MASS REARING TECHNIQUE FOR THE RICE GREEN LEAFHOPPER NEPHOTETTIX NIGROPICTUS (STAL)¹

Qazi M.A. Razzaque, Elvis A. Heinrichs, and Herminia R. Rapusas²

To develop a successful mass rearing technique for Nephotettix nigropictus (Stal), three rice (Oryza sativa L.) varieties: Nira, Taichung Native 1, Kochoi and two weed species: Echinochloa crus-galli (Linn.) Beauv. and Leersia hexandra Sw., at different stages of growth were tested as host plants under greenhouse conditions. Nira was the most suitable host plant for rearing N. nigropictus. Nymphal survival, adult longevity, and fecundity of the insects were higher and their developmental period shorter than on the other host plants. With four-day-old Nira plants, 50 pairs of N. nigropictus can produce 3,000-5,000 individuals in one generation.

INTRODUCTION

Nephotettix nigropictus (Stål), one of the most important species of rice green leafhoppers, is a pest throughout the rice growing areas of Asia (Ghauri 1971, Dhawan and Sajjan 1976). It damages the rice plant by removing both xylem and phloem sap and by plugging the vascular bundles with stylet sheaths (Auclair et al. 1982). Although direct feeding seldom causes yield losses, severe damage is often inflicted by the insects' ability to transmit virus diseases (Ling 1972, Hibino et al. 1979).

The development of a mass rearing technique for *N. nigropictus* is important to ensure adequate supply of insects in screening rice cultivars for resistance to this pest. Likewise, studies on the biology and behavior of *N. nigropictus* and on the mechanisms of resistance in rice cultivars can be conducted if insects are available. Previous attempt to mass rear this insect using TN1 plants was not successful (Sojjan 1972).

MATERIALS AND METHODS

Life history of N. nigropictus

The life cycle of *N. nigropictus* was studied using Nira (Indica, IRRI Acc. No. 1749), Taichung Native 1 (TN1, Indica, IRRI Acc. No. 105) and *Leersia hexandra* Sw. family Poaceae (Graminae), as host plants. Four-day-old Nira and TN1 plants were confined separately in test tubes at the rate

¹Received for publication 5 July 1985 and in revised form 3 October 1985. A portion of the thesis by the senior author in partial fulfillment of the requirements for the degree of Master of Science in Entomology, UP at Los Baños, College, Laguna 3720.

²Former IRRI Scholar, Entomologist and Department Head, and Senior Research Assistant, respectively, Entomology Department, International Rice Research Institute, P.O. Box 933, Manila, Philippines. EAH's present address is Department of Entomology, Louisiana State University Agricultural Center, 402 Life Sciences Bldg., Baton Rouge, Louisiana, 70803-1710 USA.

of five plants per test tube; moist cotton was placed at the bottom and the top was covered with nylon mesh. Likewise, 30-day-old Nira plants and L. hexandra stem cuttings were planted separately in 10 cm diam clay pots and covered with mylar film cages. Each treatment was replicated 20 times. For oviposition, each test plant was infested for 24 h. with a pair of three-day-old adults. The plants were observed daily for nymphal emergence. Upon hatching, newly emerged nymphs were placed on test plants of the desired age at one insect per plant and the development of the insect was observed until adult emergence.

In studying adult longevity and fecundity, a pair of newly emerged adults was placed on each test plant. Mortality was recorded daily from one day after infestation (DAI) until the death of the last insect. Whenever necessary, the plants were changed and dissected under a binocular microscope to count unhatched eggs. During this period, hatched nymphs were also counted and removed from the plants. The total number of eggs laid per female was determined by adding the number of nymphs and unhatched eggs.

Effect of four-day-old rice cultivars on N. nigropictus growth and development

Five four-day-old plants of Nira, TN1 or Kochoi (Japonica, IRRI Acc. No. 31958) were placed separately in test tubes (2.5 cm diam and 20 cm high) with nylon mesh cover and moist cotton at the bottom. Each tube served as a replication. Treatments were replicated 20 times and arranged in a randomized complete block design (RCBD).

Nymphal survival and development. The plants in each tube were infested with five newly emerged nymphs, Nymphal survival and development were noted daily and the length of the developmental period was recorded. Food plants were changed when needed. Newly emerged adults were collected and a pair of male and female adults from each tube was placed in small vial, dried at 70°C for 48 h and weighed on an electronic balance (1 ug sensitivity). Data obtained was statistically analyzed using analysis of variance (ANOVA) and Duncan's multiple range test (DMRT).

