アワヨトウの簡易人工飼料

誌名	Applied entomology and zoology
ISSN	00036862
巻/号	114
掲載ページ	p. 278-283
発行年月	1976年12月

Appl. Ent. Zool. **11** (4): 278—283 (1976)

A Simple Artificial Diet for Mass Rearing of the Armyworm, *Leucania separata* WALKER (Lepidoptera: Noctuidae)

Kazuo HIRAI

Chugoku National Agricultural Experiment Station, Fukuyama 720, Japan

(Received June 1, 1976)

A simple artificial diet for the mass rearing of *Leucania separata* has been devised. The diet, composed of wheat bran, corn powder, dried yeast, ascorbic acid, linseed oil, agar, water and three mould inhibitors, satisfactorily supports the development of *L. separata* and *L. loreyi*.

The larval development of *L. separata* on the artificial diet did not differ from that reared on some graminaceous food plants. The weight of pupae reared on the artificial diet was heavier than that reared on the food plants. The ensuing adults laid as many eggs as did those emerged from larvae reared on the food plants.

Sorbic acid added to the artificial diet as a mould inhibitor caused pupal deformity. The rate of emergence from larvae reared on the diet containing sorbic acid fell remarkably in *L. separata*, but none of the adverse effects of sorbic acid on pupation and emergence were observed in *L. loreyi*.

INTRODUCTION

The armyworm, *Leucania separata*, occurs singly or together with *L. loreyi* in graminaceous plants in the west of Japan. The damage caused by these insect pests has been serious. The development of noninsecticidal control measures and mass rearing of these species can be facilitated by the use of a practical artificial diet. Trials of rearing *L. separata* on an artificial diet have been reported in the rearing on sterilized corn stalks (TSUTSUI, 1954) and the artificial diet for mass rearing (SATO, 1965), but no artificial diet which could completely rear newly hatched larvae to adults has been reported.

The purpose of this experiment was to devise a simple artificial diet on which *L. separata* and *L. loreyi* can be reared in large numbers, together with simplifying the composition of the artificial diet for *L. loreyi* (HIRAI, 1975). At the same time, larval and adult development of *L. separata* reared on the artificial diet and various food plants is compared.

MATERIALS AND METHODS

Preparation of the artificial diet. The basic ingredients used in the artificial diet are corn seed powder and wheat bran, which were ground through a 1-mm diameter sieve. The other ingredients are listed in Table 1. Diet 2 is a modification of Diet 1 which was reported as a simple artificial diet for *L. loreyi*. Diet 3 is the same as Diet 2 except for substitution of corn powder with pinto bean powder. Diet 4 is the same as Diet 3 except linseed oil. The general method of preparation of the artificial diet

Table 1. Composition of Artificial Diets Used for Rearing *L. separata* and *L. loreyi*

Ingredient	Diet 1[a]	Diet 2
Wheat bran	100 g	180 g
Corn powder	100	90
Dried brewer's yeast	40	30
Ascorbic acid	6	6
Formalin (12.3%)	—	3
Methyl p-hydroxy benzoate	3	1.5
Sodium propionate	3	1.5
Linseed oil	4	1.5
Cellulose powder	22.6	—
Cholesterin	1	—
Water	400	400
Agar	13	11
Water	430	450

[a] Diet 1 is for the previous *L. loreyi* method.

is mostly the same as that described by Shorey and Hale (1965). Each of the ingredients of, Diet 2 with the exception of agar, is blended with 400 ml water. The agar is separately dissolved in 450 ml water. The agar solution is cooled to below 50°C, and mixed with the other blended ingredients. The diet is poured to a depth of 7 cm in 1-liter jars. The stock of diets was kept at 5°C in a refrigerator.

General rearing. Insects of the second generation reared on the artificial diet were used in this experiment. An egg mass which had turned dark brown was palced in a plastic culture dish (90 mm in diameter and 20 mm in depth) containing a slice of the artificial diet or the food plant. (Fresh young plants, about 30 cm in height, of sweetcorn, sorghum, sudangrass and grain sorghum were used.) Larvae from the newly hatched instar to the 2nd instar were reared in gregarious condition. The larvae after the 3rd instar were reared two insects per dish on the diet, but were reared singly on the food plant. A small water cup was placed in the dish in order to prevent from drying. The artificial diet was renewed every two days. The food plant was replenished daily. The pupae were weighed individually within about 12 hours after pupation, and the moth energences were recorded daily. One pair of newly emerged adults was introduced into a plastic cup (95 mm in diameter and 55 mm in depth) and supplied with a small cup containing a piece of cotton moistened with 10% aqueous honey solution, and with a folded paraffin paper for egg deposition. The number of eggs was counted daily. Larval and adult rearing were performed in a constant chamber maintained at 25°C with 16-hr light and 9-hr darkness. The relative humidity of the chamber was not controlled.

