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AN ARTIFICIAL DIET AND A METHOD FOR REARING THE TURNIP MOTH, *AGROTIS SEGETUM* SCHIFF. (LEPIDOPTERA: NOCTUIDAE)

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Kun-Jun Wu, Pei-Yu Gong and Xiu-Zhen Li (1992) An artificial diet and a method for rearing the turnip moth, *Agrotis segetum* Schiff. (Lepidoptera: Noctuidae). *Bull. Inst. Zool., Academia Sinica* **31**(2): 121-129. An artificial diet with lamb's quarters, *Chenopodium album* L. and wheat germ as its major ingredients was developed to rear larvae of the turnip moth, *Agrotis segetum* Schiff. for 16 successive generations. During the rearing period, 87-96% of the neonate larvae successfully pupated, of which less than 5% failed to emerge. Egg production of moths from diet-fed caterpillars averaged 1,000, with about 90% of the eggs hatching. A rearing technique for assuring a high survival rate, and performances of turnip moths under different rearing conditions are described.

Key words: *Agrotis segetum*, Artificial diet, Cyclic mating, Rearing method.

The turnip moth, *Agrotis segetum* Schiff., is a polyphagous pest which seriously infests the seedlings of many crops and vegetables in northern China. A year round supply of caterpillars is necessary for the investigation of their physiology and ecology, as well as for the propagation of pathogenic microbes for their control. Maintaining these moths on fresh food plants is tedious work, especially during cold weather seasons. The development of artificial diets simplifies laboratory rearing procedures.

There have been several published papers concerning artificial diets for turnip moths. However, those diets so far formulated were not satisfactory for rearing moths for successive generations. They either caused lower larval survival (Khlistovskii and Uspenskaya, 1969), reduced adult fecundity (Uspenskaya and Kozhaeva, 1974) or became too expensive for practical use (Ekbom, 1977). In addition, the number of surviving successive generations raised on these diets has been small, usually less than four (Gomaa, 1978; Skopina and Boyarkina, 1975), or in the best case, eight (Uspenskaya and Kozhaeva, 1974). Although Sherlock (1979) claimed that over 20 successive generations of turnip moths survived on his diet, moths from the field had to be annually introduced into his colony in order to prevent a decline in viability.

This paper describes a new artificial diet for rearing turnip moths, and describes their performances under different rearing conditions.

MATERIALS AND METHODS

Insect colony

Agrotis segetum Schiff. adults were collected using light traps in the field. F_1 caterpillars were fed fresh lamb's quarters leaves, *Chenopodium album* L.; the experiment started with F_2 neonate larvae.

Diet preparation

The components of our proposed diet are shown in Table 1. Young lamb's quarters leaves were collected near our Institute in the spring, then dried under sunlight, ground into powder, and passed through a 60 mesh sieve. The wheat germ and soybean powder mixture was prepared according to procedures described in a previous paper (Wu, 1985).

Diet ingredients, in grams per kilogram (Table 1), were mixed as follows: (1) agar was added to 400 ml distilled water in container 1 and heated until the agar went into solution; (2) ascorbic acid was dissolved in 50 ml water in container 2, then linoleic acid was added to the same container; (3) the remaining ingredients were thoroughly mixed in container 3, then the remaining 350 ml distilled water was added and mixed thoroughly; (4) we poured container 1 into container 3, constantly stirring this mixture; after cooling the contents of container 3 to below 45°C, (2) was added and continuosly stirred until the mixture solidified. The prepared diet should be stored uncovered at room temperature for about 1 h to allow for evaporation, then covered and kept refrigerated until used.

Rearing method

Larvae were reared in groups of 15, 10, and 5 individuals in the first, 2nd and 3rd instars, respectively, in 25 ml beakers lined at the bottom with filter paper and covered with a white cloth and plastic sheet. Fourth and later instar larvae were reared individually in identical containers. For air exchange and the escape of excessive moisture a few holes in each cover were punched with a needle. Mature larvae were separated into clean beakers containing moist soil with bits of the diet placed on filter paper on top of the soil; filter paper and larval feces were removed when pupation occurred.

