Artificial Rearing and Nutritional Physiology of the Planthoppers and Leafhoppers (Hemiptera: Delphacidae and Deltocephalidae) on a Holidic Diet

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Introduction

Among the planthoppers and leafhoppers, some species, such as *Nilaparvata lugens* Stål and *Sogatella furcifera* Horvath, have been considered to come to Japan from foreign countries every year because they are unable to hibernate in Japan, while other species like *Laodelphax striatellus* Fallén and *Nephotettix cincticeps* Uhler are able to hibernate in Japan. In spite of heavy damage of rice crop caused by them, many aspects of basic problems such as their nutritional requirements or growth physiology have not been clarified yet.

With the purpose of making clear the nutritional requirements of these insects, we have developed a method of artificial rearing on a synthetic diet which consists of only known chemical substances. By employing this method, nutritional requirements and particular substances which promote or inhibit egg-laying have been made clear.

Up to the present, three species of Deltocephalidae[3,4,9], *N. cincticeps*, *Recilia dorsalis* Motshulsky and *Macrosteles fascifrons* Stål, and four species of Delphacidae[10,16,22-24,29,30], *L. striatellus*, *N. lugens*, *S. furcifera*, and *Sogatella longifurcifera* Esaki and Ishihara, were successfully reared from the 1st instar to adult on synthetic diets without contacting host plants.

Apparatus for artificial rearing

1) *Rearing vessels*

Fig. 1 A shows a vessel for planthoppers and B for leafhoppers. For a film to hold a synthetic diet, which is sucked by insects

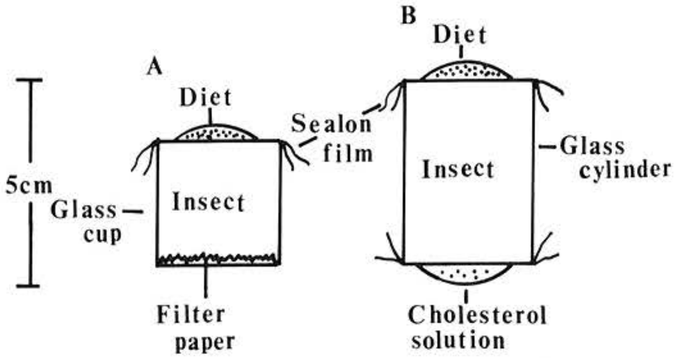

Fig. 1. Rearing vessels for planthoppers and leafhoppers

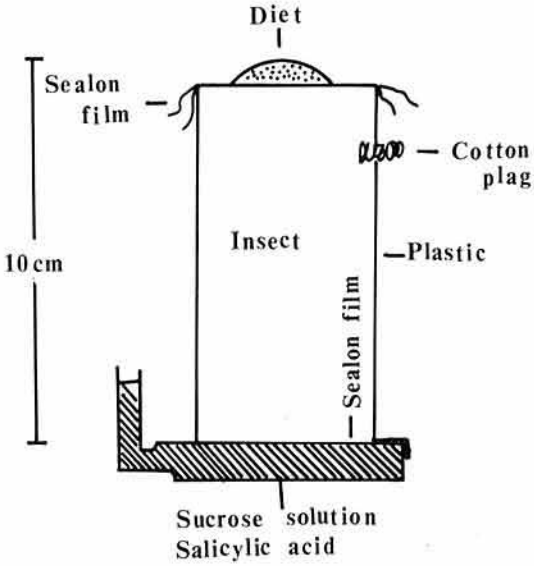

Fig. 2. An apparatus for oviposition

through the film, Parafilm M or Fuji Sealon film is used.

2) An apparatus for oviposition

This apparatus is made up as follows (Fig. 2): An L-shaped plastic tube is inserted into a small hole bored in the wall of a plastic-made Petri-dish, and fixed with adhesive. A plastic cylinder to be used as the insect cage is connected to the Petri-dish through a large opening bored in the lid of the Petri-dish. A small hole is bored in the wall at the upper part of the cylinder for the use in putting insects into the cage.

3) Other necessary apparatus and tools

Sterilized filtration system for sterilization of artificial diets, and heat-tolerant screw-capped glass tubes (20–25 *ml* of capacity) for preservation of sterilized artificial diets. In addition, pipetes, small writing brushes, etc. are needed.

