Nutritional Physiology of the Brown Planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae)

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ABSTRACT

The effect of sugars on larval growth of *N. lugens* was examined. The insects were reared on a diet containing 0-50% sucrose as the only sugar. When sucrose was eliminated completely, all the insects died in the 1st instar by the third day after the experiment was started. Insects fed on a diet containing 1% sucrose grew to some extent, but none of then reached the 4th instar. On a diet containing 3% sucrose, a few insects developed into adults, and on diets with more than 3%, and up to 50% sucrose more insects developed into adults. However larvae fed on a diet containing 5% sucrose had the shortest development time and grew the fastest. The greater the concentration of sucrose from 10 to 50%, the longer the development time of *N. lugens* larvae. When 5% glucose, fructose, trehalose, maltose or raffinose was used in the synthetic diet in place of sucrose, all the insects died in the 1st or 2nd instars. But by adding 1% sucrose to diets containing glucose, fructose or maltose, the hatched larvae developed into adults. All of the incects fed on diets containing trehalose or raffinose died in their 1st instar even when sucrose was supplemented.

The amino acids essential for the growth of larvae of *N. lugens* was examined. The insects were reared on MED-1 diet from which one of the amino acids were eliminated. The insects could grow up to the adult stage in the absence of any one of the 23 amino acids contained in the MED-1 diet. This leads us to consider that the amino acid synthesizing activity of *N. lugens* is large or that symbiotic microorganism in the insects supply amino acids to *N. lugens*. However, the insect could not grow on a diet containing no amino acids, suggesting that some amino acid substitutes for the missing amino acid when only one is missing.

N. lugens was reared on the synthetic diet MED-1 from which vitamins were eliminated one by one, in order to examine the vitamins essential for the growth of the larvae. The hatched larvae developed into adults when either riboflavin, nicotinic acid, folic acid, inositol, choline chloride, biotin or ascorbic acid was eliminated from the diet. However, all of the insects died before the 3rd instar when they were grown on a diets lacking thiamine, pyridoxine or pantothenic acid. Therefore, these three vitamins were considered to be essential for the growth of *N. lugens* larvae.

Introduction

The identification of nutritional requirements, substances inhibitory or promotive for egg laying, and stimulators or inhibitors of feeding of planthoppers and leafhoppers is extremely important as a fundamental study to understand the mechanisms of the population dynamics of planthoppers and leafhoppers, to select varieties of plants resistant to these insects and to improve the efficiency of chemical control. Therefore, we have developed a synthetic diet consisting of only known substances to study nutritional requirement of the *N. lugens*.

To date, three species of Deltocephalidae, *Nephotettix cincticeps* Uhler (Koyama, 1973), *Recilia dorsalis* Motschlsky (Koyama, 1973) and *Macrosteles fascifrons* Stål (Hou and Brooks, 1975) and four species of Delphacidae, *Laodelphax striatellus* Fallén (Mitsuhashi and Koyama, 1971), *Nilaparvata lugens* Stål (Koyama, 1979). *Sogatella furcifera* Horvath (Koyama and Mitsuhashi, 1980), and *Sogatella longifurcifera* Esaki et Ishihara (Koyama *et al.*, 1981) has been successfully reared from the 1st inster to adult on synthetic diets without contact with a host plant.

Materials and Methods

The *N. lugens* used in our experiment, has been reared on rice seedling in our laboratory at 25°C under long day conditions, for successive generation in a small test tubes since May, 1975. Rearing containers were a 30mm long glass tube which had an inner diameter of 25mm; moistend filter paper was placed on the bottom. The eggs were collected in Mitsuhashi's egg collector (Fig. 1). *N. lugens* seldom lays eggs on pure sucrose solutions, but lays some eggs on sucrose solutions containing 0.004M salicylic acid adjusted to pH 6.5. The eggs are laid in solution through a membrane, and are removed using a pipette and preserved in distilled water. The embryo in the egg develops normally even in water. Just before hatching the eggs were placed on moistened filter paper to allow hatching.

