JARQ 60 (1), 33-38 (2026) https://www.jircas.go.jp

Rearing the Rice Stink Bug, *Niphe elongata* (Hemiptera: Pentatomidae), on Mixed Grains of Brown Rice, Wheat, and Foxtail Millet

Takahiro SETOGUCHI[1]* and Takuya SHIBA[1,2]

[1] Institute for Plant Protection, National Agriculture and Food Research Organization, Tsukuba, Japan

Abstract

The rice stink bug, *Niphe elongata* (Dallas) (Hemiptera: Pentatomidae), is a re-emerging pest that has seriously infested rice over a wide area of western and central Japan since the 2000s. This study developed a novel method for rearing this insect using mixed grains of brown rice, wheat, and foxtail millet supplemented with solution of vitamin C. While the body size of emerged adults reared using this method was smaller than that of field-collected ones, 39.2% of individuals developed from first instar nymph to adult at 28°C under a 16:8 h L:D photoperiod, had an average developmental period of 25.4 days. We maintained a strain of the insect for eight generations over 14 months without the addition of field-collected individuals. The diet was easy to prepare throughout the year. This convenient technique for rearing *N. elongata* will promote field and laboratory studies of the biology of this pest.

Discipline: Agricultural Environment
Additional key words: *Lagynotomus assimulans*, *Lagynotomus elongatus*

Introduction

The rice stink bug, *Niphe elongata* (Dallas) (Hemiptera: Pentatomidae), is a pest that infests rice and is distributed throughout Japan, Taiwan, China, the Philippines, Myanmar, and India (Chen et al. 2011, Ishikawa et al. 2012). It pierces and feeds on rice panicles from the heading stage to grain maturity, causing sterile or pecky rice (Hirae 2021, Tanaka et al. 2022, Tomokuni et al. 1993) and reducing trade prices and yields. In Japan, this insect was a significant rice pest until the 1950s. Its occurrence and damage were inconspicuous from the 1960s to the 1990s, but since the 2000s, it has again been reported over a wide part of western and central Japan (Honda et al. 2021, Kitano & Masuda 2022, Matsuda et al. 2022, Ota et al. 2020, Yasue et al. 2022). Its re-emergence is likely related to the prolonged use of rice paddies suitable for its reproduction due to the recent extension of rice planting season (Ishijima et al. 2020, Ota et al. 2020). To prevent the occurrence of sterile rice caused by this pest, another insecticide application must be added to the conventional application (Honda et al. 2021, Kitano & Masuda 2022), at further economic and environmental costs. There is currently no practical measure to predict the occurrence of *N. elongata*, which varies by location and year (Honda et al. 2021, Kitano & Masuda 2022, Matsuda et al. 2022, Ota et al. 2020, Yasue et al. 2022). Thus, developing effective management measures to predict and control its occurrence is essential.

To develop effective management measures, it is necessary to improve our understanding of stink-bug biology, which has been partially revealed by observational field studies (Higuchi 2020, Honda et al. 2021, Ishijima et al. 2020, Ouchi 1954, 1956, Torikai & Higuchi 2023) but remains largely unknown. Experimental studies using homogeneous populations of insects are important to such research. However, it is not possible to obtain such populations continuously from the

*Corresponding author: setoguchi.takahiro409@naro.go.jp

[2] Present address: Faculty of Agriculture, Ryukoku University, Otsu, Japan

Received 18 November 2024; accepted 15 April 2025; J-STAGE Advanced Epub 20 October 2025.
https://doi.org/10.6090/jarq.24J19

field because *N. elongata* passes only one or two generations per year, and its reproductive season is limited to July and August (Honda et al. 2021, Ishijima et al. 2020, Torikai & Higuchi 2023). Thus, using laboratory-reared individuals obtained through successive generations is particularly important to promote biological studies of this insect.

There is no convenient, standard method for doing this. Ishizaki & Hirae (2021) proposed using frozen and thawed rice panicles from the milk- to dough-ripe stages as a diet for year-round rearing. However, this method is available only to researchers who can obtain and store enough panicles for year-round use. In addition, the quality of the diet is crucial because the preference of rice spikelets for feeding by *N. elongata* can vary with their ripening (Takeuchi et al. 2004), and detached rice panicles easily go moldy. A recent study reported that overwintering adults of *N. elongata* can oviposit when fed on grains of brown rice, wheat, and foxtail millet (Torikai & Higuchi 2022). This finding suggests the suitability of these mixed grains, which can be bought year-round with consistent quality and less risk of going moldy than detached rice panicles, for the successive rearing of this insect. In addition, Yushima et al. (1991) reported that supplementation with a vitamin C solution stabilizes the development of several hemipteran species reared on grains.

This paper reports on a convenient, standardized method for successive rearing of *N. elongata*. To this end, we successively reared this insect on grains of brown rice, wheat, and foxtail millet, which are readily available throughout the year, with a supplemental solution of vitamin C.