Preparation of the synthetic diet

The composition of the diet is given in Table 1. Since it takes a lot of time and labor to add one by one these substances in the table, the following method is adopted. All the amino acids are put together and preserved as a two times concentrated mixture. As cystine and tyrosine hardly dissolve in neutral water, they are first dissolved in the small amount of 1N hydrochloric acid, and then distilled water is added. Then, remaining amino acids are dissolved. Vitamines are kept as a ten times concentrated mixture. As riboflavin is not easily soluble in cold water, it is dissolved into warmed (50°C) water. Biotin, which hardly dissolves in neutral water, is dissolved into the small amount of alkalin solution and used. Sodium ascolbate is added at the final stage of the diet preparation, because of auto-oxidation occurring even at frozen condition after it is dissolved. Trace-metals are kept in a refrigerator as a 100 times concentrated solution. The concentrated mixtures of amino acids and of vitamines are kept by freeze storage at −20°C. The diet prepared is finally sterilized by passing through a sterilizing filter, distributed into screw-capped glass tubes, and is stored at −20°C until use. Aqueous solution of cholesterol should be prepared by adding 100 mg of cholesterol to 100 *ml* distilled water, and stored in a refrigerator.

Methods of artificial rearing

1) Methods of collecting eggs

Planthoppers and leafhoppers lay their eggs in the tissue of host plants, and at the time of oviposition, they suck plant sap. Therefore, in the study of artificial rearing from generation to generation or in that of nutritional physiology, artificial induction of egg-laying in places other than rice plants or taking out of eggs laid in rice plants has to be adopted. To get oviposition in the apparatus for oviposition (Fig. 2), the Petri-dish is covered with a stretched Para-film or Sealon film which is fixed to the wall of the dish with a rubber band. This film allows the insect to lay eggs into the solution supplied into the Petri-dish through the L-shaped side tube. For *L. striatellus* and *R. dorsalis* 5% sucrose solution is used. *N. lugens* seldom lays eggs on pure sucrose solution, but lays some eggs on

Table 1. Composition of some holidic diets for planthoppers and leafhoppers (mg/100 ml)

Ingredient	MED-1	MED-4	MMD-1
L-Alanine	100	150	100
γ-Aminobutyric acid	20	—	—
L-Arginine hydrochloride	400	—	270
L-Asparagine	300	450	550
L-Asparatic acid	100	150	140
L-Cysteine	50	80	40
L-Cystine hydrochloride	5	—	—
L-Glutamic acid	200	300	140
L-Glutamine	600	900	150
Glycine	20	—	80
L-Histidine	200	300	80
DL-Homoserine	800	—	—
L-Isoleucine	200	300	80
L-leucine	200	300	80
L-Lysine hydrochloride	200	300	120
L-Methionine	100	150	80
L-Phenylalanine	100	—	40
L-Proline	100	—	80
DL-Serine	100	150	80
L-Threonine	200	300	140
L-Tryptophan	100	—	80
L-Tyrosine	20	—	40
L-Valine	200	—	80
Thiamine hydrochloride	2.5	2.5	2.5
Riboflavin	5.0	5.0	0.5
Nicotinic acid	10.0	10.0	10.0
Pyridoxine hydrochloride	2.5	2.5	2.5
Folic acid	1.0	1.0	0.5
Calcium pantothenate	5.0	5.0	5.0
Inositol	50.0	50.0	50.0
Choline chloride	50.0	50.0	50.0
Biotin	0.1	0.1	0.1
Sodium L-ascolbate	100.0	100.0	100.0
Sucrose	5000	5000	5000
$MgCl_2 \cdot 6H_2O$	200	200	—
$MgSO_4 \cdot 7H_2O$	—	—	123
KH_2PO_4	500	500	—
K_2HPO_4	—	—	750
$FeCl_3 \cdot 6H_2O$	2.228	2.0	2.228
$CuCl_2 \cdot 2H_2O$	0.268	0.3	0.268
$MnCl_2 \cdot 4H_2O$	0.793	0.8	0.793
$ZnCl_2$	0.396	0.4	1.188
$CaCl_2 \cdot 2H_2O$	3.115	3.0	3.115
pH	6.5	6.5	6.5

sucrose solution, containing 0.004 M salicylic acid, adjusted to pH 6.5[36]. By this method, *L. striatellus* and *N. lugens* laid eggs in the solution, while *R. dorsalis* laid eggs in both of the diet and the sucrose solution. However, *N. cincticeps* does not lay eggs at all, and *S. furcifera* was hardly possible to lay eggs. The eggs laid are transferred into water in the small Petri-dish and preserved. The eggs developed normally in water. Just before hatching, the eggs are placed on moistened filter paper to allow hatching. When the hatching time has to be adjusted, the eggs in water can be preserved at low temperature[7].

