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A chemically defined diet enables continuous rearing of the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae)

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Abstract
Continuous rearing of the brown planthopper, *Nilaparvata lugens* (Stål), was achieved on a chemically defined diet D-97 developed in this laboratory. The performance of *N. lugens* reared on D-97 was much better than that on a previously reported artificial diet MMD-1, although it was inferior to the susceptible rice variety TN1. *N. lugens* can not finish a complete generation on MMD-1 due to poor fecundity and unhatchability of eggs, but it has been successfully reared for more than 6 generations on D-97. In comparison with TN1-reared *N. lugens*, D-97-reared insects had a longer nymphal period (more than 3 days longer for males and 5 days longer for females), lighter weight (over 25% lighter for males and 35% lighter for females) and lower fecundity (over 50% less). Of the six generations on D-97, the first two generations had a significantly higher rate of emergence and hatchability. The possible factors influencing rearing efficiency and the potential applications of this diet are also discussed.

Key words: *Nilaparvata lugens*, continuous rearing, chemically defined diet

INTRODUCTION

Artificial rearing of insects on chemically defined diets potentially provides a convenient approach to the study of their nutrition, symbiosis, and many other aspects of the physiology as well as insect-plant interactions. In addition, a successful artificial diet would also facilitate the evaluation of the toxicity of potential pesticides or products of candidate genes to be used in transgenic engineering.

Great progress has been made in the rearing of plant-sucking insects on artificial diets since 1962 when Mittler and Dadd (1962) developed the "Parafilm M" membrane technique to hold artificial diet. According to Singh (1985), up to 71 homopteran insects have been reared on artificial diets, including 50 species within the Aphididae family. Indeed, the artificial rearing of aphids represents the most successful case among the Homoptera. The current artificial diets for planthoppers were actually formulated based on those for aphids, including MED-1 and MMD-1, the two common chemically defined diets for planthoppers which were derived from just a minimal modification of the Ehrhardt's aphid diet and Mittler and Dadd's aphid diet, respectively (Mitsuhashi, 1974). Those artificial diets are suitable for rearing the small brown planthopper, *Laodelphax striatellus*, from generation to generation (Mitsuhashi, 1974), but they only enable *N. lugens*, one of the most destructive rice pests in Asia, to develop through several nymphal stages to adult (Koyama, 1979, 1985; Gatehouse et al., 1996). Until now, no reports have shown that *N. lugens* could be reared continuously on artificial diets.

N. lugens is a monophagous insect with host plants quite different from those of aphids, it is possible that the nutritional balance of aphid diets is unsuitable for the rearing of *N. lugens*. With this consideration in mind, we have developed a chemically defined diet D-97 by optimizing the balance of nutritional components, which has been proved to be effective for continuous rearing of *N. lugens*.

MATERIALS AND METHODS

Insects. The brown planthopper, *N. lugens*, was collected during the autumn of 1997 from paddy fields at Fuyang County, Zhejiang Province, China. They were then maintained for 8 generations on a susceptible rice variety TN1 under the conditions

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Table 1. The composition of two chemically defined diets D-97 and MMD-1[a]

Contents	D-97	MMD-1[b]	Contents	D-97	MMD-1[b]
I. Amino acids			II. Vitamins		
Glycine	30	80	Biotin	0.05	0.1
L-Alanine	130	100	Calcium pantothenate	5.0	5.0
L-Arginine hydrochloride	175	270	Choline chloride	50.0	50.0
L-Asparagine	230	550	Folic acid	0.5	0.5
L-Aspartic acid	100	140	Inositol	50.0	50.0
L-Cysteine	80	40	Nicotinic acid	15.0	10.0
L-Cystine hydrochloride	20	0	Pyridoxine hydrochloride	2.5	2.5
γ-Amino butyric acid	10	0	Riboflavin	0.5	0.5
L-Glutamic acid	250	140	Thiamine hydrochloride	2.5	2.5
L-Glutamine	240	150	Ascorbic acid	100.0	100.0[c]
L-Histidine	80	80	III. Inorganic salts		
L-Methionine	70	80	$CaCl_2 \cdot 2H_2O$	3.115	3.115
L-Isoleucine	100	80	$CuCl_2 \cdot 2H_2O$	0.268	0.268
L-Leucine	240	80	$FeCl_3 \cdot 6H_2O$	2.228	2.228
L-Proline	100	80	$MnCl_2 \cdot 4H_2O$	0.793	0.793
L-Lysine hydrochloride	200	120	$ZnCl_2$	0.396	0.396
L-Phenylalanine	200	40	$MgCl_2 \cdot 6H_2O$	200	200
L-Serine	400	80	KH_2PO_4	500	500
L-Threonine	130	140	IV. Others		
L-Tryptophane	105	80	Sucrose	9,000	5,000
L-Tyrosine	10	40	pH	6.8	6.5
L-Valine	300	80			