Materials and methods

1. Apparatus

Clear cylindrical plastic containers (100 mm diameter, 40 mm high; SPL Life Sciences, Gyeonggi-do, Korea) with a mesh ventilation hole in the lid were used (Fig. 1), and a filter paper (90 mm diameter, Advantec, Tokyo, Japan) lined the bottom of the container. Grains of brown rice (*Oryza sativa* ‘Koshihikari’) and wheat (*Triticum aestivum* ‘Nishinokaori’) in a ratio of 1:1 (vol/vol) were attached to gaffer tape rolled into a tube with the adhesive side out (15 mm diameter, 25 mm long; Askul Corporation, Tokyo, Japan). To fill the gaps among the grains on the tape, grains of foxtail millet (*Setaria italica* ‘German Strain R’) were attached to the outer adhesive side. Pieces of tape with grains were placed in the container (Torikai & Higuchi 2022). A 3 cm × 6 cm bellows-folded filter paper acted as an oviposition substrate for adults. A cotton pad (4 cm square, ca. 1 g) holding ca. 10 mL of vitamin C solution (0.05% [w/v] sodium l-ascorbate + 0.025% [w/v] l-cysteine hydrochloride monohydrate; Nacalai Tesque, Kyoto, Japan; Noda & Kamano 2002) was placed over the ventilation hole in the lid as a water supply. To maintain high humidity (≥ 95%) in the container, another plastic container was placed upside down over the lid. A humidity logger (TR-74Ui, T&D Corporation, Tokyo, Japan) monitored relative humidity.

2. Colony founders

Adult bugs were collected from mondo grass, *Ophiopogon japonicus* (Asparagaceae), at the National Agriculture and Food Research Organization (NARO), Tsukuba, Ibaraki (36° 01′ 32.8″ N, 140° 06′ 55.8″ E), in February 2023. Egg masses were collected from 20 containers, with three pairs of males and females being mated in each container at 25 ± 1°C under a photoperiod of 16:8 h L:D. The containers, cotton pad, and food were changed twice weekly to maintain cleanliness. After two to three months, laid egg masses (ca. 14 eggs per mass) were collected as the first generation of the laboratory strain.

3. Procedure

One container was used for each egg mass until adult emergence and for up to three mating pairs at the adult stage. The diet was supplied from the second instar stage because we confirmed that the first instar nymphs

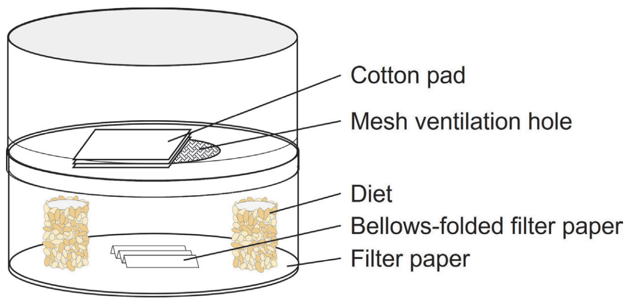

Fig. 1. Container for rearing *Niphe elongata*
Mixed grains of brown rice, wheat, and foxtail millet were attached to gaffer tape rolled into a tube with the adhesive side facing out (15 mm diameter, 25 mm long) and provided food in the container on a base of filter paper. A bellows-folded filter paper was placed in the container for adults as an oviposition substrate. As a water supply and humidifier, a cotton pad containing vitamin C solution was placed on the mesh ventilation hole in the lid with another container placed upside down over the top.

molt without feeding. From the first to the fourth generations, feed grains were added every two to three days when the number of individuals was ≤ 5 and every one to two days when the number was ≥ 6. From the fifth generation, one or two feedings were added three times a week. Moldy feed was removed immediately. The containers were replaced once or twice a week to maintain cleanliness, except that the initial container was used from the egg to the end of the first instar nymph to minimize mortality from handling. To reduce inbreeding depression and retain the vigor of the strain, individuals hatched from different egg masses were mated in the same containers, and nymphs exhibiting retarded growth and rearing containers with low survival rates were removed from the third generation onward. All adults and nymphs were reared under laboratory conditions of 28 ± 1°C and a photoperiod of 16:8 h L:D, which showed the highest nymphal survival on detached rice panicles (Yatsuzuka et al. 2023).

4. Performance of reared insects

In the second generation, we measured the duration of each stage and total development, the number of eggs per mass, and pre-mating and pre-oviposition periods. The duration of each developmental stage was based on the date when at least half of the individuals in the container reached the respective stage. Pre-mating and pre-oviposition periods were examined for three weeks in containers with three mating pairs that emerged on the same day. We measured the pronotum width and body length of laboratory-reared and field-collected adults using a micrometer with a precision scale of 0.01 mm under a stereomicroscope. The field-collected insects were collected in paddy and weedy fields at NARO, Tsukuba, Ibaraki (36° 01′ 29.6″ N, 140° 06′ 24.8″ E) in September 2023.