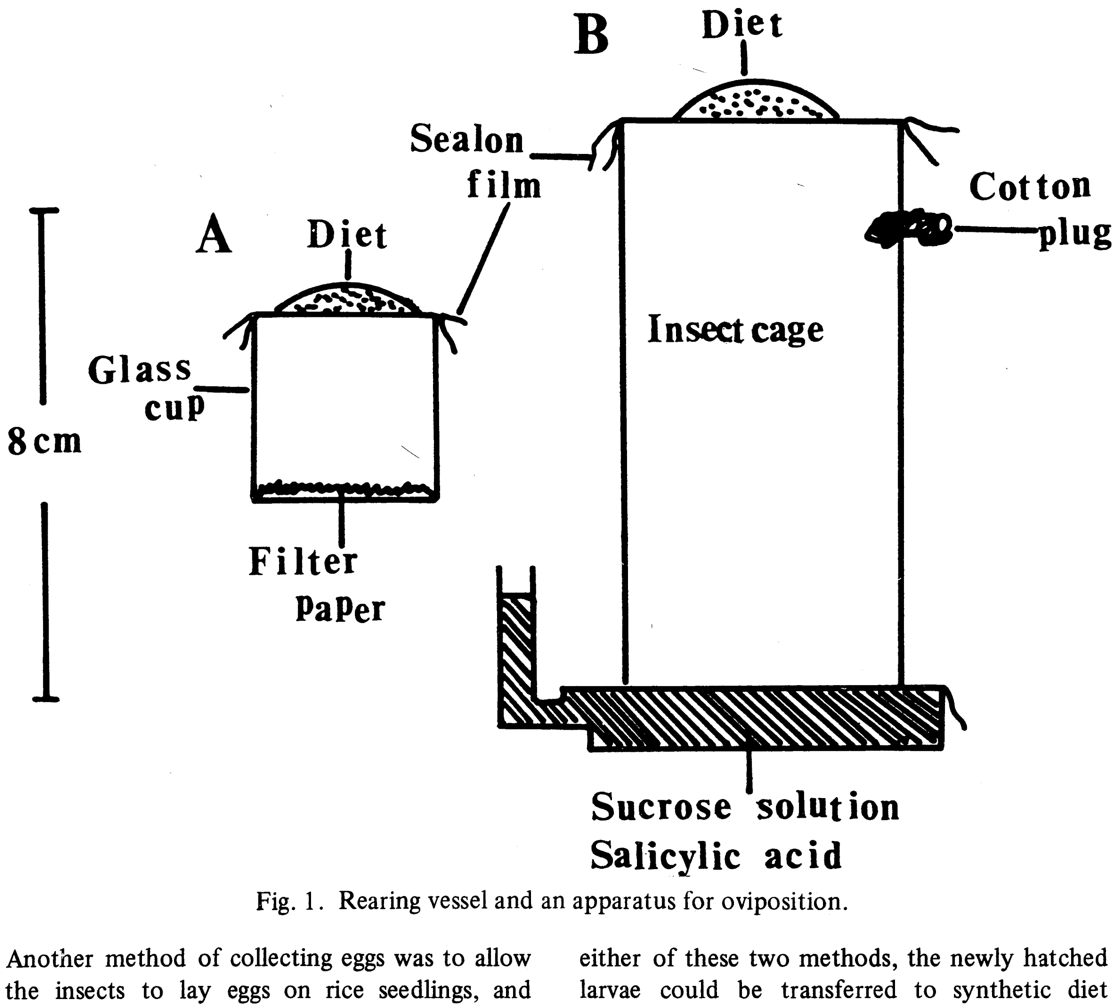

Fig. 1. Rearing vessel and an apparatus for oviposition.

Another method of collecting eggs was to allow the insects to lay eggs on rice seedlings, and remove the eggs just before hatching with needles under a dissecting microscope and allow the eggs to hatch on moistened filter paper. By using either of these two methods, the newly hatched larvae could be transferred to synthetic diet without contact with the host plant. The synthetic diet was a liquid containing amino acids, vitamins, sugar, inorganic salts and micro-

Table 1. Composition of MED-1 Diet (mg per 100 ml)

L-Alanine	100	Thaimine hydrochloride	2.5
γ-Aminobutyric acid	20	Riboflavin	5.0
L-Arginine hydrochloride	400	Nicotinic acid	10.0
L-Asparagine	300	Pyridoxine hydrochloride	2.5
L-Asparitc acid	100	Folic acid	1.0
L-Cysteine	50	Calcium pantothenate	5.0
L-Cystine hydrochloride	5	Inositol	50.0
L-Glutamic acid	200	Choline chloride	50.0
L-Glutamine	600	Biotin	0.1
Glycine	20	Sodium L-ascorbate	100.0
L-Histidine	200		
DL-Homoserine	800	Sucrose	5,000
L-Isoleucine	200		
L-Leucine	200	$MgCl_2 \cdot 6H_2O$	200
L-Lysine hydrochloride	200	KH_2PO_4	500
L-Methionine	100		
L-Phenylalanine	100	$FeCl_3 \cdot 6H_2O$	2.228
L-Proline	100	$CuCl_2 \cdot 2H_2O$	0.268
DL-Serine	100	$MnCl_2 \cdot 4H_2O$	0.793
L-Threonine	200	$ZnCl_2$	0.396
L-Tryptophan	100	$CaCl_2 \cdot 2H_2O$	3.115
L-Tyrosine	20		
L-Valine	200	pH	6.5

elements (Table 1). The insects were allowed to suck the synthetic diet through a Fuji Sealon film that was stretched out. The film sachet was replaced every other day. The number of animals per an experimental group was 100, and the insects were reared individually to observe their growth.

1. Effects of Sugars on Nymphal Development

MED-1 (Mitsuhashi and Koyama, 1971; 1972) was used as a basal diet. MED-1 contained only 5% sucrose as sugars. To investigate the effects of sucrose concentration on the growth of the larvae, the concentration of sucrose in the basal diet was varied ranging from 0 to 50%. In addition in order to examine the effect of sugars other than sucrose, synthetic diets were prepared by substituting sucrose with glucose, fructose, maltose, raffinose, or trehalose in a concentration of 5% for sucrose in MED-1. Synthetic diets containing 1% sucrose and 5% of one of sugars listed above were also prepared and used in other experiments. The synthetic diet was fed through Fuji Sealon films and was changed every other day. Replicates of one hundred individuals were reared to moulting, adult emergence or death.

Results

Effects of sucrose concentration on larval development

The larvae were reared on concentrations of sucrose in MED-1 diet ranging from 0 to 50%. Rearing on the 0% sucrose diet all individuals died in the 1st-instar within 3 days after hatching. On the diet containing 1% sucrose, the larvae grew to some extent but all died before the 4th-instar. On diets containing 3% or more sucrose, the larvae grew to adults to a considerable extent (Fig. 2). Within these diet, the shortest nymphal period was obtained with a sucrose concentration of 5%. This indicates that the best growing conditions were attained at this sucrose concentration (Fig. 3). The nymphal period was longer on sucrose concentrations over 10%. The mean nymphal period was longest, *i.e.* 44 days, on the diet containing 40% sucrose. This was about twice the duration of the shortest period, which was on the diet containing 5% sucrose. Adult body-size was not affected by the duration of the nymphal period (date not shown). Percentage of adult emergence showed the highest value of 51% on the diet containing 25% sucrose, while it was very low, less than 5%, on diets containing either 3% or 50% sucrose (Fig. 2).