[a] Unit: mg/100 ml.
[b] After Koyama (1988).
[c] Using sodium ascorbate instead.

of 26–30°C, over 80% RH, and a photoperiod of 12–14 h. The present study was initiated with one-day-old 9th generation *N. lugens* nymphs.

Diets. To develop a chemically defined diet for continuous rearing of *N. lugens*, about 40 nutrient components of artificial diet were optimized by testing 252 diets designed from tables of orthogonal arrays. The results showed that the balance of some components, including sucrose, most amino acids and some vitamins, is essential for the rearing of *N. lugens* (data not shown). After making some modifications on the balance of those components in MMD-1 (Koyama, 1988), we developed a chemically defined diet D-97 suitable for rearing *N. lugens* (Table 1).

To prepare the diet, all the amino acids, vitamins (except ascorbic acid), and trace metals (except $FeCl_3 \cdot 6H_2O$) were prepared as stock solutions of 2×, 10×, and 100×, respectively and stored at −20°C if not used at once. To a beaker were added amino acid stock, sucrose, KH_2PO_4, $MgCl_2 \cdot 6H_2O$, and $FeCl_3 \cdot 6H_2O$, and the mixture was stirred until the compounds were completely dissolved. Then, vitamin and trace metal stocks as well as ascorbic acid solution (separately prepared as 10× stock solution before use) were added. The diet solution was adjusted to pH 6.8 with 4% KOH, and diluted to the working concentration with distilled water. And finally, the solution was filter-sterilized through a Millipore disposable filter (0.45 μm), and stored at −20°C prior to use.

The chemically defined diet MMD-1 (Koyama, 1988) (Table 1) and susceptible rice variety TN1 were used as controls.

Rearing procedure. For the rearing on artificial diets, glass cylinders (Fig. 1A), 15.0 cm in length and 2.5 cm in diameter, were used as feeding chambers. The artificial diets were held between two layers of stretched Parafilm M (about four times of the original area) which were located at one open end of the chamber and renewed every two days. The opposite end of the chamber was enclosed with a piece of nylon mesh after test insects had been introduced. The chambers were covered

with a sheet of wet black cotton cloth, but the end with the artificial diet was exposed to light. Insects could feed on diets by piercing through the inner Parafilm M of the diet sachet. Twenty-five one-day-old nymphs were infested into each chamber and four replicates were used. Mortality data were recorded every two days until the first adult emerged. Newly emerged adults (within 24 h after emergence) were counted every day and weighed individually on a 0.01-mg sensitivity balance (Mettler AE240).

N. lugens to be reared on artificial diets never had contact with rice plants after being infested onto these diets. Adults after emergence were reared for another three days on artificial diet in the feeding chambers before being transferred to the oviposition chambers (one female and one male per chamber) (Fig. 1B) to measure the fecundity of individual females. The oviposition chambers had the same diameter as the feeding chambers but were only 3.0 cm long. One open end of the chamber was covered with the artificial diet sachet and the other with the sachet including the oviposition medium (5% sucrose, 0.004 M salicylic acid, pH 6.5) prepared according to Koyama (1988), by which insects could feed on the artificial diet and lay eggs in the oviposition medium. Eggs were removed from the medium every two days, kept in distilled water for 6 days and incubated on moist filter paper. Their hatching was recorded. Newly hatched nymphs (no less than 100 individuals) were reared on the same artificial diet as their parents.