Adult longevity and fecundity. One pair of newly emerged male and female adults was used to infest the plants in each test tube. Dead insects were counted daily starting at one DAI until the death of the last insect. The sex of the dead insects was noted. Food plants were changed when needed and dissected under a binocular microscope to count the number of eggs laid. During each observation, the hatched nymphs were counted and removed from the test tubes. Total number of eggs laid per female was determined by adding the number of nymphs and unhatched eggs. Data was statistically analyzed as above.

Effect of plant age on N. nigropictus growth and development

Three rice cultivars: Nira, TN1 and Kochoi and two weed species: L. hexandra Sw., and Echinochloa crus-galli (Linn.) Beauv., family Poaceae

(Graminae), were tested. Seeds of the rice varieties and E. crus-galli were sown in seedboxes on staggered basis (10 day interval) to obtain plants of three different ages simultaneously. Five stem cuttings of L. hexandra were planted in 10 cm diam clay pots at the same time that seeds of the other test plants were sown.

Seven days after sowing (DAS), the seedlings were transplanted in 10 cm diam clay pots with 5 seedlings per pot. The pots were placed in a waterfilled iron tray, arranged in RCBD and covered with nylon mesh cage to protect them from insects pests and other arthropods. All treatments were replicated four times, one pot representing a replication.

Nymphal survival and development. When the test plants reached the desired ages (10, 20, and 30 DAS) the plants in each pot were enclosed with a mylar film cage (10 cm diam and 90 cm high). Ten newly hatched N. nigropictus nymphs were introduced into each cage. Nymphal survival, development and dry weight of the adults were observed and determined following the procedure discussed elsewhere in this paper. Data gathered were statistically analyzed by ANOVA and treatment means tested for significance using DMRT.

Adult longevity and fecundity. Test plants at 10, 20 and 30 DAS were also caged as described previously. One pair of newly emerged male and female adults was released into each cage for oviposition. Longevity and fecundity were determined following the procedure discussed elsewhere in this paper.

RESULTS AND DISCUSSION

Life history of N. nigropictus

In studying the life history of N. nigropictus on TN1, Nira and L. hexandra, 4 DAS Nira plants were found to be the most suitable host (Table 1). N. nigropictus laid more eggs, had faster development and higher longe-

Table 1. Life history of N. nigropictus on three host plants at different ages. IRRI 1983.1

| Life stages | Host plants at different stages | | | | | | |
|-------------------------------------|---------------------------------|-------|-------------|---------------------|--|--|--|
| | TN1 | N | L. hexandra | | | | |
| | 4 DAS ² | 4 DAS | 30 DAS | 10 DAT ³ | | | |
| Egg/female (no.) | 46 | 110 | 68 | 34 | | | |
| Incubation (days) | 6 | 6 | 6 | 6 | | | |
| Nymphal developmental period (days) | 16 | 14 | 16 | 20 | | | |
| Male longevity (days) | 16 | 20 | 16 | 18 | | | |
| Female longevity (days) | 17 | 20 | 18 | 20 | | | |

Based on 20 replications.
Days after sowing.

³Days after transplanting.

vity on Nira than on TN1 and L. hexandra. L. hexandra was the least suitable of the three hosts especially in relation to fecundity and nymphal development. This may have been due to physical factors or chemical constituents in L. hexandra. Viswanathan and Kalode (1981) reported that the high level of phenolic compounds and low amino acid concentrations in L. hexandra may result in low fecundity and slow development of N. nigropictus.

Effect of four-day-old rice cultivars on N. nigropictus growth and development

In comparing the nymphal survival, development period, dry weight, adult longevity and fecundity on four DAS seedlings of Nira, TN1 and Kochoi, Nira proved to be the most suitable host (Table 2). Four-day-old Nira seedlings appeared to be slightly better than 30-day-old plants. Sugimoto (1977) suggested the use of highly susceptible young seedlings in the mass rearing of *N. cincticeps* Uhler.

Table 2. Adult longevity, fecundity, nymphal survival, development and dry weight of N. nigropictus on four-day-old plants of three rice cultivars. IRRI 1983. 1

| | Host plant | | | | |
|-----------------------------|------------|-------|--------------|--|--|
| Insect responses | Nira | TN1 | Kocho | | |
| Adult longevity - Male | 18 a | 17 ab | 15 b | | |
| - Female | 18 a | 17 ab | 16 b | | |
| Fecundity/female | 102 a | 56 b | 54 b | | |
| Nymphal survival (%) | 95 a | 78 b | 72 b 15 b | | |
| Developmental period (days) | 15 b | 16 a | | | |
| Mean dry weight/pair (mg) | 3 a | 2.6 b | 2 b | | |

¹Average of 20 replications. Means in a row with the same letter are not significantly different at the 5% level by DMRT. The nymphs and adults were placed on the plants at 4 days after sowing.

