Response to Selection for Virulence of *Nephotettix virescens* (Homoptera: Cicadellidae) on Resistant Rice Cultivars

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**ABSTRACT**

Rate of response to selection for *Nephotettix virescens* (Distant) virulence was studied for 20 generations on five rice cultivars with different levels of resistance. Rate of response to selection on all cultivars varied depending on the measurement criteria. Survival, growth, progeny production, and weight increased, and developmental period decreased, with selection time. Based on survival, the *N. virescens* population became highly virulent within one to four generations, whereas high virulence as measured by progeny production generally required more generations of selection. Virulence as measured by the ability to vector tungro virus also increased with selection time.

**KEY WORDS** Insects, rice, leafhoppers, biotypes

**THE LEAFHOPPER** *Nephotettix virescens* (Distant) is a major threat to rice, *Oryza sativa* L., production in south and southeast Asia because it is an efficient vector of tungro virus (Rivera & Ou 1965). Because of the cost and lack of effectiveness of insecticides, *N. virescens*-resistant rice cultivars have been bred and released for commercial cultivation.

Breeding for *N. virescens* resistance has been a major objective since the rice breeding program was established at the International Rice Research Institute in the early 1960s. 'IR 8,' the first cultivar released by the institute, has a moderate level of *N. virescens* resistance. 'IR 8' was followed by the release of a number of other cultivars, of which 'IR 36,' also a moderately resistant cultivar, has been the most widely planted (IRRI 1983).

Evidence of biotypic differences between *N. virescens* populations was first observed in 1967 when the newly introduced cultivar, 'IR 8,' which was resistant to *N. virescens* in the Philippines, was reported as susceptible in laboratory tests in Bangladesh (Karim & Pathak 1982). However, selection for *N. virescens* virulence on a commercially grown cultivar had not yet been observed at that time.

'IR 36' was released in 1976 and in a few years occupied most of the lowland rice area in the Philippines. A survey in 1979 indicated that natural selection for a virulent population of *N. virescens* on 'IR 36' had not occurred (Rapusas & Heinrichs 1982). However, the survey was repeated in 1984 and indicated the presence of *N. virescens* populations virulent on 'IR 36' in some locations (Rapusas & Heinrichs 1986).

Because the commercial IR cultivars do not have resistance to tungro virus, selection for *N. virescens* virulence on resistant cultivars is expected to result in high tungro incidence. A study was conducted to determine the fitness of artificially selected *N. virescens* colonies based on feeding and development. The ability of the adults to transmit tungro virus in rice with different levels of *N. virescens* resistance also was examined (Heinrichs & Rapusas 1984). The study indicated that after selection on highly resistant 'Pankhari 203' and moderately resistant 'IR 8,' 'Ptb 8,' 'TAPl 796,' and 'Moddai Karuppan' for 19 generations, survival increased and duration of the nymphal period decreased on all cultivars. However, there were distinct differences among cultivars in the percentage of tungro virus-infected plants that indicated possible differences in levels of tungro virus resistance.

In that study, the various parameters used to measure the level of *N. virescens* virulence were determined after the 19th generation of selection. By that time, selection had progressed to a level where *N. virescens* was as well adapted to the resistant cultivars as to the susceptible check 'Tai-chung Native 1' ('TN 1'). Therefore, a follow-up study was conducted to determine the rate at which responses to selection occur on these same cultivars by measuring several parameters in generations 0 to 10 and in the 15th and 20th generations. The results of this study are reported herein.

**Materials and Methods**

About 100 pairs (males and females) of *N. virescens* adults were collected from each of 15 rice fields in different locations in the central and southern Philippines. All of the field collections were combined and were reared on 'TN 1' in the greenhouse. To obtain *N. virescens* populations that had been reared on the respective test cultivars for various numbers of generations, the insects were trans-

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ferred from 'TN 1' to the cultivars on a staggered
basis over time. The population to be tested after
20 generations of selection was transferred from
'TN 1' to the cultivars after two generations
on 'TN 1' and continuously reared on the respective
cultivars for 20 generations. The population to be
transferred after 15 generations of selection on the test
cultivars was transferred from 'TN 1' to the test
cultivars five generations after the transfer of the
20-generation population. This process was continued
until the generation-1 population was transferred.
Separate cages each containing one of the
following cultivars was established; 'Pankhari 203'
(Glh 1, gene for N. virescens resistance), 'IR 8'
(Glh 3 gene), 'IR 8' (glh 4 gene), 'TAPL 796' (Glh 6
gene) or 'Moddai Karuppan' (Glh 7 gene). For
each generation, 500 pairs of N. virescens were
introduced into each cage. A single population was
selected on each rice cultivar. Rearing cages were
made of wood frames with nylon mesh sides and
topped and measured 50 by 50 by 10 cm (width, length,
height).