We reared a strain of *N. elongata* without the addition of other field-collected individuals for 14 months. We measured egg hatchability and nymphal survival in the second, third, and fourth generations. For calculating nymphal survival, individuals removed from the strain because of poor development were counted dead. We also recorded the number of eggs per female and the survival of adults during the first three weeks after adult emergence in containers with three mating pairs that emerged on the same day in the second and third generations. In the eighth generation, when almost a year had passed since rearing on the mixed grains started, we counted the number of emerged adults per container to confirm the reproductivity of the strain.

5. Statistical analysis

A two-way ANOVA was used to assess the effects of insect origin (reared or field-collected) and sex on adult body size. Statistical significance was set at $P < 0.05$. All analyses were performed with R software v. 3.6.1 (R Core Team 2019).

Results

Niphe elongata completed their life cycle under the new method without needing rice panicles, their main diet in the field. In the second generation, the eggs hatched with a developmental duration of 4.2 ± 0.4 days (mean ± SD, n = 413; Table 1), and the nymphs developed into adults with a developmental duration of 25.4 ± 2.2 days (mean ± SD, n = 28; Table 1). Adults first mated at 6.8 ± 1.7 days (mean ± SD, n = 8) after emergence and

Table 1. Developmental periods of *Niphe elongata* reared in this study

Developmental stage	Number of individuals	Developmental period (days ± SD)
Each stage		
Egg	413	4.2 ± 0.4
First instar nymph	384	2.9 ± 0.4 (29) [a]
Second instar nymph	279	5.7 ± 0.7 (28) [a]
Third instar nymph	221	4.7 ± 0.9 (28) [a]
Fourth instar nymph	179	5.3 ± 0.9 (28) [a]
Fifth instar nymph	162	6.9 ± 1.0 (28) [a]
Oviposition to adult	162	30.1 ± 3.0
Nymph	162	25.4 ± 2.2 (28) [a]

[a] The developmental period was monitored in each container because nymphs in the same container cannot be distinguished. The duration of each developmental stage was based on the date when at least half of the individuals in the container reached the next developmental stage. Numbers in parentheses represent the number of containers monitored.

oviposited at 12.6 ± 3.3 days (mean ± SD, n = 8). During the three weeks after adult emergence, we obtained 17.7 ± 12.3 (mean ± SD, n = 8) eggs per female. Egg number per mass had a mean (± SD) of 16.4 ± 5.4 and a mode of 14.

Rearing on the mixed grains reduced adult body size compared with field-collected individuals (Fig. 2). The pronotum widths of reared adults were 5%-8% smaller than those of field-collected ones (n = 20). The effects of origin and sex on pronotum widths were significant (origin: df = 1, F = 68.31, P < 0.001; sex: df = 1, F = 6.79, P = 0.011), without a significant interaction (df = 1, F = 1.61, P = 0.208). The body lengths of reared adults were 2%-6% smaller than those of field-collected ones (n = 20). The effects of origin and sex on body lengths were significant (origin: df = 1, F = 24.51, P < 0.001; sex: df = 1, F = 23.60, P < 0.001), with a significant interaction (df = 1, F = 4.57, P = 0.036).

The new method maintained the strain of *N. elongata* for eight generations over 14 months without field-collected individuals being added. In the second, third, and fourth generations, egg hatchabilities were over 80% (Table 2) and nymphal survival was over 30%. The number of eggs per female and adult survival between the second and third generations did not differ remarkably. In the eighth generation, we obtained 3.5 adults on average from each container (n = 15).