The survival and development of *N. lugens* on rice plants were tested on 45-day-old TN1 which were enclosed in cylindrical plastic cages (8 cm in diameter, 50 cm in height) with a nylon mesh top and two nylon mesh side windows. Twenty-five one-day-old nymphs were placed in each of the four cages. The nymphal mortality, adult emergence and adult weight were recorded as those on artificial diets.

To test female fecundity and egg hatchability on TN1 plants, newly emerged adults were transferred into similar plastic cages (one female and one male per cage). The "oviposition mark" in TN1 plants was monitored daily to estimate the preoviposition period of each female. The fecundity of each female and the hatch of eggs were determined by counting the numbers of newly emerged nymphs every day and the unhatched eggs ten days after the female died.

All rearing experiments were carried out at 27.0±0.5°C, over 90% RH, with a 12–14 h photoperiod.

RESULTS

N. lugens, which could not complete a generation on diet MMD-1 (Gatehouse et al., 1996), could now be continuously reared for more than 6 generations on our diet D-97.

The survivorship of the first generation *N. lugens* raised on D-97 was much better than that on MMD-1, and was similar to that on TN1 (Fig. 2). The rate of adult emergence at the first generation on D-97 was also markedly higher than that on MMD-1 ($p<0.01$), although it was somewhat lower than that on TN1 ($0.05<p<0.10$). In the successive rearing on D-97, the emergence rate was highest in the first generation, decreased slightly in the second generation (no significant difference, $p>0.05$), but declined markedly after the third gen-

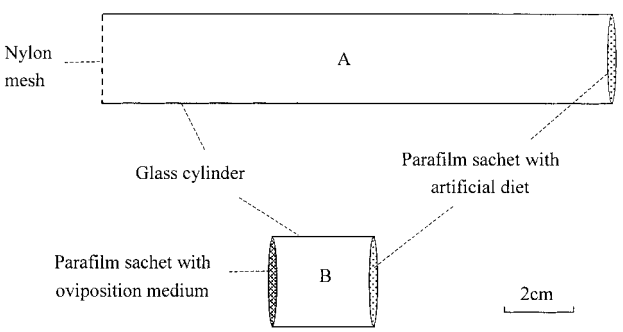

Fig. 1. Schematic diagram of feeding chamber (A) and oviposition chamber (B) for rearing *Nilaparvata lugens* on artificial diets.

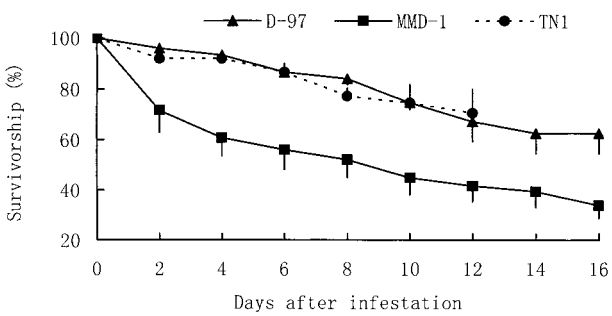

Fig. 2. Survivorship of the first generation of *Nilaparvata lugens* reared on chemically defined diets D-97 and MMD-1. Values are expressed as means±SE ($n=4$).

eration ($p<0.01$) (Fig. 3).

Both brachypters and macropters could emerge on the two artificial diets and TN1 as well (Fig. 3). For brachypters, the average ratio of each generation on D-97 ranged from 28.9% to 33.3%, which was markedly lower than that on TN1 (83.7%) ($p<0.01$), but higher than that on MMD-1 (9.4%) ($0.05<p<0.10$).

In comparison with TN1, nymphs reared on D-97 and MMD-1 were retarded in development (Table 2). The nymphal duration of diet-reared insects was at least 5 days longer for females and 3 days longer for males. However, there was no significant difference among nymphal periods of each generation on D-97 except the fourth generation which was prolonged, possibly due to the lower environmental temperature during renewal of the diet (Table 2).