Effect of plant age on N. nigropictus growth and development

Nymphal survival, developmental period, dry weight of adults, adult longevity, and fecundity on the five host plants differed significantly. Nira was the most favorable host plant as shown by the higher percentage survival of both nymphs and adults, greater number of eggs laid and shorter developmental period (Tables 3 and 4). Among the three plant ages (10, 20 and 30 DAS), differences were generally not significant. These observations partially agreed with Viswanathan and Kalode (1981) where the plant ages tested did

Table 3. Nymphal survival, development and dry weight of N. nigropictus on five test plants at three ages, IRRI 1983.

| Sources | Days after sowing | Test Plants | | | | |
|----------------------------|-------------------|-------------|----------|-----------|---------------|-------------|
| | | Nira | TN1 | Kochoi | E. crus-galli | L. hexandra |
| Nymphal | | | | | | |
| survival ² (%) | 10 | 80.0 abc | 75.0 abc | 50.0 de | 76.5 bcde | 82.5 ab |
| | 20 | 87.0 ab | 75.0 bcd | 52.5 cde | 70.0 bcd | 97.5 a |
| 3 | 30 | 80.0 abc | 40.0 e | 65.0 bcde | | 97.5 a |
| Mean ³ | | 82.5 b | 63.3 c | 55.8 с | 70.0 bc | 92.5 a |
| Developmental | 10 | 15.9 cd | 15.7 cd | 18.9 ab | 17.9 ab | 18.0 ab |
| period (days) ² | 20 | 15.4 de | 18.5 ab | 17.1 bc | 17.3 bc | 18.3 ab |
| Mean ³ | 30 | 14.7 d | 18.0 ab | 17.8 ab | 12.2 ab | 19.7 a |
| | | 15.3 b | 17.4 ab | 17.9 ab | 17.8 ab | 18.6 a |
| Dry weight of a | 10 | 2.7 ab | -2.5 bc | 2.7 ab | 2.7 ab | 2.3 bc |
| pair (mg) ² | 20 | 2.7 ab | 1.7 с | 2.6 ab | 1.9 bc | 2.4 bc |
| 1 | 30 | 3.3 a | 1.7 с | 2.7 bc | 2.3 bc | 1.9 bc |
| Mean ³ | | 2.9 a | 2.0 с | 2.6 ab | 2.3 bc | 2.2 c |

Plant age refers to days after transplanting of stem cuttings.

²Means within a source followed by a common letter are not significantly different at the 5% level by DMRT.

 $^{ ilde{3}}$ Means in a row, followed by a common letter are not significantly different at the 5% level by DMRT.

Table 4. Adult longevity and fecundity of N. nigropictus on five test plants at three plant ages. IRRI 1983.

| Sources | Days after | 200000 | r de | | | |
|--------------------------------------|------------|---------------------|-----------------------|---------------------|----------------------|--------------------|
| | sowing | Nira | TN1 | Kochoi | E. crus-galli | L. hexandra |
| Male longevity (days) ² | 10 20 | 12.0 a 14.3 a | 13.3 a 12.5 a | 11.3 a 17.3 a | 12.3 a 16.3 a | 15.5 a 20.5 a |
| Mean ³ | 30 | 17.5 a 14.6 a | 26.3 a 14.0 a | 26.0 a 14.9 a | 15.3 a 14.6 a | 16.5 a 17.5 a |
| Female longevity (days) ² | 10 20 | 13.0 cd 18.8 abc | 14.0 bcd 16.3 abcd | 13.5 bcd 19.8 ab | 13.8 bcd 18.8 abc | 17.8 abc 21.3 a |
| Mean ³ | 30 | 18.8 abc 16.9 ab | 15.8 abcd 15.4 b | 10.0 d 14.4 b | 18.6 abc 17.1 ab | 19.5 abc 19.5 a |
| Fecundity/female ² | 10 20 | 67.8 ab 63.8 abc | 23.3 de 38.3 bcd | 31.0 cd 31.0 cd | 15.0 e 43.3 bcd | 30.3 cd 32.5 cd |
| Mean | 30 | 99.8 a 77.1 a | 23.8 de 28.5 b | 24.8 de 28.9 b | 41.3 bcd 33.2 b | 34.3 cd 32.4 b |

¹Plant age refers to days after transplanting of stem cuttings.