Five pairs of newly emerged moths were placed in a cage made of plastic gauze (25×25×25 cm) for 3 days. The

Table 1
Components of an artificial diet for rearing larvae of the turnip moth

Function	Ingredient	Weight in g per kg
Plant material	Lamb's quarters, *Chenopodium album* L.[a]	74.0
Nutrients and vitamins	Wheat germ[b]	50.0
id.	Brewer's yeast (Medical)[c]	34.0
Protein	Soybean powder[b]	20.0
Vitamin	Ascorbic acid	2.0
Lipid	Linoleic acid	1.0
Antiseptic	Methyl parahydrobenzoate	2.0
id.	Sorbic acid	1.0
Inner carrier	Agar	16.0
id.	Distilled water	800 (ml)

[a] Whole young leaves were used; see text for details.
[b] Prepared in the laboratory following the procedures described in a previous paper (Wu, 1985).
[c] Jiankangpai, Shanghai Yeast Factory, Shanghai, China.

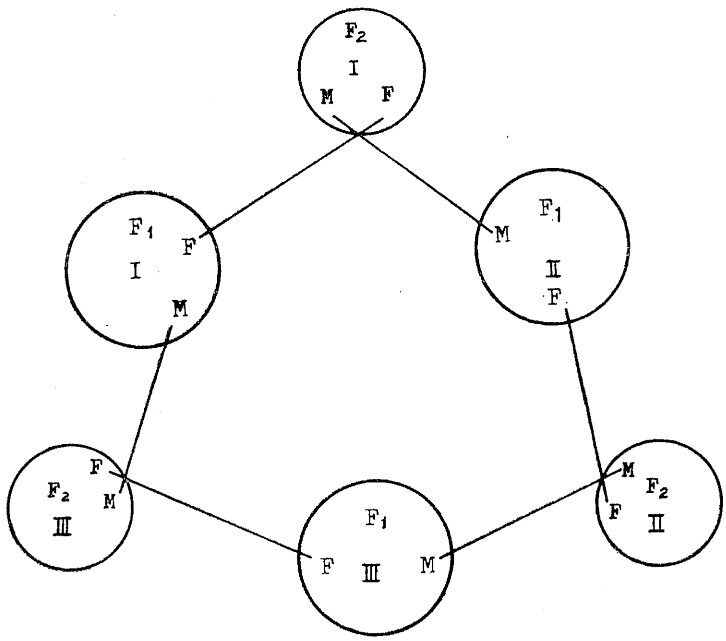

Fig. 1. Cyclical mating program for the turnip moth. F_1, F_2, ··· refers to the 1st, 2nd, ··· generation, respectively.
I, II, III—separated groups; F—female, M—male.

pairs were then reared separately in glass lamp chimneys (10×10 cm) covered on both sides with gauze; another piece of identical gauze was placed inside the chimney to capture eggs. The chimney was put on a wide-mouth jar containing some water; a 10% bee honey solution was supplied as adult food.

The turnip moths were then separated into three groups, and a cyclical mating procedure was followed throughout maintenance of the colony to reduce excessive inbreeding (Fig. 1).

Rearing experiments were conducted at cyclic diurnal temperatures ranging from 23° to 28°C under a 14L:10D photoperiod. To block microbial infection, eggs were disinfected with a 10% formalin solution for 20 min; beakers were disinfected with the same solution for 1 h before use. Cages and other equipment were irradiated with ultraviolet rays for 2 h, twice a week.

Determination of food utilization efficiences

Newly hatched larvae were raised on the artificial diet; fresh plant leaves were used as control. One-day-old penultimate instar larvae were selected and weighed for determination of food utilization efficiencies. It was observed that defecation in all caterpillars almost completely ceased within 1 h following food withdrawal. Therefore, caterpillars were deprived of food for 1 h before and after feeding experiments. After 72 h of feeding, larvae, uneaten diet or leaves, and feces were collected; these were then oven-dried at 80°C until a constant weight was reached. Food ingestion was calculated as the difference between initial food weight and unconsumed food weight. In the case of fresh plant leaves, ingestion was corrected for weight loss due to respiration (Schroeder, 1976). Similarly, increases in larval biomass during the feeding experiments were recorded.

