Mass-rearing of the Onion and Seed-corn Flies, *Hylemya antiqua* and *H. platura* (Diptera: Anthomyiidae), on an Artificial Diet with Antibiotics¹

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A simple and inexpensive artificial diet was developed for mass-rearing the onion fly, *Hylemya antiqua* MEIGEN and the seed-corn fly, *H. platura* M. The diet consists of a commercial food for guinea pigs, defatted soybean flour, dehydrated yeast, sugar, agar, micro-nutrients and antibiotics. A large amount of cellulose powder was also included to improve the physical quality of the diet. The advantages of this diet are: 1) no sterilization of egg surface or disinfection of the rearing container prior to inoculation is required; 2) growth of the larvae is fairly good and uniform; 3) yield is considerably high (over 65%) and pupal size is comparable to those reared on onions; 4) the rearing condition is quite hygienic and the diet discharges no unpleasant odor as do onion and other artificial diets. Screening of several antibiotics showed that kanamycin sulfate singly, and the mixtures of neomycin-chloramphenicol and spiramycin-chloramphenicol effectively suppress the frequently observed larval diseases. A neomycin-chloramphenicol mixture was used in the standard diet since these are less expensive than the other antibiotics.

INTRODUCTION

A half-cut onion has been used for rearing the onion fly, *Hylemya antiqua* MEIGEN, though this method has such disadvantages as emission of an unpleasant odor from the culture and accidental death of the larvae by residual insecticides in onion. Thus, development of a good alternative diet for the onion fly has long been sought.

Various artificial diets including a holidic one were developed (ALLEN and ASKEW, 1970; FRIEND, SALKELD and STEVENSON, 1959; McCLANAHAN, 1966; TICHELER, 1971). None of these diets, however, have proved satisfactory because each has some problems such as unavailability of ingredients, low or unstable yield of the adults, or difficulty in preparation or rearing procedure.

Recently in Japan, artificial diets for several species of Noctuidae, Tortricidae and Pyralidae have been successively developed utilizing commercial food for guinea pigs (GC-4®, Oriental Kobo Co. Ltd.) as a basal ingredient (KOJIMA and NAKAYAMA,

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1979; OYAMA and KAMANO, 1976). As this guinea pig food is well standardized, nutritionally balanced and available at low cost, the success in the above lepidopteras prompted us to adopt this as constituent of the artificial diet for rearing the onion fly and another *Hylemya* fly, the seed-corn fly, *H. platura* MEIGEN.

MATERIALS AND METHODS

Insects. The onion flies used, *Hylemya antiqua* MEIGEN, were of laboratory culture originating from Hokkaido National Agricultural Experiment Station and had been reared on onion bulbs by the method of MATSUMOTO and THORSTEINSON (1967) for several years. Seed-corn flies, *Hylemya platura* MEIGEN, were collected at the university farm on the Yayoi campus with 2-phenyl-ethanol-baited traps (ISHIKAWA, in preparation) and reared on soybean seeds according to the method of MATSUMOTO and SUGIYAMA (1967).

Ingredients. Neomycin sulfate, kanamycin sulfate, erythromycin and tetracycline hydrochloride were products of Sigma Chemicals. Chloramphenicol and spiramycin were obtained from Sankyo Pharmaceuticals Co. Ltd. Suppliers of the other ingredients are shown in Table 1.

Adult culture. In a 30 cm cubic cage 200 to 300 flies were confined with a supply of water, cube sugar and Difco® yeast extract. Flies were reared at $23 \pm 1^\circ\text{C}$, R.H. over 60% under a 16L8D photoperiod. To obtain eggs, some onion pieces for the onion fly or macerated soybean seeds for the seed-corn fly were placed as oviposition stimulants on moistened sand (10 to 30 mesh) in a plastic cup. Eggs laid on the sand were sieved after addition of water and gentle stirring. More than 1,000 eggs per day were easily obtained.

Preparation of the artificial diet. Ingredients 1 to 6, 9 and 12 listed in Table 1 were weighed in an aluminum container (120×170×55 mm) and blended thoroughly. The mixture was covered, steamed for 20 min, and again well stirred until it cooled below 60°C. Meanwhile, choline chloride, ascorbic acid and antibiotics or formalin were dissolved in 10 ml of water in a small vial. Then this antibiotic solution was added to the diet in small portions so that it was homogeneously incorporated. The diet could be stored over one month in a refrigerator.