Tests for levels of virulence for each generation
from 0 to 10, 15, and 20 were conducted simulta-
neously. The parameters measured were adult
recovery (survival of nymphs to the adult stage),
developmental period, growth index, population
growth, weight of adults, and efficiency in transmit-
ting rice tungro virus. A colony that had been reared
on susceptible 'TN 1' for >50 generations
was tested on 'TN 1' and included as the control.
In all tests, treatments were replicated five times
and arranged in a randomized complete block de-
Sign. Data were subjected to an analysis of variance
(P < 0.05), and treatment means were separated
using Duncan's (1955) multiple range test.

Adult Recovery, Developmental Period, Growth
Index, and Weight. Five-day-old seedlings of the
test cultivars were planted in clay pots (5 cm di-
ameter) at two seedlings per pot. Five pots were
planted for each replication. Ten days later, the
two plants in each pot were enclosed with one
Mylar film cage (4 cm diameter, 40 cm high), and
pots were arranged in a metal tray half-filled with
water. At 20 d after sowing, the plants were in-
fested by introducing two newly emerged N. vi-
rescens nymphs from the respective colony into
each cage (10 insects per replication). The insects
in each cage were observed daily. Emerging adults
were counted, collected, placed in plastic vials, and
weighed individually with a Mettler ME-30 elec-
tronic balance (1 \mu g sensitivity) (Mettler Instru-
ment Corporation, Hightstown, N.J.).

The number of insects that survived to the adult
stage and the length of developmental period (from
infestation to adult emergence) were determined.
Percentage of adult recovery (survivability) was
computed by the equation: number of adults count-
ed/number of nymphs infested \times 100. The growth
index was then computed by the equation: % adult
recovery/mean developmental period (in days).

Population growth. Seven-day-old seedlings of
the test cultivars were transplanted in clay pots (10
cm diameter) with five seedlings per pot. One pot
represented one replicate. One week after trans-
planting, the pots were arranged in a metal tray
half-filled with water, and the plants in each pot
were enclosed with Mylar film cages (10 cm di-
ameter, 90 cm high). When the plants were at 30
d after sowing, they were infested by introducing
into each cage five pairs of 3-d-old N. virescens
adults coming from the respective colony. At 30 d
after infestation, the hoppers in each cage were
counted.

Rice Tungro Virus Infection. Five-day-old seed-
lings of the test cultivars were transplanted in clay
pots (5 cm diameter) at one seedling per pot. Five
pots were planted for each replication. Pots were
randomly arranged in a metal tray in the green-
house, after which the plants in each pot were
enclosed with a Mylar film cage (4 cm diameter,
40 cm high). Ten days after transplanting, the plants
were inoculated with the virus by introducing a
pair of viruliferous N. virescens adults into each
cage. Viruliferous hoppers were obtained by allow-
ing virus-free N. virescens adults to feed on tungro-
infected 'TN 1' plants for 48 h. They were then
allowed a 24-h inoculation period on the test cul-
tivars. Twenty-five days after inoculation, tungro-
infected plants were counted (based on visual dam-
age symptoms), and percentage of tungro infection
was computed by the equation: number of plants
infected/number of plants inoculated \times 100.

Results

'Pankhari' 203. Adult recovery was low (18%)
in generation 0 (reared on 'TN 1') but increased
to 40% with just one generation of selection on
'Pankhari 203' (Fig. 1). By generation 5, recovery
was not significantly different from the control.
The development period decreased from gener-
ation 3 to 5, being equal to the control in the fifth
generation. The growth index was similar to the
control by the sixth generation. Population growth
was low (15 progeny per female) in generations 0
and 1. It took nine generations of selection before
the population was not significantly different from
that of the control. Both female and male weight
decreased slightly in the second generation but in-
creased thereafter. Female weight was equal to the
control in the sixth and male weight in the fifth
generation. Except for a low percentage (<10%) in
generations 3 and 5, biotype selection did not
result in a change in percentage of tungro-infected
plants.