Effects of glucose on the growth of the larvae

Larvae reared on MED-1 basal diet in which 5% glucose was substituted for sucrose, died within 6 days after hatching (Fig. 4). Addition of 1% of sucrose to this diet, however, resulted in survival greater than that obtained on 1% sucrose diet, and the larvae grew to adults. Nymphal period was longer and adult emergence lower than those on the diet containing 5% sucrose (Fig. 5).

Effects of fructose on the growth of the larvae

All larvae died by day-3 when reared on

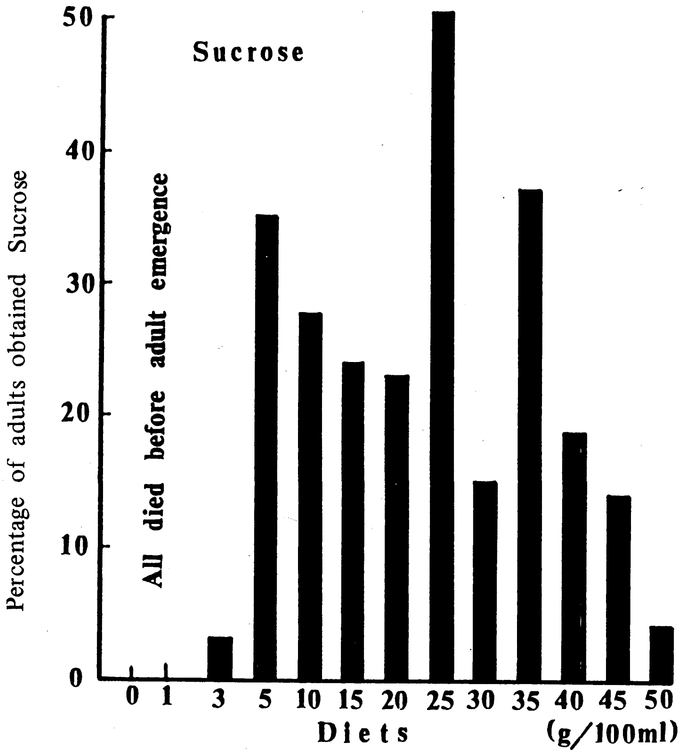

Fig. 2. Effect of sucrose concentration in MED-1 diet on percentage of adult emergence.

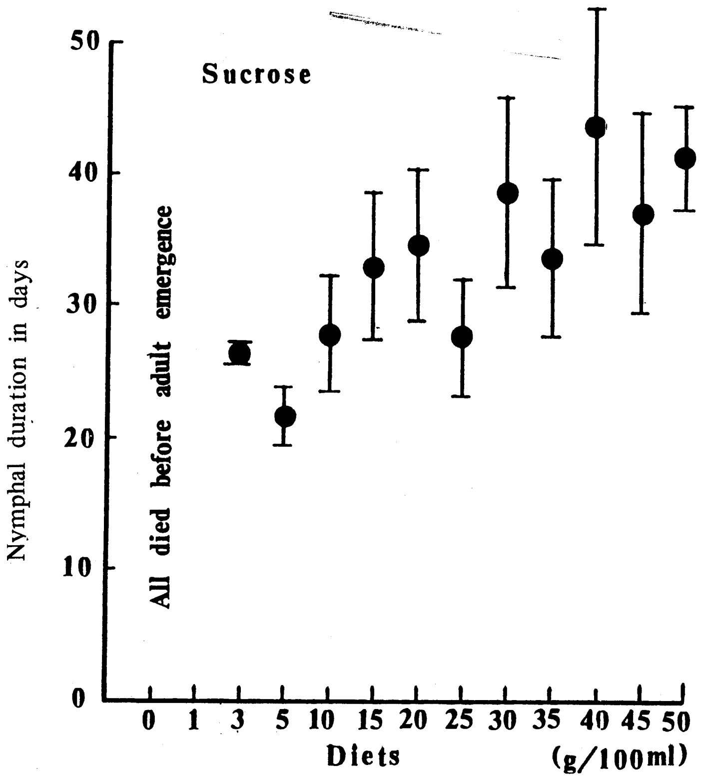

Fig. 3. Effect of sucrose concentration in MED-1 diet on nymphal period.
:Average±standard deviation.

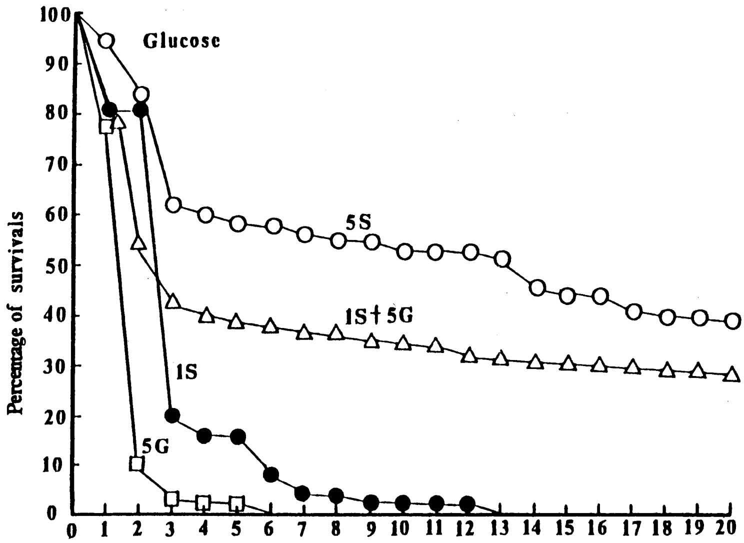

Fig. 4. Effect of the concentration of glucose and sucrose in MED-1 diet on nymphal survival.
1S: 1g/100 ml Sucrose 5S: 5g/100 ml Sucrose
5G: 5g/100 ml Glucose 1S+5G: 1g Sucrose, 5gGlucose/100ml