Parameters of food utilization by larvae in terms of approximate digestibility (AD), efficiency of conversion of ingested food into larval biomass (ECI), and the efficiency of conversion of digested food into larval biomass (ECD), were estimated on the basis of dry weight (Waldbauer, 1968). Parameters for energy and nitrogen utilization were derived from dry matter budgets, with energy and nitrogen content of the experimental materials determined using a Phillipson microbomb calorimeter (Ser. 416, Gentry Inst. Inc.) and following the semimicro-Kjeldahl procedure (McKenzie and Wallace, 1954), respectively.

Data analysis

A Student-Newman-Keul's test (Steel and Torrie, 1960) was applied for data analysis with significance at the 5% level.

RESULTS

Performances of turnip moths on different diets

Performances of the first generation of insects reared on the artificial diet, lamb's quarters leaves, or young cotton leaves are shown in Table 2. Cutworms fed the artificial diet not only survived better, grew significantly faster, and produced larger pupae, but they also showed greater adult fecundity in comparison with those raised on the two types of plant leaves.

Rearing of successive generations

Sixteen successive generations of turnip moths were reared on the artificial diet. Fig. 2 shows that in our laboratory population, 87–96% of newly hatched larvae successfully pupated, of which less than 5% failed to emerge. Female pupae in these generations weighed 383.5 mg on average, 19% more than first generation moths reared on *Chenopodium album* leaves.

Egg production for six generations examined averaged about 1,000/female, with a range of 681 to 1,175; the egg hatching percentage varied between 87.9% and 98.2%, with a mean of 94.2%.

Food utilization

Of nine nutritional indices determined, seven indices in caterpillars raised on the artificial diet were significantly greater than those measured in the larvae raised on young cotton leaves (Table 3). The

Table 2
Performances of turnip moths reared on different foods at 25±1°C (M±SE)*

	Artificial diet	Lamb's quarters leaves	Cotton leaves
No. of larvae tested	100	100	100
Larvae			
% Survival	96.0	78.0	94.0
Duration (day)	23.1±0.3[a]	26.2±0.3[b]	33.7±0.3[c]
Instar number	6	6	6-7
Pupal weight (mg)			
Female	462.3±6.8[a]	323.6±5.2[b]	283.5±4.0[c]
Male	395.3±5.1[a]	299.0±4.7[b]	273.5±3.7[c]
No. of eggs laid per female	1,174.8±104.2[a]	893.4±155.7[b]	1,120.9±72.7[a]

* Means followed by different letters differ significantly at $p<0.05$.

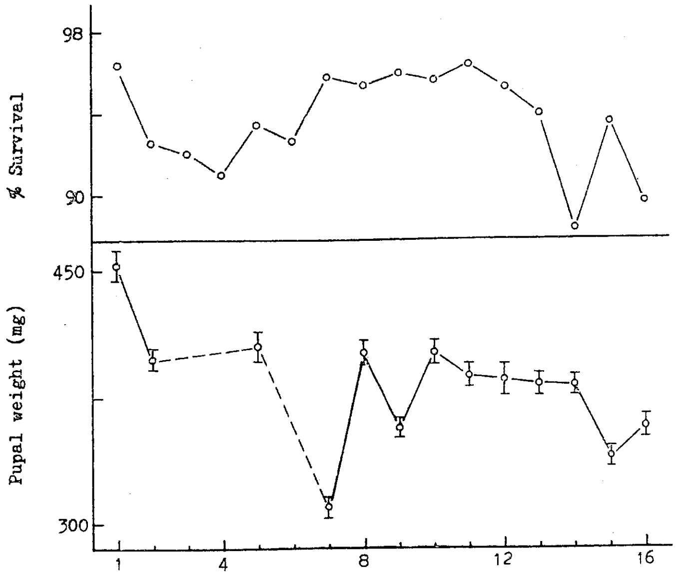

Fig. 2. Larval survival percentages and female pupal weights of turnip moths successively reared on the artificial diet.