Selection of effective antibiotics. Effectiveness of several antibiotics and formalin were tested separately or in combinations to suppress epidemic diseases of the larvae. The test agents shown in Table 2 were incorporated into the diet without any changes of other components. The additives were evaluated by reproducibility of pupation rates among the repeated tests in addition to their ability to suppress the larval disease.

Larval culture of the onion fly. Fine sand (40 to 60 mesh, 600 g) was dried in an oven at 120°C for 30 min. After cooling, the sand was put in a plastic container (140 mm in diam.×80 mm in depth), and 8 w/w % quantity of distilled water was added to moderately moisten the sand. At the center on the sand was put a 20-g slice of the artificial diet (10×50×15 mm), on which 500 eggs were placed. The diet was covered with a small petri dish (9 cm in diam.) to prevent dessication.

In the experiments selecting antibiotics, 500 newly-hatched larvae in place of the eggs were inoculated to accurately determine the pupation rate. All the rearing experiments were carried out at $23 \pm 1^\circ\text{C}$. Under this condition, eggs hatched within two days and the larvae began feeding immediately. An appropriate amount of fresh

Table 1. Composition of the standard artificial diet for the onion and seed-corn flies

Ingredients	Quantity	Cost ^a
1) Guinea pig food ^b (RC-4®, Powder, Oriental Kobo Co.)	8 g	1.68 yen
2) Defatted soybean flour ^c (Prorich®, Ajinomoto Co.)	14	7.00
3) Cellulose powder (Pulp Flock®, Sanyo pulp Co.)	14	4.90
4) Dehydrated yeast powder (Ebios®, Ebios Co.)	4	3.76
5) Sucrose	4	1.12
6) Agar powder (Nakarai Chemicals Co.)	4	21.60
7) Choline chloride (Wako Pure Chemicals Co.)	300 mg	3.96
8) Ascorbic acid (Takeda Pharmaceuticals Co.)	300	1.80
9) Methyl p-hydroxy benzoate	600	2.00
10) Chloramphenicol (Chloromycetin® sol intramuscular, Sankyo)	0.6 ml ^d	16.80
11) Neomycin sulfate (Sigma Chemicals)	150 mg	23.40
12) Distilled water	200 ml	—
Total	250.0 g	88.02

^a 1981 Cost.

^b GC-4®, a formula for pregnant animals, is also usable.

^c This can be substituted by Ajipron®, a product of the same company.

^d 150 mg equivalent.

diet was added to the previous portion as the larvae consumed it. Their consumption increased exponentially as they grew. Seven days after inoculation, the petri dish was removed and the entire container was covered with a sheet of tissue paper to prevent the entry of other insects.

Larval culture of the seed-corn fly. Seed-corn flies were reared on an artificial diet of the same recipe as the onion fly. For rearing larvae, a smaller container (100 mm in diam. × 60 mm in depth) was used and 200 eggs were inoculated on the diet. Other rearing conditions were similar to those of the onion fly.

Separation of the pupae. Twenty days after inoculation of the eggs, the tissue paper cover on the container was removed to let the diet dessicate. The dessicated diet was then carefully removed and water was added to the sand. After gentle stirring, the pupae floated were sieved and stored in a refrigerator at 5°C until adults were required. The pupae started growing when they were restored to room temperature and adults emerged in 2 weeks.

Rearing experiment in a petri dish. As it was difficult to accurately evaluate larval growth in the large scale culture described above, another rearing experiment was made in a petri dish. In a dish 9 cm in diameter was placed a disk of the diet (1.5 cm in diam. × 1 cm in height), onto which 20 newly-hatched larvae were placed. Four days after inoculation, almost all larvae had reached the 2nd instar. Afterwards, they

were transferred daily to a new disk diet with a soft brush so that the numbers in each larval instar and the pupae could be recorded.

RESULTS

Composition of the artificial diet

Ingredients of the standard diet are shown in Table 1. Major components are the guinea pig food, defatted soybean flour (Prorich®) and cellulose powder. Prorich® was included as a protein supplement instead of kidney bean flour which was in the diet for lepidopteran species (OYAMA and KAMANO, 1976). A large quantity of cellulose powder was used to provide the diet with appropriate moisture and to improve its physical property so that it became highly porous after larval feeding; thus the diet itself provides a suitable habitat for the larvae. We have tested several other artificial diets of different constitutions and quantities of micro-nutrients, however, that shown in Table 1 gave the most reliable and highest yield.