On the other hand, *N. cincticeps*, *S. furcifera*, and *S. longifurcifera* are allowed to lay eggs on rice seedlings, and the eggs are collected just before hatching onto moistened filter paper, and allow them to hatch.

2) Methods of rearing larvae

For planthoppers, the following procedure has been established. A drop of distilled water is given to round-shaped filter paper placed on the bottom of the rearing vessel. Newly hatched larvae are transferred into the vessel one by one using a small writing brush, and the top of the vessel is covered with a stretched film. A drop of synthetic diet is placed on the film, and then another film is spread on it. The larvae of planthoppers suck the diet through the film. Larvae of *N. cincticeps* and *R. dorsalis* are given a synthetic diet from one side and cholesterol solution from the other side (Fig. 1 B). The rearing by this method at 25°C and 16 hr of illumination causes deterioration of the diet mostly by microorganisms, because no antiseptic or antibiotic substance is contained in the diet. Therefore, the diet is renewed every two days. Although the rearing from the 1st instar to adult can be done continuously in a vessel, the vessel is renewed when the inside of the vessel is spoiled with insect excreta, etc.

Development of synthetic diet and nutritional physiology

1) Sugars

Sugars are very important not only as energy sources for planthoppers and leafhoppers, but also as stimulators to oviposition or to feeding. The most suitable kind of sugars and its concentration for the survival of *L. striatellus* were examined. The result showed that sucrose gave the highest survival rate, followed by glucose, fructose, and maltose in that order. Treharose and raffinose showed the survival rate lower than that of the control (distilled water)[28]. *R. dorsalis* prefers sucrose to glucose and fructose (Table 2)[6]. The number of eggs laid by *L. striatellus* into sugar solutions was the greatest with sucrose, followed by glucose, raffinose and fructose in that order[32]. Hatched larvae of *N. lugens* grew up to adult on the synthetic diets containing 3 to 50% of sucrose. S.icrose at 5% showed the shortest larval period, indicating that it was favorable to larval growth and development. When sucrose content was 0–1%, all the larvae died at an initial stage after hatching[11-13].

Table 2. Multiple choice experiment with *Inazuma dorsalis* using water and aqueous solution of different sugars

Choice	Time (hr)				
	1	3	5	7	24
Distilled water	5	4	4	2	3
10% sucrose	8	12	12	8	20
10% glucose	3	6	3	0	1
10% fructose	4	1	2	3	0

Numbers of larvae found on four liquids at each time are shown. The result is the total of 5 repeated experiments, using each a total of 20 nymphs.

2) Amino acids

It was made clear that cystine and methionine are essential to the growth of larvae of *L. striatellus*[18], while larvae of *N. lugens* require no indispensable amino acids (Table 3)[12,14]. It indicates that planthoppers require only few essential amino acids[1,25,26], as compared to *Myzus persicae* and *Aphis fabae*, both are hemipterous insects whose nutritional requirement was already known. For *M. persicae*, methionine, histidine and isoleucine are indispensable, and for *A. fabae*, alanin, cysteine, histidine, methionine, phenylalanine, proline, serine, and tryptophan are indispensable. The fact that planthoppers require very few kinds of amino acids, compared with other insects, is regarded attributable to symbiotic microorganisms (present in the insect body) which are supposedly able to produce various substances required by the host insect[2,14,18].

Effect of 23 kinds of amino acids on ovi-

Table 3. Essential amino acids for the larval growth of the hemipterous insects

	Nilaparvata lugens	*Laodelphax striatellus*	*Myzus persicae*	*Aphis fabae*
Alanine	−	−	−	+
Aminobutyric acid	−	−	−	−
Arginine	−	−	−	−
Asparagine	−	−	−	−
Asparatic acid	−	−	−	−
Cystine (Cysteine)	−	+	−	+
Glutamic acid	−	−	−	−
Glutamine	−	−	−	−
Glycine	−	−	−	−
Histidine	−	−	+	+
Homoserine	−	−	−	−
Isoleucine	−	−	+	−
Leucine	−	−	−	−
Lysine	−	−	−	−
Methionine	−	+	+	+
Phenylalanine	−	−	−	+
Proline	−	−	−	+
Serine	−	−	−	+
Threonine	−	−	−	−
Tryptophan	−	−	−	+
Tyrosine	−	−	−	−
Valine	−	−	−	−

+: Essential, −: Nonessential.