ECI for nitrogen in moths raised on the actificial diet was also greater, but the difference between the two groups was not significant. The only exception was the AD for nitrogen, which was smaller in artificial diet group than in cotton leave group. These determinations indicate that moths larvae utilized the artificial diet more efficiently than their natural plant food (young cotton leaves).

Effects of rearing conditions

1. *Density*

Cannibalism rarely occurred during earlier instars on the present diet, while later instar larvae became cannibalistic

Table 3
Digestion and utilization of different foods by turnip moth larvae at 25±1°C (M±SE)*

	Efficiency (%)		
	AD	ECI	ECD
Artificial diet			
Dry matter	50.36±1.47	21.69±1.16	42.52±1.26
Energy	54.07±1.34	27.90±1.50	51.19±1.64
Nitrogen	62.01±1.13	40.89±2.24	65.30±2.45
Cotton leaves			
Dry matter	45.65±1.26	13.84±0.37	31.19±1.55
Energy	50.63±1.14	15.20±0.39	30.01±1.36
Nitrogen	67.52±0.75	35.97±1.15	53.56±1.98

* 25 larvae were determined for each group during their penultimate instars, *i.e.* 5th and 6th instars for the cases of the medium and cotton leave, respectively (see Table 2). For definition of AD, ECI and ECD, see text (section Materials and Methods).

Table 4
Performances of the turnip moth under different rearing densities at 25±1°C (M±SE)*

	Individuals per container				
	1	2	4	8	16
Number of larvae tested	84	12	24	48	96
Larvae					
% Survival	100	100	83.3	66.7	46.9
Duration (day)	24.5±0.2[a]	24.3±0.6[a]	25.0±0.3[ab]	25.7±0.4[bc]	26.6±0.3[c]
Pupal weight (mg)					
Female	387.8±9.7[a]	387.6±11.4[a]	338.9±9.1[b]	326.1±8.6[b]	327.2±9.1[b]
Male	366.0±6.5[a]	360.6±13.3[ab]	329.2±8.9[bc]	311.2±6.9[c]	278.9±5.2[d]

* The experiments started with early 4th instar larvae. Glass jars of 9 cm in diam. were used as rearing containers for all groups except the first one (25 ml beakers). Means followed by different letters differ significantly at $p<0.05$.

to a greater extent when raised in higher density groups. Table 4 shows that caterpillar survival rates decreased, developmental times were prolonged, and pupae became smaller with increasing rearing density.

2. *Soil substratum*

Agrotis segetum is a subterranean pest; it would seem that this would make them unsuitable for rearing on artificial diets because soil can easily be contaminated with microbes. As a matter of fact, larvae on the actificial diet did not need substratum soil during most larval instars. On the contrary, a lower survival rate was observed when soil was added too soon. However, successful pupation was impossible if no soil was provided during the late sixth instar, as shown in Table 5.

3. *Time for providing soil substratum*

Active feeding by sixth instar larvae lasted about six days under the above-described rearing conditions. Adding substratum soil on day one made nearly half of larvae fail to pupate; adding substratum soil later increased pupation percentages. Mean water content of the

Table 5
Effects of substratum soil on survival and growth of turnip moths at 25±1°C (M±SE)*

	Time of soil added		
	Early 4th instar	Late 6th instar	No soil added
No. larvae tested	84	84	84
% Pupation	86.9	98.8	21.4
% Eclosion	82.1	96.4	10.7
Larval duration (day)	23.2±0.2[a]	23.6±0.2[a]	24.8±0.7[b]
Pupall weight (mg)			
Female	393.9±4.0[a]	390.4±6.9[a]	402.2±13.5[a]
Male	368.1±6.6[a]	342.5±6.5[b]	347.9±17.0[ab]

* Initial water content in soil was about 5.5%. Means followed by different letters differ significantly at $p<0.05$.