Selection of antibiotics

No pupa was obtained when only methyl *p*-hydroxy benzoate, an antifungal agent, was incorporated into the diet because of diet deterioration and frequent infection of the larvae with diseases (Table 2). In addition to the antifungal agent, several antibiotics were incorporated into the diet to evaluate their ability to suppress the diseases. The results are shown in Table 2. The two replications with chloram-

Table 2. Effect of anti-microbial agents in the artificial diet on the pupation rate of the onion fly

Anti-microbial agent ^{a, b}	Pupation rate (%)				
	I	II	III	IV	V
None	0.0 ^c	0.0 ^c	—	—	—
Spiramycin	45.6	0.0 ^c	—	—	—
Chloramphenicol	77.4	1.2 ^c	—	—	—
Neomycin sulfate	72.6	0.0 ^c	—	—	—
Tetracycline	0.0 ^c	0.0 ^c	0.2 ^c	—	—
Erythromycin	14.4 ^c	12.8 ^c	—	—	—
Kanamycin sulfate	55.4	61.3	47.0	33.8	—
Formalin ^d	61.8	33.2	11.4 ^c	9.0 ^c	—
Tetracycline + Chloramphenicol	0.0 ^c	3.4 ^c	—	—	—
Erythromycin + Chloramphenicol	14.4 ^c	6.2 ^c	0.0 ^c	0.0 ^c	—
Spiramycin + Chloramphenicol	65.0	59.8	57.4	31.8	—
Neomycin sulfate + Chloramphenicol	57.6	56.6	89.2	64.5	64.2

^a 150-mg equivalent each/250-g diet.

^b Methyl *p*-hydroxy benzoate was incorporated into all diets as a fungicide.

^c Disease epidemic occurred. All larvae left the diet and began wandering.

^d 0.8 ml of 37% formalin solution/250-g diet.

phenicol and neomycin sulfate showed pupation rates of 77.4 and 1.2%; 72.6 and 0.0%, respectively.

These large fluctuations indicate that the accidental infection with the diseases was fatal to the whole culture and suggest that the low yield was not caused from the deficiency of nutrients in the artificial diet. The latter supposition is supported by the fact that the average weight, length and width of the pupae obtained from any diet with antibiotics did not differ significantly, in spite of the large fluctuation of pupation rates.

Both tetracycline hydrochloride and erythromycin failed to suppress the diseases and thus only a few pupae were obtained. Only kanamycin sulfate gave a moderate pupation rate, an average of 49.3% in 4 replications; the high price of this material, however, prohibits its practical use.

Formalin, a common anti-microbial agent in the diet for lepidoptera (KOJIMA and NAKAYAMA, 1979; OYAMA and KAMANO, 1976), was also tested, but gave an unsatisfactory result. Moreover, the larvae clearly avoided the formalin-incorporated diet when a choice between diets with and without formalin was offered. The fly larvae were probably more affected by formalin gas than lepidopteran larvae because the former feed deeply into the food whereas the latter feed outside.

As shown from these results, the treatment with a single antibiotic or formalin provided no applicable diet. Therefore, mixtures of antibiotics were tested (Table 2). Addition of erythromycin or tetracycline hydrochloride to chloramphenicol did not improve the diet performance compared with chloramphenicol alone. However, spiramycin-chloramphenicol, and neomycin-chloramphenicol mixtures satisfactorily prevented the diseases and gave average pupation rates of 53.5% and 66.4%, respectively. As the neomycin-chloramphenicol mixture is reliable in the reproducibility of high yield and is least expensive among the effective combinations of antibiotics tested, this mixture was used hereafter in the standard artificial diet as the disease suppressant.

Growth of the larvae on the standard diet

In the petri dish experiment, the growth of the larvae was fairly uniform; they began to pupate 10 days after their inoculation and almost all had pupated by day 12. The average larval period of 11.5 ± 1.3 days did not differ significantly from the 12.2 ± 0.7 day period when reared on onions at the same temperature (ZURLINI and ROBINSON, 1978). The average weight, length and width of pupae and the pupation rates when larvae were raised on the standard diet were comparable to those when

Table 3. Comparison of pupae raised on artificial diet and halved onion

	Artificial diet	Halved onion
Pupation rate ^a	66.4% (56.6–89.2)	53.0% (28.6–65.2)
Weight ^b	12.0 ± 2.1 mg	11.8 ± 1.8 mg
Length ^b	6.1 ± 0.4 mm	6.2 ± 0.3 mm
Width ^b	2.2 ± 0.1 mm	2.2 ± 0.1 mm

^a No. pupae/No. larvae inoculated, mean of 5 replications.

