropriate containers for feeding insects. The leaf powder is usually prepared from fresh cabbage leaves by drying at 60°C for 24 h and then grinding up with a blender, The powder is then screened through a 100-mesh sieve before storing at low temperature.

Feeding procedures

Biever and Boldt (1971) found that females were able to oviposit on paper towels; therefore, eggs are very easy to collect. They glued or stapled sections of oviposited towels with about 100 eggs to the lids of 6 oz ice-cream cups containing the diet. Most larvae will pupate on the lids which are then hung on a rod in the adult cage (about 2,160 cm³) for emergence. Hsiao and Hou (1978) reared 30 newly hatched larvae on 5 ml diet placed in a glass tube (3 × 9 cm). The diet was changed every two to three days.

Growth and development of insects

The diet developed by Biever and Boldt (1971) seemed satisfactory for DBM. The developmental period from egg to adult was only 19 days; egg, 3; larva, 11; pupa, 5 days at 23 + 1°C, 60 + 5% RH. Similar results were obtained when DBM was fed on artificial diets and on host plants in Taiwan (Wu 1968; Hsiao and Hou 1978). However, Harcourt (1957) reported that it took about 30 days from larval to adult stage in Canadian populations. The mean hatchability was 90%, mean larval survival 90%, and pupal survival 86%. It was also indicated that egg viability remained stable after rearing for one year. But Hsiao and Hou (1978) could only obtain about 62% larval survival and about 59% pupation when insects were fed on the Biever and Boldt diet, while both larval survival and pupation were about 83% when insects were fed on the modified diet which in turn was similar to results when the insects were fed on cabbage leaves. Adult emergence from the larvae fed on the Biever and Boldt diet was also poor (Table 3). This difference could possibly be ascribed to variations in the method of dietary preparation and to geographic variation of the insect. Nevertheless, it is conceivable that semi-synthetic diets are useful for laboratory rearing of DBM although they are not more economical than rearing on cruciferous seedlings as will be described in the next section.

Table 3. Survival and development of DBM fed on various diets^a

Diet	Larval survival (%)	Pupation (%)	Adult emergence (%)
Biever and Boldt	61.9	58.8	26.4
Modified diet	83.4	83.4	83.4
Cabbage leaves	84.8	84.8	84.8

^a Source: Hsiao and Hou 1978.

Rearing on Cruciferous Seedlings

Chi and Sun (1975) reported a mass rearing method using cabbage heads kept in petri dishes containing water. They were able to obtain 100 mature larvae per head from 30-50 adults which were allowed to oviposit on the head. This method is rather costly and has low productivity. A simple mass rearing technique was developed using rape seedlings germinated densely in plastic vessels by Koshihara and Yamada (1976). Later, Yamada and Koshihara (1978) made some modifications and described the rearing techniques in greater details. This section is intended to summarize their rearing procedures in brief and to elicit a recent modification of this technique by Liu and Sun (1984).

Seedling preparation

Seedlings of rape or radish are used as rearing materials. To prepare the seedlings, seeds are soaked in water for 5 to 24 h, and then treated with disinfectants, for example, Benlate T, Daconil, and Homai for 30 min. Seven to 10 g disinfected seeds are transferred into a small plastic vessel (9.5 cm diam, 5.5 cm deep) with a perforated lid on top and a piece of paper at the bottom on which the seeds are sown. Each vessel is filled with 4 ml water. The seeds begin to sprout after one day, and are ready to feed larvae on their cotyledons in three days.

Rearing procedures

Three pairs of one to two day-old adults are confined in a rearing vessel. They will lay about 150-250 eggs in three days. The seedlings are usually exposed to natural light. The hatched larvae feed on the seedlings and reach 4th instar by 14 days after introduction of parental adults. Most will pupate by 17 days at 25°C, with a 16 h photoperiod, the pupation rate being over 80%. Replenishment of water is not necessary in this system, as the optimal humidity is 60- 70% RH. The mature larvae will pupate on pieces of folded filter paper placed on top of the seedlings; the pupae can be collected easily from the paper.