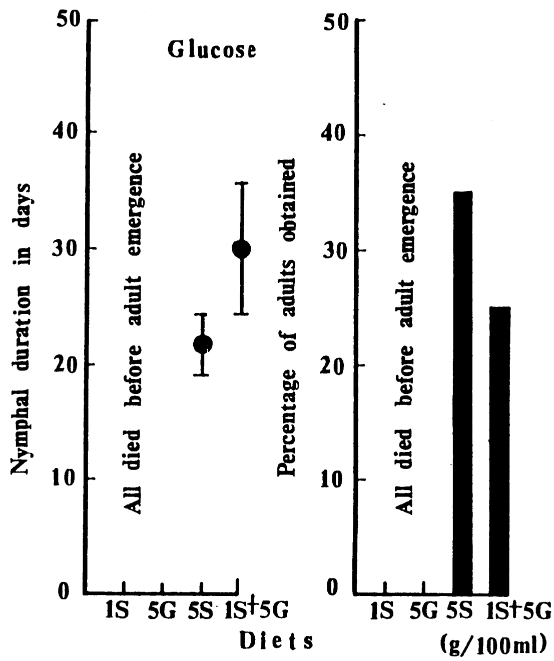

Fig. 5 Effect of the concentration of glucose and sucrose in MED-1 diet on nymphal period and adult emergence.

: Average ± standard deviation.

1S: 1g/100 ml Sucrose 5S: 5g/100 ml Sucrose
5G: 5g/100 ml Glucose 1S+5G: 1g Sucrose, 5g Glucose/100 ml

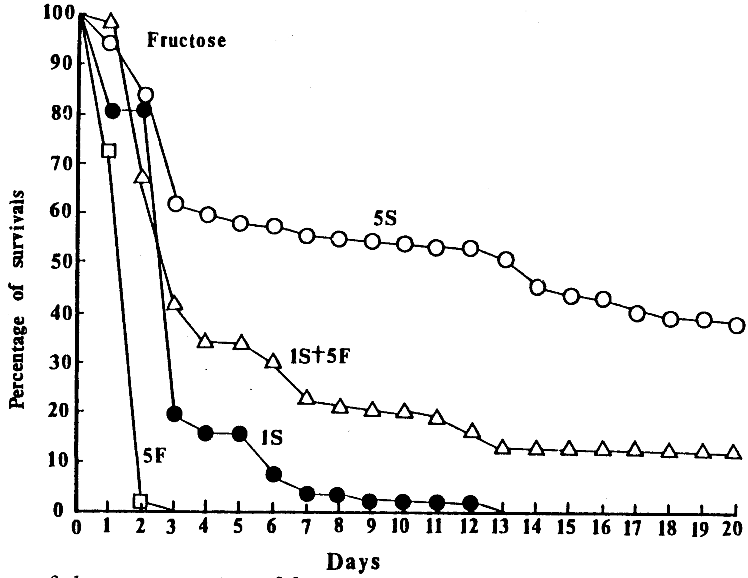

Fig. 6. Effect of the concentration of fructose and sucrose in MED-1 diet on nymphal survival.
1S: 1g/100 ml Sucrose
5F: 5g/100 ml Fructose
5S: 5g/100 ml Sucrose
1S+5F: 1g Sucrose, 5g Fructose/100 ml

MED-1 diet containing 5% fructose as an only sugar component (Fig. 6). Supplements of 1% sucrose to the diet resulted in the larval growth, the larvae grew to adults, however nymphal duration was longer than that on the diet containing 5% sucrose, and the rate of adult emergence was only around 10% (Fig. 7).

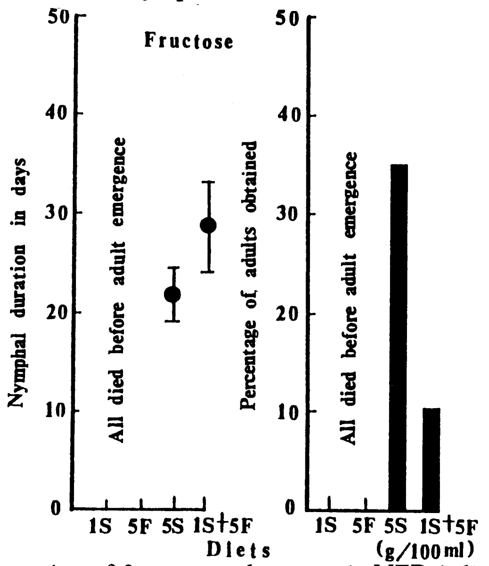

Fig. 7. Effect of the concentration of fructose and sucrose in MED-1 diet nymphal period and adult emergence. 1S: 1g/100 ml Sucrose
Ī : Average±standard deviation. 5F: 5g/100 ml Fructose
5S: 5g/100 ml Sucrose
1S+5F: 1g Sucrose, 5g Fructose/100 ml

Effects of maltose on the growth of the larvae

All larvae died within 4 days of rearing on MED-1 diet containing 5% maltose as a sole sugar component (Fig. 8). However, the larvae grew to adults when this diet was fortifying by 1% sucrose like the case of glucose and fructose. A slightly longer nymphal period and lower adult emergence rate 1% were observed compared to larvae reared on the diet containing 5% sucroce (Fig. 9).