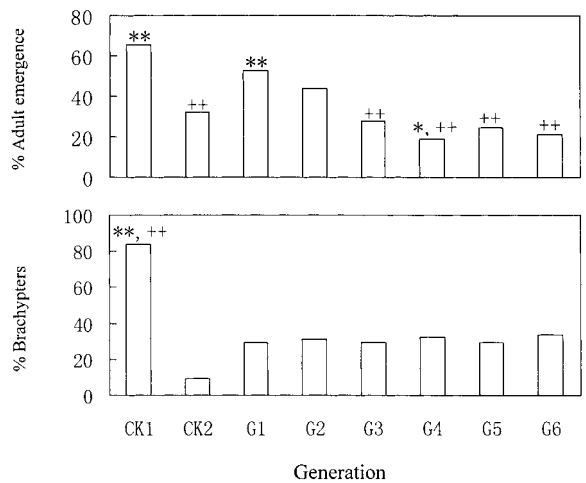

Fig. 3. Adult emergence and brachypters of *Nilaparvata lugens* reared on chemically defined diet D-97 (each generation started from no less than 100 newly hatched nymphs). CK1: control fed on TN1; CK2: the first generation on MMD-1; **, *: significantly higher or lower than the first generation on MMD-1 at the level of 0.01, 0.05, respectively (*t*-test); ++: significantly higher or lower than the first generation on D-97 at the level of 0.01 (*t*-test).

The adults emerged on D-97 and MMD-1 were similar in size, but much smaller than those on TN1 as demonstrated by the weight of newly emerged adults (Table 2). Adult males and females reared on the artificial diets were less than 75% and 65% the weights of those on TN1 plants, respectively. Among the six consecutive generations raised on D-97, the first generation females were the heaviest, partly as a result of feeding on rice plant within one day after egg hatch before being infested onto D-97.

There was no significant difference between the longevity of females raised on the two artificial diets and on TN1 ($p>0.05$). However, the fecundity of females on the artificial diets was obviously lower than that on TN1 (Table 3). Of females reared on D-97, 42.1–58.3% laid eggs, while 93.8% and 5.9% ones did on TN1 and MMD-1, respectively. Oviposition also significantly delayed on both D-97 and MMD-1, as indicated by the preoviposition period, which was about one week later on D-97 and 12 days later on MMD-1. The number of eggs laid per female on D-97 increased markedly as compared with that on MMD-1. Some females on D-97 could lay more than 200 eggs with a maximum of 275 (laid by a female of the third generation), although the average fecundity on D-97 was less than 50% that on TN1.

Table 2. The adult weight and the nymphal period of *Nilaparvata lugens* on the chemically defined diet D-97 (mean±SE)[a]

Diets	Generation	Nymphal period (days)		Weight of newly emerged adult (mg)	
		Female	Male	Female	Male
D-97	1st	19.3±0.3 b	17.8±0.4 b	1.568±0.041 b	0.994±0.043 b
	2nd	20.0±0.6 b	17.7±0.5 b	1.442±0.045 bc	0.958±0.046 b
	3rd	20.3±0.8 b	17.8±0.5 b	1.395±0.053 bc	0.987±0.028 b
	4th	22.6±0.6 c	20.1±0.6 c	1.358±0.062 c	0.949±0.029 b
	5th	19.5±0.6 b	18.5±0.5 b	1.400±0.050 bc	0.970±0.036 b
	6th	20.3±0.7 b	18.8±0.6 b	1.388±0.069 bc	0.932±0.041 b
MMD-1	1st	19.2±1.0 b	18.5±0.8 b	1.429±0.027 bc	1.029±0.046 b
TN1		14.2±0.1 a	14.1±0.2 a	2.432±0.067 a	1.388±0.041 a

[a] Means within columns followed by same letter are not significantly different ($p=0.05$, LSD test).

Table 3. Reproduction and egg hatching of *Nilaparvata lugens* on the chemically defined diet D-97[a]

Diets	Generation	Females observed	Female laying (%)	Preoviposition (days)[b]	Eggs laid per female[b]	Longevity of female (days)[b]	Egg-hatching (%)
D-97	1st	19	42.1	7.0±0.4	73.6±24.4	15.1±3.6	79.7
	2nd	12	58.3	7.8±0.3	90.9±20.2	16.8±1.8	70.8
	3rd	19	52.6	6.8±1.3	109.3±28.1	19.4±1.6	45.8*
	4th	12	58.3	11.8±1.5*	56.0±18.0	17.1±1.2	45.2*
	5th	9	55.6	7.9±1.2	103.0±22.1	21.3±1.9	51.9*
	6th	8	50.0	8.3±1.1	117.0±28.1	20.1±2.4	41.1*
MMD-1	1st	17	5.9*	15.0	3.0	18.1±0.9	0.0*
TN1		16	93.8*	2.9±0.2*	257.0±37.9*	22.1±1.7	75.1

[a] "*" showed significant difference from the first generation on D-97 at the level of 0.05 (*t*-test for percentage data, LSD test for others).
[b] Expressed as means±SE.