Mass rearing

It was found that pupation rate and pupal weight varied with the larval density in each rearing vessel. Table 4 shows that the pupation rate is over 90% when feeding 50-100 newly hatched larvae in each vessel, but it reduces drastically if the larval number is increased to 200-300. In this rearing system, 100-140 pupae can be obtained in each vessel, the pupal duration being about four to five days. For mass rearing it is practical to prepare 18 rearing vessels using about 150 g of radish seed, twice a week. By this method some 15,000 pupae will be obtained each month. Since the rate of adult emergence is about 80%, with a sex ratio of 1:1, some 6,000 pairs of adults per month can be obtained by this rearing program.

According to Yamada and Koshihara (1978), continuous rearing was carried out for two years using this technique; however, some wild adults collected from cabbage fields were introduced into the rearing colonies once or twice each year to avoid crossing between genetically related individuals. Rearing was continued for 30 generations with normal development. They did not find any distinct abnormality in pupation of the insects when investigation was carried out up to 21 generations (Table 5). This mass rearing technique has been widely used to rear colonies of DBM for studies on physiology, pathology, toxicology and other factors in Japan and elsewhere (Koshihara and Yamada

Table 4. Pupation and pupal weight of DBM fed on rape seedlings^a

No. of newly hatched larvae	Pupation (%)	Pupal weight (mg)	
		Female	Male
50	96.0	4.3	3.7
100	93.5	4.9	3.9
200	74.0	3.6	3.4
300	77.9	4.0	3.8

^a Koshihara and Yamada 1976.

Table 5. Continuous rearing of DBM on rape seedlings^a

Generation	No. of rearing vessels	No. of pupae per vessel
4	6	187.2
5	3	87.7
10	12	99.2
12	6	65.2
13	6	107.8
16	6	134.7
18	4	104.8
21	12	74.0

^a Source: Yamada and Koshihara 1978.

1980, Ishii et al 1981, Liu et al 1982, Miyata et al 1982, Tomiyama and Aoki 1982, Yamada and Kawasaki 1983, Nemoto et al 1984).

A modified rape seedling method

Recently, Liu and Sun (1984) reported a modification in the rearing DBM by Koshihara and Yamada (1976) method. In their procedure they sowed seeds in vermiculite in a rearing vessel (9 cm diam, 4 cm deep) without covering them. This, according to the authors, permitted better air circulation and lesser microbial contamination. Mass oviposition by 100 adults on the seedlings in an egg-laying cage (20×20×30 cm) was undertaken to save labor. The oviposited seedlings are transferred into a rearing cage (30×30×50 cm) for mass rearing. To collect the larvae, it is necessary only to reverse the rearing vessel and to tap the bottom of it, allowing the larvae to spin down, or they can be helped using forceps or a brush. This rearing method can be carried out without temperature control or critical lighting equipment, and is considered to be more practical and a better technique for mass rearing of the DBM in places where manpower and facilities are limited.

Concluding Remarks

Although mass rearing of DBM has been found feasible using cruciferous seedlings, it seems that microbial contamination in the course of the feeding period may still cause some practical problems. Treatment of seeds with disinfectants and improvement in air circulation by uncovering the rearing vessels containing seedlings have been attempted with satisfactory results.

The semi-synthetic diets with a wheat germ base were formulated for laboratory rearing of DBM; however, dietary composition has to be simplified to meet the

economical assessment. In addition, feeding procedures on artificial diets for the purpose of mass rearing remain to be developed to increase the productivity per unit quantity of diet and time. Theoretically, development of mass rearing methods should be directed toward feeding on artificial diets rather than on natural food, especially when aseptic rearing is essential. It is thus suggested that rearing of DBM on semi-synthetic diets should be encouraged and ought to be considered as a prospective method for mass rearing of experimental colonies.

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