²Means within a source followed by a common letter are not significantly different at the 5% level by DMRT.

Means in a row, followed by a common letter are not significantly different at the 5% level by DMRT.

not alter the degree of antibiosis to green leafhoppers. N. nigropictus nymphal period was longer on Kochoi, TN1, E. crus-galli, and L. hexandra than on Nira. Dry weight of adults was significantly higher on Nira than on the other host plants except Kochoi. Likewise, Panda and Heinrichs (1983) reported that dry weight of the brown planthopper Nilaparvata lugens (Stal) was less on resistant than on susceptible plants.

Mass Rearing of N. nigropictus

We develop a mass rearing technique for N. nigropictus by utilizing the information obtained from the above studies. To start the culture, field collected males and females (50 each) were placed on four-day-old Nira

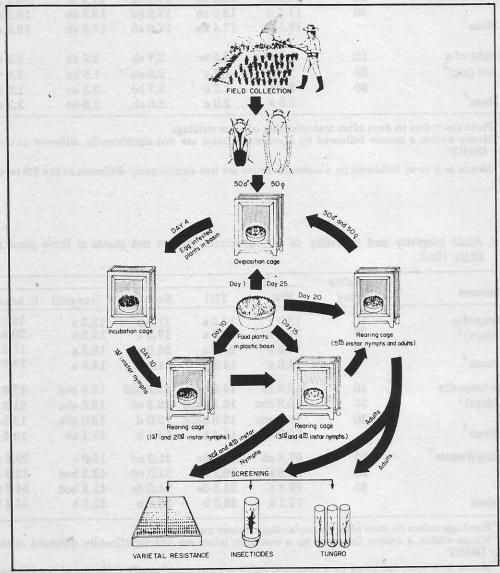


Figure 1. Procedure for rearing N. nigropictus on four-day-old Nira plants.

plants growing in a plastic basin within an oviposition cage (Fig. 1). Four days later the egg infested plants in the basin were transferred to an incubation cage. Six days later, the newly hatched nymphs were transferred to fresh plants in the rearing cage. Fresh plants were added at day 15 and 20. When the nymphs became adults 50 males and 50 females were transferred to the oviposition cage to complete the rearing cycle. Excess insects can be removed at any period in the cycle depending on the age of insects desired for a particular test. N. nigropictus could also be mass reared on TN1 and Kochoi seedlings, although the population growth is lower than on Nira.

Using the mass rearing procedure for *N. nigropictus* shown in Fig. 1, 3,000-5,000 individuals can be produced in one generation on Nira plants.

REFERENCES

- AUCLAIR, J.L., E. BALDOS and E.A. HEINRICHS. 1982. Biochemical evidence for the feeding sites of the leafhopper, *Nephotettix virescens* within susceptible and resistant rice plants. Insect Sci. Application 3: 29-34.
- DHAWAN, A.K. and S.S. SAJJAN. 1976. Biology of the rice green leafhopper Nephotettix nigropictus (Stål) Cicadellidae: Homoptera. J. Punjab Agric. Univ. 13: 379-383.
- GHAURI, M.S.K. 1971. Revision of the genus *Nephotettix* Matsumura (Homoptera: Cicadelloidae: Euscilidae) based on the type material. Bull. Ent. Res. 60: 481-512.
- HIBINO, M., N. SALEH and M.R. ROECHAN. 1979. Transmission of two kinds of rice tungro associated virus by insect vector. Phytopathology 69: 1266-1268.
- LING, K.C. 1972. Rice virus diseases. The International Rice Research Institute, Los Baños, Laguna, Philippines. 142 p.
- PANDA, N. and E.A. HEINRICHS. 1983. Level of tolerance and antibiosis in rice varieties having moderate resistance to the brown planthopper, *Nilaparvata lugens* (Stål). (Hemiptera: Delphacidae). Environ. Entomol. 12: 1204-1214.
- SAJJAN, S.S. 1972. Varietal resistance of rice to N. Nigropictus (Stål). Report on the work done during the fellowship period. International Rice Research Institute, Los Banos, Laguna, Philippines. 26 p.
- SUGIMOTO, A. 1977. A method of mass-rearing rice green leafhopper. In The Rice Brown Planthopper. Food and Fertilizer Technology Center, Asian and Pacific Region, Taipei. pp. 248-256.
- VISWANATHAN, P.R.K. and M.B. KALODE. 1981. Studies on varietal resistance and host specificity of rice green leafhoppers. Int. Rice Res. Newsl. 6(3): 7.