RESULTS

Larval and pupal durations, pupal weight and the rate of pupation and emergence are given in Table 2. Average durations of larvae fed on Diet 4, Diet 3, Diet 1, sudangrass, Diet 2, sweetcorn, sorghum and grain sorghum were: 16.8±0.7, 16.8±0.9, 17.2±1.0, 17.9±0.4, 18.2±1.1, 18.7±0.7, 18.9±0.7 and 19.1±0.4 days, respectively. The results also showed highly significant differences between durations of larvae reared

Table 2. LARVAL AND PUPAL DURATIONS, PUPAL WEIGHTS AND THE RATE OF PUPATION AND EMERGENCE OF *L. separata* REARED ON THE DIFFERENT DIETS[a]

Diet	Number used	Larval duration (days)	% of pupation	Pupal weight (mg)	% of emergence	Pupal duration (days)
Diet 1	70	17.2±1.0	98.6	302.0±32.2	82.6	11.6±0.6
Diet 2	70	18.2±1.1	97.1	341.0±40.2	94.0	11.7±0.6
Diet 3[b]	70	16.8±0.9	98.6	325.2±39.7	89.9	11.5±0.6
Diet 4[c]	68	16.8±0.7	97.1	322.7±31.8	89.4	11.8±0.6
Sweetcorn	20	18.7±0.7	90.0	302.5±18.5	94.4	11.4±0.5
Sorghum	20	18.9±0.7	100.0	253.7±30.0	90.0	11.2±0.5
Sudangrass	20	17.9±0.4	100.0	288.1±25.0	100.0	11.9±0.5
Grain sorghum	20	19.1±0.4	80.0	294.1±30.7	86.7	11.5±0.7

[a] Mean±SE was used in Table 2-4.
[b] Diet 3 is the same as Diet 2 except for substitution of corn powder with pinto bean powder.
[c] Diet 4 is the same as Diet 3 except linseed oil.

on Diets 3 and 4 versus Diet 1 ($P<0.01$), Diet 1 versus sudangrass ($P<0.01$), sudangrass versus Diet 2 ($P<0.05$) and Diet 2 versus sweetcorn ($P<0.05$).

In calculating the rate of pupation and emergence, partial pupation, deformed pupae and wing-wrinkled adults were excluded. The rate of pupation was almost the same among the different diets except grain sorghum and sweetcorn. Sex ratio for pupae was approximately 50% in every diet. The rate of emergence for pupae reared on sudangrass, sweetcorn and Diet 2 was higher than that reared on any other diets. Average weights of pupae were: 341.0±40.2, 325.2±39.7, 322.7±31.8, 302.5±18.5, 302.0±32.2, 294.1±30.7, 288.1±25.0 and 253.7±30.0 mg when the larvae fed on Diet 2, Diet 3, Diet 4, sweetcorn, Diet 1, grain sorghum, sudangrass and sorghum, respectively. The results showed highly significant differences between the weight of pupae reared on Diet 2 versus Diets 3 and 4 ($P<0.05$), Diets 3 and 4 versus sweetcorn ($P<0.01$), and sudangrass versus sorghum ($P<0.01$). There was no correlation between larval durations and pupal weights. Durations for pupae were almost the same among the different diets.

Sexual differences were not found in larval duration and pupal weight, but pupal duration of females was significantly shorter by one day than that of males ($P<0.01$).

Adult longevity and the number of eggs are given in Table 3. Many of the female moths started laying eggs on the third or fourth night after emergence and continued for

Table 3. PREOVIPOSITIONAL AND OVIPOSITIONAL PERIODS, MEAN NUMBER OF EGGS AND ADULT LONGEVITY OF *L. separata* EMERGED FROM LARVAE REARED ON THE DIFFERENT DIETS

Diet	Pairs used	Preoviposition period (days)	Oviposition period (days)	Egg number per female	Longevity (days)	
					♀	♂
Diet 2	14	2.8±0.7	9.7±2.4	1332±529	13.4±3.2	19.4±3.8
Sweetcorn	8	3.1±0.4	8.3±2.3	1239±160	11.8±2.0	21.6±5.1
Sorghum	8	2.6±0.5	7.0±0.8	885±269	9.8±0.9	22.0±3.2
Sudangrass	6	3.5±0.5	7.0±1.0	1466±371	10.7±0.5	21.0±5.7
Grain sorghum	5	3.6±0.9	7.0±2.2	1237±335	10.2±1.6	19.8±7.1

5–13 days. Mating occurred in two of the pairs of adults used from those reared on Diet 2. Only a few matings were found among the other pairs reared on the different diets, suggesting that *L. separata* cannot mate well in such a small cup as was used.