The hatchability of eggs on D-97, which was as high as the TN1 population for the first two generations (Table 3), showed a substantial decrease after the third generation ($p<0.01$). On MMD-1, however, no eggs hatched.

DISCUSSION

Modification of the nutrient balance in artificial diet is an effective approach for improving the rearing efficiency, as has been proved in the development of artificial diets for homopterans (Dadd and Mittler, 1966; Mitsuhashi and Koyama, 1969; Hou and Brooks, 1975; Kunkel, 1977; Febvay et al., 1988). In this study, we developed a chemically defined diet D-97 mainly by modifying the balance of dietary nutrients, especially that of amino acids (see Table 1). D-97, on which *N. lugens* could be reared to the 6th generation, was much more suitable than MMD-1, as no *N. lugens* could complete one generation on the latter due to poor fecundity of females and unhatchability of eggs. This implies that the significant improvement in rearing *N. lugens* could be attributed to a better balance of nutrition in this diet.

However, the growth and reproduction of *N. lugens* on D-97 were still inferior to those on the susceptible rice variety TN1. This is consistent with some earlier studies regarding the rearing of other homopterans on holidic diets (Mitsuhashi, 1974; Mittler and Koski, 1976). Even *Myzus persicae* and *Acyrthosiphon pisum*, the most successful species reared on holidic diets in Homoptera, also showed lighter weight, slower development and lower fecundity when reared on chemically defined diets (Dadd and Mittler, 1966; Akey and Beck, 1972; van Emden, 1988).

As had been discussed by many authors (Mittler and Koski, 1976; Prosser and Douglas, 1992; Sandstrom, 1994; Sandstrom and Pettersson, 1994; Liadouze et al., 1995), many factors may be related to the poor performance of homopterans reared on holidic diets. Some of these factors may be involved in the control of feeding behavior and others in post-ingestion physiology. In the present study, the feeding rate of *N. lugens* on D-97 was only 31% that on susceptible rice variety TN1 (data not shown), probably because the artificial diet lacks positive pressure to assist insects in diet uptake, and also many of the physical and chemical cues associated with natural feeding on host plants may be lacking. The evidence that only the first two generations of *N. lugens* on D-97 showed higher rates of adult emergence and egg hatchability implies that the diet still lacks, or is deficient in, certain nutrients. It may also indicate that some plant-derived nutrients or products thereof are transmitted from plant-reared insects to their off spring reared on artificial diets as in the case of aphids (Mittler and Koski, 1976), although the first generation fed on rice plant for less than one day before being infested onto artificial diets. In addition, it is possible that D-97 is still imperfect in nutrient balance, and insects reared on such an unbalanced diet may incur a metabolic cost to provide compensation (Liadouze et al., 1995). Clarification of these uncertainties would be essential for further improvement of *N. lugens* artificial rearing.

The D-97 diet provides an effective tool for studying physiology such as nutritional requirements of both nymphs and adults and the planthopper-rice interactions relative to the resistant mechanism among rice varieties. This is the first example of successive rearing of *N. lugens* on a chemically defined diet, leading to the possibility of studying the physiology of *N. lugens* over continuous generations. In addition, though generally used in bioassaying the toxicity of potential pesticides and evaluating the candidate genes possibly used in transgenic plants, the previous diets such as MMD-1 are not suitable due to poor survivorship (Powell et al., 1993; Gatehouse et al., 1996). D-97 is not only suitable for *N. lugens* as already verified in this investigation (Figs. 2 and 3), but also for *Sogatella furcifera*, where good survivorship and an emergence rate of over 70% were recorded (data not shown).

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