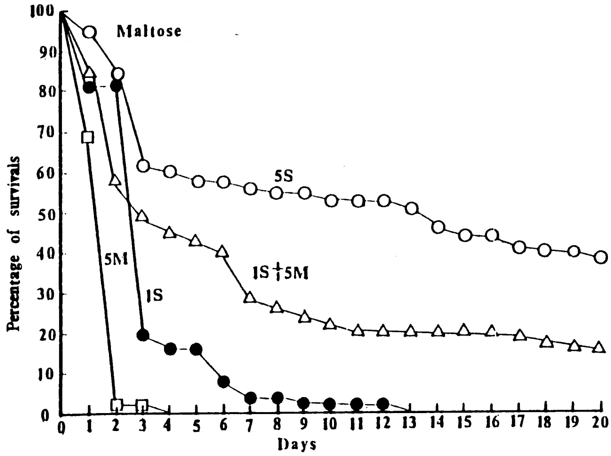

Fig. 8. Effect of the concentration of maltose and sucrose in MED-1 diet on nymphal survival.
1S: 1g/100 ml Sucrose
5S: 5g/100 ml Sucrose
5M: 5g/100 ml Maltose
1S+5M: 1g Sucrose, 5g Maltose /100 ml

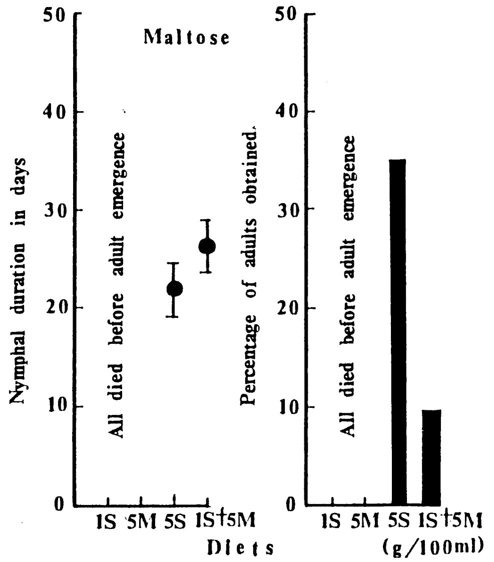

Fig. 9. Effect of the concentration of maltose and sucrose in MED-1 diet on nymphal period and adult emergence.
Ī : Average±standard deviation.
1S: 1g/100 ml Sucrose
5S: 5g/100 ml Sucrose
5M: 5g/100 ml Maltose
1S+5M: 1g Sucrose, 5g Maltose/100 ml

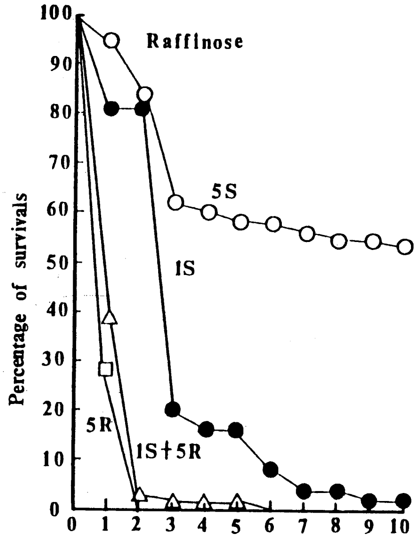

Fig. 10. Effect of the concentration of raffinose and sucrose in MED-1 diet on nymphal survival.
1S: 1g/100 ml Sucrose 5S: 5g/100 ml Sucrose
5R: 5g/100 ml Raffinose 1S+5R: 1g Sucrose, 5g Raffinose/100 ml

Effects of raffinose on the growth of the larvae

All larvae died by day-2 when reared on MED-1 diet containing 5% raffinose. All larvae died by day-6 when reared on the diet containing 1% sucrose and 5% raffinose (Fig. 10).

Effects of trehalose on the growth of the larvae

All the larvae died by day-3 when reared on MED-1 diet containing 5% trehalose as the sole sugar source. Addition of 1% sucrose to the diet did not significantly improve the situation and all the larvae died within 6 days after hatching (Fig. 11).

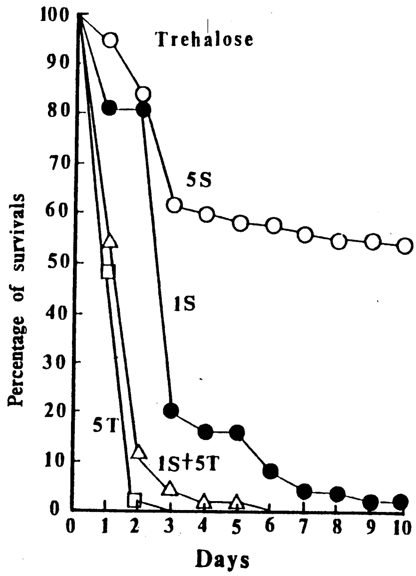

Fig. 11. Effect of the concentration of trehalose and sucrose in MED-1 diet on nymphal survival.
1S: 1g/100 ml Sucrose 5S: 5g/100 ml Sucrose
5T: 5g/100 ml Trehalose 1S+5T: 1g Sucrose, 5g Trehalose/100ml

Discussion

Larvae of brown rice planthoppers were reared on the basal MED-1 diet supplemented with one of the following sugars, sucrose, glucose, fructose, maltose, raffinose, and trehalose. All larvae died within several days of rearing on all sugar sources except sucrose. However, the larvae grew to adults on diets containing either glucose, fructose, or maltose if they were supplemented with 1% sucrose. Since the larvae could not grow to adults on the MED-1 diet containing 1% sucrose alone, it is presumed that glucose, fructose, and maltose can function as nutrients in the presence of sucrose. For the growth of the larvae of brown rice planthopper, the present study showed that the nymphal period was shortest on the diet containing 5% sucrose. Although the reason why nymphal period increased on 10-50% sucrose is not clear at present, but it is likely that quantitative balance between sucrose and other nutrients in the basal diet might be correlated closely with the growth rate of the larvae. In the previous studies (Koyama, 1981), it was shown that sucrose concentration itself in the diet would also play an important role on the growth of the larvae, since administration of higher concentration of sucrose to the 2nd-instar larvae resulted in the longer survival period.