At first, the eggs laid by mated females are yellowish while those laid by unmated females are white. As hatching time nears, the former turn dark brown but the latter dry up. Since there is no significant difference between the number of eggs laid by mated and unmated females, the average number of eggs was calculated for each of the different diets irrespective of mating. The number of eggs laid by females reared on sorghum was significantly smaller than that laid by females reared on the other diets ($P<0.05$).

Preovipositional periods of females emerged from pupae reared on grain sorghum and sudangrass were longer than those reared on the other diets. There was no significant difference in the ovipositional periods and the adult longevity among the different diets. Male longevity of the moths was about 1.4 to 2.2 times that of the female. The data in Table 3 indicate that larval diet did not affect the longevity of ensuing moths. This did not agree with the effects of larval diets on the longevity of moths of *Heliothis zea* reported by ABDALLAH and SALAMA (1975).

Larval and adult development of *L. separata* and *L. loreyi* reared on the diet containing sorbic acid as a mould inhibitor is shown in Table 4. In *L. separata*, although the larval development and the pupal period were not significantly different from those reared on Diet 2, the rate of pupation was lower and the pupal weight was significantly smaller ($P<0.01$). The rate of emergence was under one-fourth of that reared on Diet 2. On the other hand, the development of *L. loreyi* was not affected by sorbic acid.

Table 4. A COMPARISON OF REARING *L. separata* AND *L. loreyi* ON THE ARTIFICIAL DIET CONTAINING SORBIC ACID AS A MOULD INHIBITOR[a]

Species	Number used	Larval duration (days)	% of pupation	Pupal duration (days)	Pupal weight (mg)	% of emergence
L. separata	60	18.4±1.2	38.9	11.8±0.4	303.6±36.5	20.4
L. loreyi	60	24.7±3.0	91.4	13.8±0.8	298.6±45.1	100.0

[a] Diet composition (g) : wheat bran 180, corn powder 90, dried Brewer's yeast 30, ascorbic acid 6, sodium propionate 1.5, sorbic acid 0.8, linseed oil 1.5, agar 11.0 and water 870.

DISCUSSION

Larval duration of *L. separata* reared on sweetcorn was shorter than that previously reported (HIRAI, 1975). The difference is considered to depend on the number of instars: all larvae in this experiment had six instars, yet 85.2% of the insects previously used had seven instars. The proportions of the six-instar type of larvae reared on sudangrass, sorghum and grain sorghum were: 100%, 85% and 0%, respectively. Moreover, larvae reared on paddy rice or upland rice had seven to nine instars. Thus, there may be some correlations between the toughness of leaves and the appearance of extra moults.

This species usually has six instars, but individuals having extra moults occur at times when the larvae are reared on unfavourable diets or under adverse conditions. Thus the number of larval instars can be used as a criterion in devising the artificial diets.

For the artificial diets, the proportions of the six-instar type among larvae reared

on Diet 2, Diet 3 and Diet 4 were: 14.3%, 60.3% and 62.9%, respectively. Considering larval instars and durations, the bean-powder-based Diets 3 and 4 seem to be favorable, but the resulting pupae were smaller and the rate of emergence was lower. This was partly due to mould gathering on both diets, so Diets 3 and 4 must be improved by further addition of mould inhibitor. Diet 2 was more suitable, since pupae reared on Diet 2 were heavier than those reared on Diets 3 and 4, and the ensuing adults emerged better.

Although Diet 1 was devised for *L. loreyi*, it was also tested for rearing *L. separata*, however, the pupal weight was lighter and the rate of emergence was lower than those reared on Diet 2. Moreover, the ingredients of Diet 1 are complicated, making it unfavorable for mass rearing. On the other hand, as Diet 2 gave good development of *L. loreyi* also, this is considered more suitable for mass rearing of both species.