^b Mean of 150 pupae.

Table 4. Rearing of the seed-corn flies on standard artificial diet

Replications	Pupation ^a %	Weight ^b (mg)	Length ^b (mm)	Width ^b (mm)
I	68.8	6.4±0.7	5.0±0.1	1.8±0.1
II	50.6	5.7±0.8	4.8±0.2	1.8±0.1
III	50.0	6.3±0.8	4.9±0.3	1.8±0.1
IV	65.0	6.6±0.6	5.0±0.2	1.8±0.1

^a No. pupae obtained/No. larvae inoculated.^b Mean of 30 measurements.

reared on halved onions (Table 3). These figures suggest that nutritional balance of the diet is optimal for the onion fly.

Rearing tests of the seed-corn flies

The standard artificial diet for the onion fly was also tested for the seed-corn fly since the latter is known to have similar nutritional requirements (FRIEND, SALKELD and McCLANAHAN, 1958). The result is shown in Table 4. The average pupation rate of four experiments was 58.6%, showing that this diet is appropriate for the rearing of the seed-corn fly as well.

DISCUSSION

Diseases of larvae caused by an overly rich bacterial population in the alimentary canal are often observed when insects are reared on artificial diets. For instance, digestion becomes disturbed in the silkworm, *Bombyx mori* L. because of an extraordinary proliferation of *Streptococcus* sp. in the alimentary canal when they are reared on an artificial diet with addition of only sorbic acid and propionic acid as antimicrobial agents (HORIE, 1981). This bacterial indigestion in the silkworm is prevented by the addition of chloramphenicol to the diet (HORIE, 1981). Our preliminary observation of onion fly larvae under a microscope showed that the diseases were also caused by the extraordinary multiplication of pathogenic bacteria in the alimentary canal. However, incorporation of chloramphenicol alone was insufficient in the onion fly (Table 2). The synergistic effect of spiramycin and neomycin sulfate, which were ineffective individually, to chloramphenicol for preventing indigestion suggests that the diseases of the onion fly larvae were not caused by a single bacterial species but by many species (Table 2).

The chemical characters of the antibiotics used here varied diversely from amino-glucosides (kanamycin, neomycin), macrolides (spiramycin, erythromycin), tetracyclines (tetracycline) to benzo-derivatives (chloramphenicol). However, all these are known to have a broad spectrum in their antibiotics. There seemed to be no clear correlations between the chemical property of the antibiotics and the effectiveness in suppressing the diseases.

Generally, in evaluating the effectiveness of antibiotics against a disease, it is desirable to use the isolates of bacteria from larvae as SIKOROWSKI et al. (1980) did with the boll weevil, *Anthonomus grandis* BOHEMAN. If these experiments had been done with bacterial isolates from the onion fly, more rational and detailed explanation could

have been made in selecting the antibiotics and determining their effective concentrations than the present conventional random screening allowed.

Though antibiotics are indispensable for preventing some diseases of insects, their possible adverse effects cannot be overlooked. The effect of antibiotics on insects over the long term should be thoroughly studied, particularly when beneficial symbionts are known to be present within their bodies, because any effect on the symbionts necessarily affects the welfare of the host insects. The presence of symbionts is not known in the larvae of onion fly, although they grow faster under septic conditions (ZURLINI and ROBINSON, 1978). At present, a study on the long-term effects of antibiotics such as fecundity and longevity of adults over several generations is under way.

The conditions desirable for an artificial diet and a mass-rearing of insects are: easy supply and high quality of ingredients, low cost of the diet, and minimum labor in the preparation and insect rearing. The present diet, at a cost of 90 yen per 250 g (Table 1), can easily raise over 300 pupae, a cost per pupa of less than 0.3 yen. This value is fairly reasonable compared with ca. 0.6 yen when onion fly larvae are reared on onion bulbs (300 pupae/1 kg onion). As for the labor required, the most time-consuming and labor-requiring disinfection process of the rearing containers is unnecessary. In all these regards, the artificial diet newly developed here is suitable in practical use not only for the onion fly but also for the seed-corn fly. Many other dipteran species will also be raised on this diet after a slight modification.

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