The complete synthetic diets for leafhoppers and planthoppers so far described contained 5% sucrose as a sugar source; for the small brown planthopper, (Mitsuhashi and Koyama, 1971); for brown rice planthoppers, (Koyama, 1979); for white-backed rice planthoppers, (Koyama and Mitsuhashi, 1980); for *Sogatella longifurcifera* Esaki et Ishihara, (Koyama et al., 1981); for green rice leafhopper and zig-zag rice leafhoppers, (Koyama, 1973); for aster leafhoppers, (Hou and Brooks, 1975). The present study showed that the optimal sucrose concentration in the diet was also 5% for brown rice planthoppers, and that glucose, fructose, and maltose have nutritive value and would be utilized for studies on nutritional physiology in combination with sucrose.

2. Essential Amino Acids for the Nymphal Development

Results

Fig. 12 shows the average larval period for *N. lugens* reared on the MED-1 diet from which each amino acid had been eliminated one by one. This figure shows that the larvae developed completely in the absence of any one amino acid so far as only one amino acid had been eliminated at one time. However the larval period longer when the larvae were reared on a diet lacking cystine, histidine or methionine. The percentege of larvae developing into adults tended to decrease with an increase in the larval period, except for the larvae grown without arginine (Fig. 13). Most of the deaths during the larval period occurred immediately after hatching. *N. lugens* could survive for only 15 days on a diet that lacked all amino acids.

Discussion

From the experiment in which individual amino acids were eliminated one at a time, it is concluded that no amino acid was essential for the larval growth of *N. lugens*. This is very different from other insects whose nutritional requirements have been studied. In many other insect, essential amino acids are the following 10 amino acids, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophane and valine, similar to manmmals. Even another species in the same family, *L. striatellus* required cystine or cysteine, and methionine as essential amino acids for the growth of larvae (Koyama and Mitsuhashi, 1975).

Comparison of the amino acid requirement of *N. lugens* with *L. striatellus* shows that the larvae of *L. striatellus* can not develop into adults in the absence of either cysteine or cystine, whereas *N. lugens* can develop into adults even when both cysteine and cystine are removed from the diet. Methionine is also essential for the growth of the larvae of *L. striatellus* but not for *N. lugens*. All of the larvae of both species, however died at the 1st instar when these three sulfur-containing amino acids were eliminated from the diet together. These results clearly show that sulfur-contraining amino acids are important for the growth of *L.*

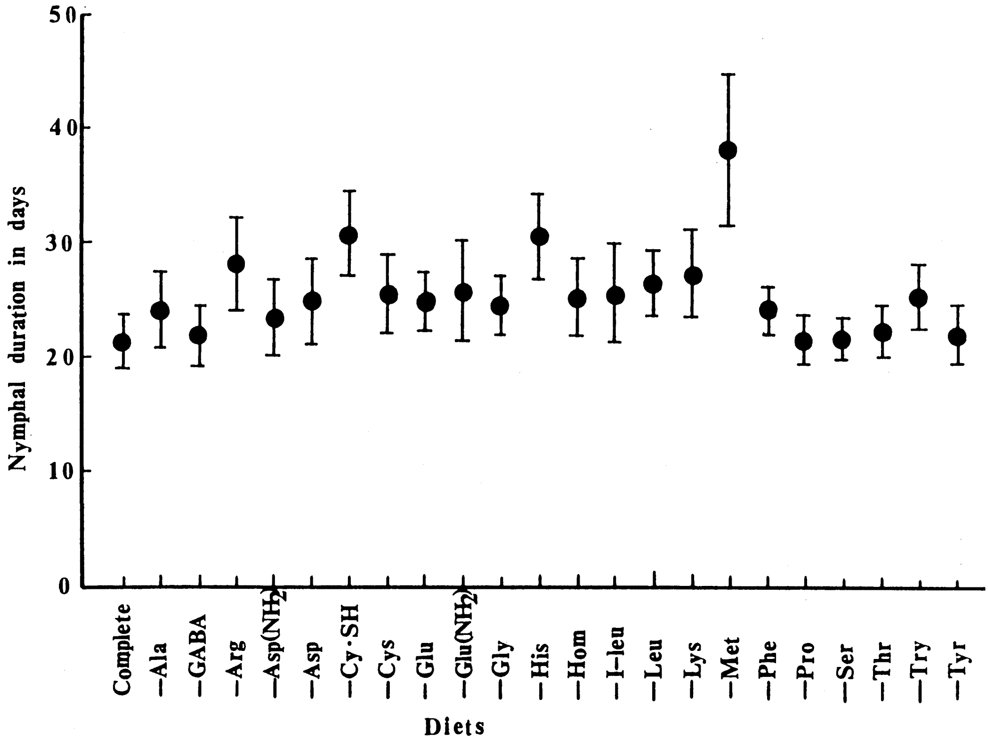

Fig. 12. Nymphal period of *N. lugens* reared on MED-1 diets lacking single amino acids.
$\bar{\Phi}$: Average±standard deviation.