Table 4. Essential vitamins for the larval growth of the hemipterous insects

	Nilaparvata lugens	*Laodelphax striatellus*	*Myzus persicae*	*Riptortus clavatus*
Thiamine	+	+	+	−
Riboflavin	−	−	−	+
Nicotinic acid	−	−	+	−
Pyridoxine	+	+	−	−
Folic acid	−	−	−	−
Pantothenate	+	+	+	−
Inositol	−	−	−	−
Choline chloride	−	−	−	−
Biotin	−	−	−	−
Ascorbate	−	−	−	−

+: Essential, −: Nonessential.

position of *L. striatellus* was examined: arginine, glutamic acid, tyrosine, and valine were inhibitory, while cystine was stimulative[19,33].

3) Vitamins

Essential vitamins to the larval growth of *L. striatellus* and *N. lugens* were thiamine, pyridoxine, and pantothenate[12,15,20]. Of hemipterous insects except planthoppers and leafhoppers, only aphids and true bugs were examined for vitamin requirements. Aphids (*M. persicae*) require thiamine nicotinic acid, and pantothenate, and true bugs (*Riptorus clavatus*) only riboflavin as essentail vita-

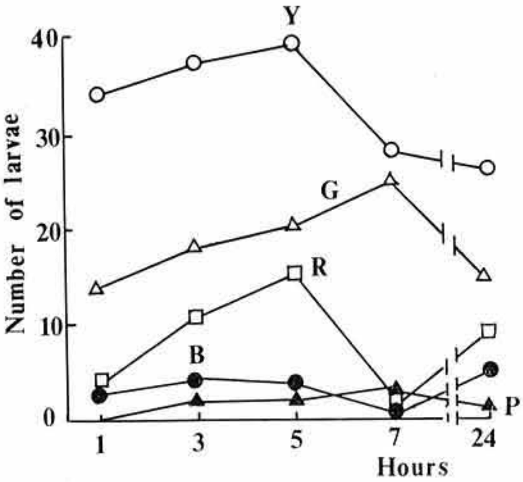

Fig. 3. Multiple choice experiments of *Inazuma dorsalis* using different colors
Y: yellow, G: green, R: red, B: blue, P: purple.
A total of 100 larvae were used.
Number of larvae found on diffrent colors at various times is shown.

mins[2,35]. Like this, insects even of the same order differ in vitamin requirements with different species. It was also presumed that vitamin-balance influences wing form of the red-eye strain of *L. striatellus*[17,31].

4) *Inorganic salts*

It was made clear that K, Mg, P, Fe and Zn are indispensable to larval growth of *L. striatellus*. Removal of Mn alone or of Ca alone allowed the larvae to grow up to the adults[21].

5) *Sterol*

One of the characteristics of insect nutrition is sterole requirement. Although it is said generally that insects are unable to synthesize sterol by themselves, *L. striatellus*, *N. lugens*, *S. furcifera*, and *S. longifurcifera* can grow from the initial larval stage to the adults on the synthetic diet lacking sterol[10,16,22-24,29,30]. As the reason, sterol synthesis by symbiotic microorganisms in the insect body is considered. On the other hand, *N. cincticeps*, *R. dorsalis*, and *M. fascifrons* require sterol like other ordinary insects[3,4,9].

6) *Color*

It seems that by coloring artificially the synthetic diet with the color which attracts planthoppers and leafhoppers, better result of artificial rearing will be obtainned. In an experiment of color choice, *R. dorsalis* likes yellow most of all, followed by green, but don't like red, blue, and purple (Fig. 3)[8].

Conclusion

The method of artificial rearing of planthoppers and leafhoppers, important insect pests to rice crop, using the synthetic diet has been established. By adopting this method, it becomes possible to investigate in detail the nutritional requirements and growth physiology of these insects, as well as metabolism in the insect body. In this connection, it is anticipated that the role of symbiotic microorganisms inhabiting the insect body will be clarified, when those microorganisms can be removed. Furthermore, the research in this field will definitely be able to contribute to the selection of resistant varieties and development of chemical control methods, and also it is applicable to various experiments and researches such as oral inoculation of virus, mycoplasma, etc., and oral administration of pesticides. At present, this rearing technique is successfully utilized for the artificial rearing of predacious mites, natural enemies of thrips and mites, which are pests for vegetables, and examination of nutritional requirements is going on[5,34].

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