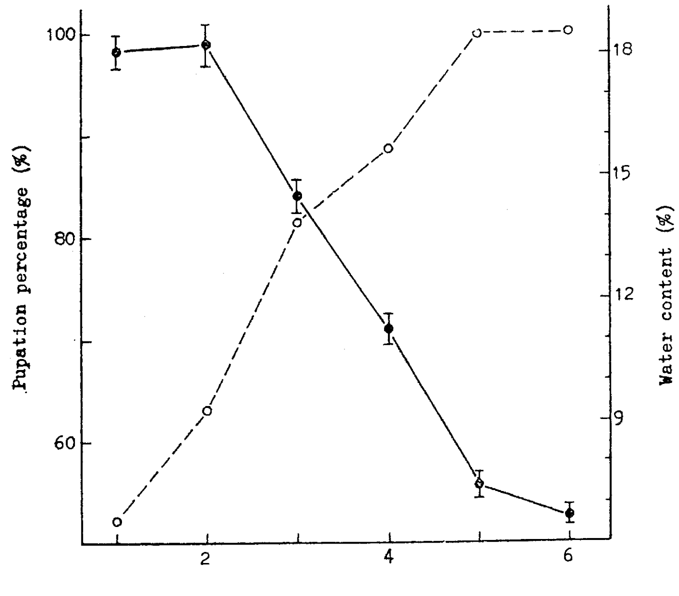

Fig. 3. Pupation percentages for 6th instar larvae (open cycle) and water content in soil after pupation (solid cycle) in relation to the time soil was added. The initial water content of the soil was about 5%.

substratum soil measured on the day of pupation changed adversely (Fig. 3), suggesting that drops in pupation success rates in cases where soil was added too early resulted from the high water content of the soil.

4. *Storage at low temperature*

In the field, turnip moths overwinter during their larval stage; as an experimental insect, moth growth occasionally needs to be controlled. Therefore, a series of low temperature storage experiments were conducted. Our results in Table 6 show that the final instar larval stage was the stage most suitable for storage at 5-8°C, although all prepupae showed that they could successfully pupate after being kept at identical temperatures for one month. About 80% of sixth instar larvae tested survived and succeeded in entering their next stage after up to four months of storage at this range of temperatures. Our observations were that caterpillars still fed at a very low frequency during storage

Table 6
Effects of low temperature storage on turnip moth survival rates*

Instar or stage	No. insects tested	Stored period (day) 30	60	90	120	150
		% Survival				
Larval instar						
4th	172	75.0	15.1	1.2	0	
5th	140	92.1	88.6	68.6	56.4	42.1
6th	129	100.0	100.0	89.1	78.3	51.9
Prepupae	112	100.0	67.0	55.4	51.8	33.0

* Insects were refrigerated at 5-8°C.

period, which indicates that the artificial diet should be provided for stored larvae.

CONCLUSION

A new artificial diet has been developed for rearing larvae of the turnip moth, *Agrotis segetum*. On this diet, 16 successive generations of turnip moths were successfully reared with a 87-96% larval survival rate and an average fecundity rate of about 1,000/female. Both rates were either better than or comparable to those of first generation moths raised on natural diets of fresh plant leaves.

This diet has fewer ingredients than artificial diets reported by other authors. Moreover, its major ingredients are easily available. Lamb's quarters is a species of weed found in large areas of China in late spring and early summer. Wheat germ and soybean powder can be easily prepared in the laboratory.

For good results special care should be taken with rearing methods. In addition to routine sterilization of eggs and rearing equipment, full air circulation must be maintained, particularly during early caterpillar instars. Also, a cyclical breeding procedure should be followed.

Providing moist substratum soil at the proper time is very important for mature larvae to pupate. The most suitable time to add the soil is during the the last two days of the final instar.

If need be, last instar larvae can be stored at 5-8°C for up to four months with about an 80% survival rate. The artificial diet should be offered during storage, and examinations at regular intervals should be made.

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黃地老虎 (*Agrotis segetum* Schiff.) 的人工飼料和飼養方法

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本文報導黃地老虎 (*Agrotis segetum* Schiff.) 幼蟲的一種人工飼料和飼養方法，該飼料的主要成分是灰藜 (*Chenopodium album* L.) 葉粉和麥胚粉。已用這種人工飼料連續飼養黃地老虎 16 個世代，幼蟲化蛹率在 87～96%之間，95%以上的蛹均能正常羽化。成蟲平均產卵千粒左右，卵的孵化率約 90%。同時，還研究了不同飼養條件對黃地老虎的影響。