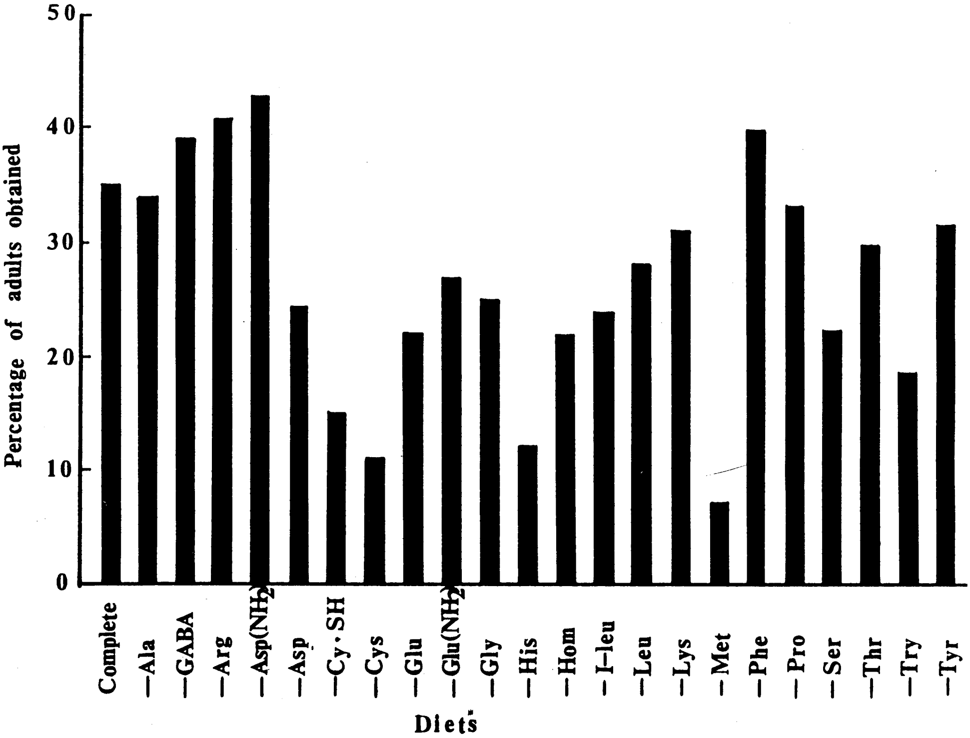

Fig. 13. Adult obtained of *N. lugens* reared on MED-1 diets lacking single amino acids.

striatellus and *N. lugens*. The amino acid requirement of aphids has been examined for *Myzus persicae* and *Aphis fabae*. In *M. persicae*, methionine, histidine and isoleucine have been reported to be essential (Dadd and Krieger, 1968), and for *Aphis fabae*, alanine, histidine, methionine, proline, serine, cysteine, phenylalanine and tyrosine are essential (Leckstein and Llewellyn, 1973. 1974). This also shows that amino acid requirements vary even among the insects of the same family. Generally the nutritional requirement of hemiptera is qualitatively smaller than insects of other orders. This might be because of the presence of symbiotic microorganisms which produce the substances essential to the host insect. In *N. lugens*, symbiotic yeast-like organisms are present in the mycetocytes in its abdomen. These organisms may be able to produce various substances necessary for the growth of *N. lugens*. Also, it may be possible that some amino acids can be replaced by other amino acids. Lastly the balance of each amino acid may be important. The elucidation of these points as well as the role of the symbiotic organisms are problems to be solved in the future.

3. Essential Vitamins for the Nymphal Development

Results

Fig. 14 shows the duration of the nymphal period of the brown rice planthopper when reared on MED-1 diets lacking one vitamin. When fed on diet lacking thiamine, pyridoxine or calcium pantothenate, nymphs grew to the 4th, 3rd and 4th instr respectively, however they did not reach the adult stage. The nymphal period were greatly enlongated by the deletion of

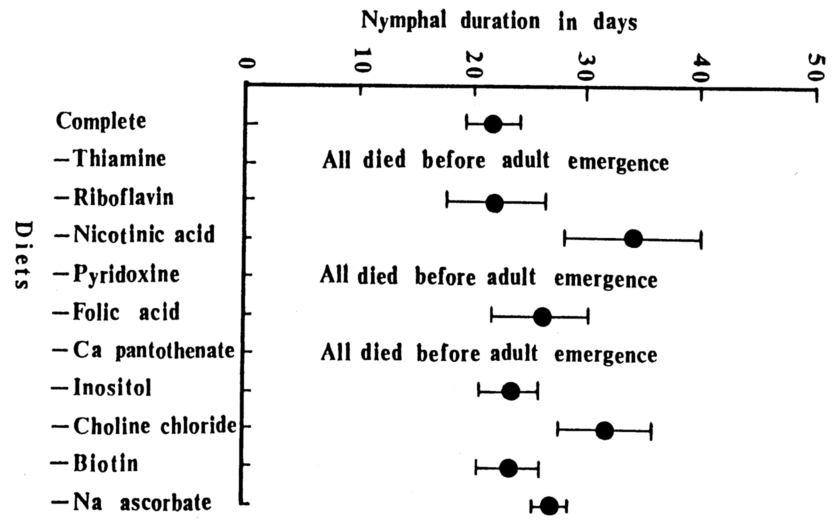

Fig. 14. Nymphal period of *N. lugens* reared on MED-1 diets lacking single vitamins.
Ɨ : Average±standard deviation.