Sorbic acid has been used as a mould inhibitor in diets for noctuid species (SHOREY and HALE, 1965), *Spodoptera litura* (YUSHIMA et al., 1972). *S. littoralis* (KHALIFA et al., 1973), etc., but it caused deformity of pupae and adults in rearing *L. separata*. Larvae of *L. separata* grew well on the diet containing 0.24% sorbic acid and the newly hatched larvae preferred the diet to one containing p-methyl hydroxy benzoate in two-choice experiments, but the ensuing pupae were not fully formed in the outer surface around the mid- and hind-legs. Adults also could not emerge normally; some emerged with wrinkled wings, other died in pupae. On the other hand, no obvious effects of sorbic acid were observed in pupation and emergence of *L. loreyi*. Since the effects of sorbic acid depend on the species, and symbioticidal and embryocidal effects on insects have also been reported (LEVINSON, 1975), care is needed in using sorbic acid as a mould inhibitor in artificial diets.

The fact that linseed oil has a function in spreading the wings of adults was clarified in the small tea tortrix, *Adoxophyes orana*, (TAMAKI, 1961) and in *L. loreyi* (HIRAI, 1975). The same fact was recognized in *L. separata*. Feeding linseed oil at 0.08% was enough for wing spreading of *L. loreyi* (unpublished), and this result was applied in adding the oil to the diet of *L. separata*. Since Diets 3 and 4, without linseed oil, also gave normal wing formation, pinto beans may contain the same components found in linseed oil.

The cost of Diet 2 is approximately 216 yen per kg, excluding personnel expenses. Since a larva of *Spodoptera litura* consumes 10-g diet in its duration (OKADA, unpublished), rearing of one larva costs 2.16 yen. To date, five generations of *L. separata* and over 20 generations of *L. loreyi* have been reared on Diet 2, with no cannibalism. In mass rearing, it is necessary to keep the receptacles clean and particularly, to reduce the high humidity in both species. The rearing receptacles are soaked in 1.5% hyperchlorite solution for one night to disinfect. The newly hatched larvae to the 2nd instar are reared in the culture dish described above, the 3rd to 4th instars are reared in a 0.45-liter case and the 5th to 6th instars are reared in a 2.5-liter case, with 30 insects per container.

REFERENCES

ABDALLAH, S. and A. E. SALAMA (1975) Biological studies on the American cotton bolloworm, *Heliothis zea* (BODDIE) in Egypt. *Z. angew. Ent.* **79** : 370—376.

HIRAI, K. (1975) Rearing the loreyi armyworm, *Leucania loreyi* DUP. on a simple artificial diet. *Kinki Chugoku Arg. Res.* **50** : 62—64. (In Japanese).

HIRAI, K. (1975) The influence of rearing temperature and density on the development of two *Leucania*

species, *L. loreyi* DUP. and *L. separata* WALKER (Lepidoptera : Noctuidae). *Appl. Ent. Zool.* **10**, : 234—237.

KHALIFA, A., H. S. SALAMA and A. F. ELSHARABY (1973) Rearing the cotton leafworm, *Spodoptera littoralis* BOISD. on a semi-artificial diet. *Z. angew. Ent.* **73** : 129—132.

LEVINSON, H. Z. (1975) Possibilities of using insectistatics and pheromones in pest control. *Naturwissenschaften* **62** : 272—282.

SATO, Y. (1965) The artificdial diet for mass rearing of the tobacco cutworm, *Prodenia litura* FABRICIUS and the common armyworm, *Leucania separata* WALKER. *Jap. J. appl, Ent. Zool.* **9** : 99—107.

SHOREY, H. H. and R. L. HALE (1965) Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. *J. econ. Ent.* **58** : 522—524.

TAMAKI, Y. (1961) Studies on nutrition and metabolism of the smaller tea tortrix, *Adoxophyes orana* (FISCHER von ROSLERSTAMM). 2. An essential factor for adult emergence. *Jap. J. appl. Ent. Zool.* **5** : 58—63.

TSUTSUI, K., A. SATO, K. TANAKA and S. TANIMOTO (1954) On the rearing method of rice stem borer and other some insects by sterilized host plant media. *Bull. Tokaikinki Nat. Agr. Exp. Stn* **1** : 68—86. (In Japanese).

YUSHIMA, T., H. NOGUCHI, Y. TAMAKI, N. FUKAZAWA and T. SUGIMOTO (1973) Mating and sex pheromone of *Spodoptera litura* F. (Lepidoptera : Noctuidae) : An introductory report. *Appl. Ent. Zool.* **8** : 18—26.