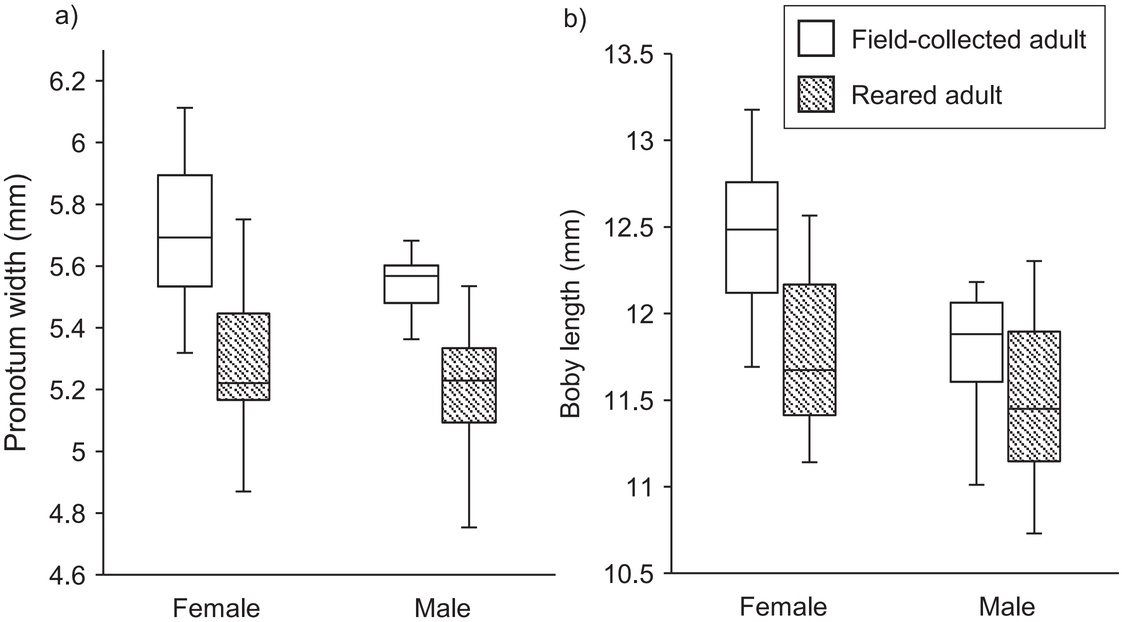

Fig. 2. Pronotum width (a) and body length (b) of *Niphe elongata* female and male adults collected in the field (*n* = 20) and reared in the laboratory (*n* = 20)
The effects of origin and sex on pronotum widths were significant (two-way ANOVA: origin, P < 0.001; sex, P = 0.011), without a significant interaction (P = 0.208). The effects of origin and sex on body lengths were significant (two-way ANOVA: origin, P < 0.001; sex, P < 0.001), with a significant interaction (P = 0.036).

Table 2. Comparative performance of *Niphe elongata* reared in this study from the second to the fourth generation

Generation	Egg hatchability (%)	Nymphal survival (%)	Number of eggs per female [a] (mean ± SD)	Adult survival [a] (female/male) (%)
Second	85.0 (486)	39.2 (413)	17.7 ± 12.3 (8)	70.8 (24) / 100 (24)
Third	84.4 (506)	38.4 (427)	32.7 ± 10.7 (6)	94.4 (18) / 94.4 (18)
Fourth	81.0 (332)	33.5 (269)	-	-

Numbers in parentheses represent the numbers of individuals or containers monitored.
[a] The number of eggs per female and adult survival during the first three weeks after adult emergence in containers with three mating pairs that emerged on the same day was recorded.

Discussion

This study demonstrated that mixed grains of brown rice, wheat, and foxtail millet can be used as a diet for the successive rearing of *N. elongata*. This new method can maintain a strain of *N. elongata* for over one year without adding field-collected individuals. The method allowed us to maintain a sufficient population during the first six months because of comparable egg hatchability and nymphal survival from the second to fourth generations (Table 2). The grains used in the method are available throughout the year, unlike rice panicles used in the previous rearing study (Ishizaki & Hirae 2021). This is a significant advantage for laboratory experiments using *N. elongata*, as researchers begin rearing at any time by buying the feed from the market, regardless of season.

The pronotum width and body length of the reared adults were significantly smaller than those of field-collected adults (Fig. 2), as by the previous rearing method (Ishizaki & Hirae 2021). The reduced body size of adults usually indicates inadequate environmental resources during nymphal or larval development (Callier & Nijhout 2013, Nijhout 2003, Nijhout et al. 2014). This inadequacy can affect the duration of nymphal development and the fecundity and survival of adults (Beukeboom 2018, Calvo & Molina 2005, Kohno & Ngan 2004). Given these findings, the reared insects' development may differ from that of field-collected ones. The data presented here, such as the developmental duration of nymphs and the fecundity and survival of adults, should be carefully compared against actual field occurrence data. Alternatively, further improvements in the rearing method, such as optimization of the grain composition and adding supplementary nutrition, may help.

In conclusion, we developed a convenient method for rearing *N. elongata* throughout the year. The diet used in our method is easy to prepare at any time, and this innovative method will promote field and laboratory studies of the biology of this pest.

Acknowledgements

We sincerely thank Dr. Hiroya Higuchi for showing us how to prepare the diet using this rearing method. We thank Dr. Masahiro Hirae of NARO for his help in providing *N. elongata* for the preliminary experiments. We also thank Mr. Hijiri Kanazawa of NARO for his assistance with this study. We thank Dr. Keiichiro Matsukura of NARO for his valuable comments on the manuscript and his help.

This work was supported by a grant from the Ministry of Agriculture, Forestry and Fisheries of Japan (a MAFF-commissioned project study on the "Development of rice diseases and pests forecasting system for labor-saving IPM" [Grant Number JPJ011280]).

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