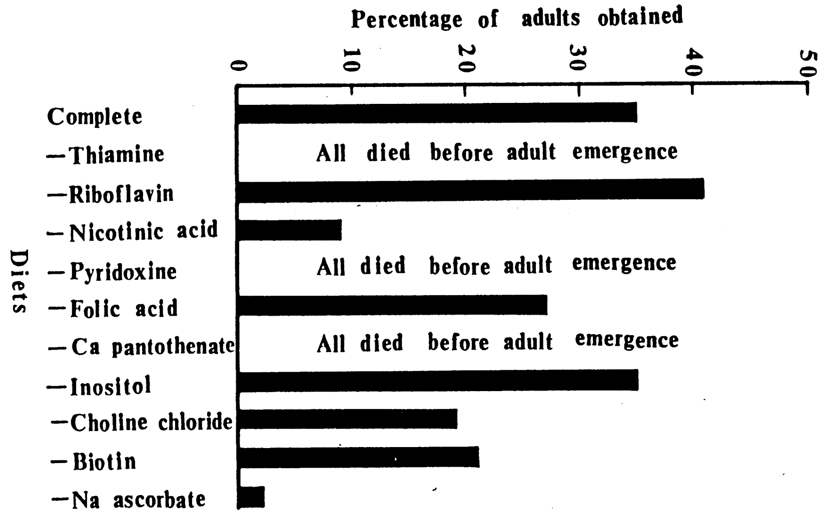

Fig. 15. Adult emergence of *N. lugens* reared on MED-1 diets lacking single vitamins.

nicotinic acid or choline chloride, and somewhat enlongated by the deletion of sodium ascorbate. Absence of other vitamins did not significantly effect the duration of the nymphal period. The deletion of sodium asocrbate or nicotinic acid decreased the rate of adult emergence considerably (Fig. 15). Mortality during the nymphal period was found to be high immediately after the initiation of rearing in every case (Figs. 16 and 17).

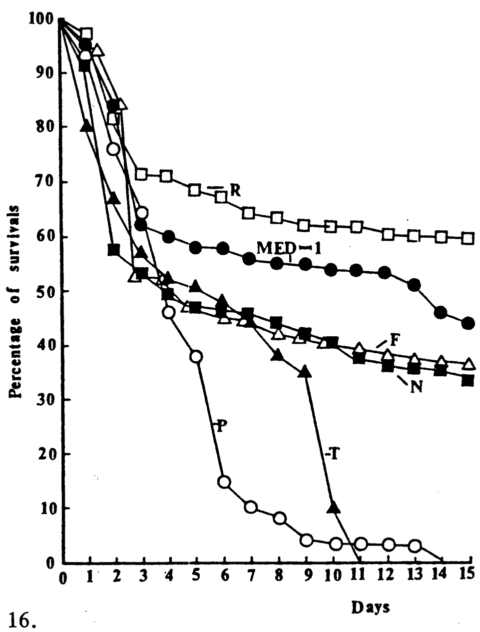

Fig. 16.
Survival of *N. lugens* within 15 days after hatching.
MED-1: Complete
T: MED-1 diet lacking thiamine hydrochloride
R: MED-1 diet lacking riboflavin
N: MED-1 diet lacking nicotinic acid.
P: MED-1 diet lacking pyridoxine hydrochloride
F: MED-1 diet lacking folic acid

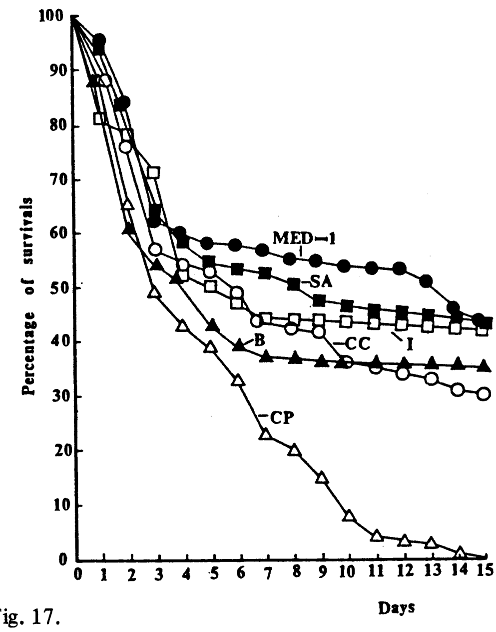

Fig. 17.
Survival of *N. lugens* within 15 days after hatching.
MED-1: Complete
CP: MED-1 diet lacking calcium pantothenate
I: MED-1 diet lacking inositol
CC: MED-1 diet lacking choline chloride
B: MED-1 diet lacking biotin
SA: MED-1 diet lacking sodium L-ascorbate

Discussion

These experiments showed that essential vitamins for nymphal development of the brown rice planthopper were thiamine. Pyridoxine and pantothenic acid, similar to the smaller brown planthopper, *Laodelpax striatellus* Fallén (Koyama and Mitsuhashi, 1977). It was impossible to determine the optimum concentration of these vitamins exactly because there were no large differences in nymphal development and survival over a wide range of concentration.

Aphids and coreidoid bugs are the only other hemipterous insects whose vitamin requirement have been studied. In *Myzus persicae,* the essential vitamins for nymphal development are thiamine, nicotinic acid and pantothenic acid (Dadd et al., 1967). *In Riptorus clavatus,* riboflavin is only one essential vitamin for the nymphal development (Noda and Kamano, 1983). These results show that the vitamin requirements of hemipterous insects depend on the species.

The nutritional requirement of hemipterous insects is lower than those of other insects probably because the intracellular symbionts in hemiptera produce and provide many essential substances for the host. The brown rice planthopper seems to own a considerable part of its essential nutrition to such microorganisms, which may synthesize essential vitamins for their host insect. However the balance among the vitamins as a group also seems to be important, Therefore, it is also a problem to be studied in future like the role of the symbionts.

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