'IR 8.' Based on adult recovery, the 'IR 8' colony
took only three generations for virulence to develop
(Fig. 2). The nymphal development period was
longest in generation 1, then decreased gradually
until generation 6. Developmental period from
generations 6 to 20 did not differ significantly from
Fig. 1. Percentage of adult recovery (survival), developmental period, growth index, population growth, tungro infection, weight of female and male adults, and average weight of adults of *N. virescens* reared on 'Pankhari 203' for up to 20 generations and on susceptible 'TN 1' (control). Bars within each parameter with the same letter are not significantly different (*P* < 0.05, Duncan's [1955] multiple range test).
Fig. 2. Percentage of adult recovery (survival), developmental period, growth index, population growth, tungro infection, weight of female and male adults, and average weight of adults of *N. virescens* reared on 'IR 8' for up to 20 generations and on susceptible 'TN 1' (control). Bars within each parameter with the same letter are not significantly different (*P* < 0.05, Duncan's [1955] multiple range test).
Fig. 3. Percentage of adult recovery (survival), developmental period, growth index, population growth, tungro infection, weight of female and male adults, and average weight of adults of *N. virescens* reared on 'Pt 8' for up to 20 generations and on susceptible 'TN 1' (control). Bars within each parameter with the same letter are not significantly different \( (P < 0.05, \text{Duncan's } 1955) \) multiple range test.)
Fig. 4. Percentage of adult recovery (survival), developmental period, growth index, population growth, tungro infection, weight of female and male adults, and average weight of adults of *N. vitescens* reared on 'TAPL 796' for up to 20 generations and on susceptible 'TN 1' (control). Bars within each parameter with the same letter are not significantly different (*P* < 0.05, Duncan's [1955] multiple range test).
Fig. 5. Percentage of adult recovery (survival), developmental period, growth index, population growth, tungro infection, weight of female and male adults, and average weight of adults of *N. virescens* reared on 'Moddai Karuppan' for up to 20 generations and on susceptible 'TN 1' (control). Bars within each parameter with the same letter are not significantly different (*P* < 0.05, Duncan’s [1955] multiple range test).
that of the control. Growth index was lowest in generations 0 and 1, increased in generation 2, and continued to increase in generation 3, when it did not differ significantly from that of the control. By the 15th generation, population growth was similar to that of the control.

Weight of female adults varied little among the generations except in generations 1 and 2, which were significantly lower than the control. A similar trend was also observed in the weight of males, except those from generations 0 to 4 were lighter than the control.

The ability to transmit tungro virus increased with the increase in the number of generations through which the insects were reared on 'IR 8' and equaled the control in generation 4. Virus infection reached 100% in generation 15.

"Ptb 8." Adult recovery was high and similar to that of the control in all generations (Fig. 3). This parameter indicated an absence of resistance in 'Ptb 8.' Developmental period decreased with selection except in generation 15. Growth index was similar in all generations. Population growth fluctuated. Average weight of adults was similar in all generations.

Biotype selection on 'Ptb 8' significantly increased the efficiency of the insect to transmit tungro virus. Tungro infection was low (45-55%) in generations 0 to 5 and increased to almost 100% in generation 6 and remained high to the 20th generation.

"TAPL 796." Nymphs reared on 'TN 1' and transferred to 'TAPL 796' (0 generation) had 58% adult recovery on 'TAPL 796.' Survival increased to almost 80% after one generation of selection on 'TAPL 796' and remained high through generation 20 (Fig. 4). Developmental period fluctuated. Progeny per female was low until generation 10. Weight of adults, both male and female, increased significantly in generation 15.

Insects reared on 'TN 1' (generation 0) were not able to transmit tungro virus to 'TAPL 796.' However, an increase in infection of 15% was observed from generations 1 to 3. Tungro infection ranged from 45 to 65% in generations 6 to 20, never reaching the control.

"Moddai Karuppan." Adult recovery, developmental period, and growth index did not vary much among the various generations on 'Moddai Karuppan' and the control (Fig. 5). Progeny production fluctuated. There was no significant increase in female and male weight until generations 15 and 20, respectively.

Tungro infection was initially high (65% in generation 0). This increased to 90% in generation 1 but decreased again in generation 2. Infection in generations 4-20 remained high (range, 82-100%).

**Discussion**

Selection of *N. virescens* on resistant cultivars is manifested by an increase in the survival, growth index, progeny production, weight, and a subsequent decrease in the period of development from the nymph to the adult stage. As this study indicated, the rate of selection for virulence to some extent varies with the level of *N. virescens* resistance in the cultivar on which the population is selected and depends on the criteria that are being used to measure virulence.

The five test cultivars differed in their level of resistance to *N. virescens* as indicated by their response in generation 0 (before selection). Differences were most obvious in adult recovery, where it was 18, 45, 75, 59, and 92% on 'Pankhari 203,' 'IR 8,' 'Ptb 8,' 'Moddai Karuppan,' and 'TAPL 796,' respectively (Fig. 1-5). Selection for survival to the adult stage (adult recovery) occurs at a more rapid rate than does the ability to produce progeny. For example, survival of the population selected on 'TAPL 796' was already similar to that of the susceptible control in generation 1, whereas population growth on 'TAPL 796' did not reach the level of the control until generation 10 (Fig. 4). Insect weight also increased at a slower rate than did survival.

Percentage of tungro virus-infected plants is a function of the level of resistance of the cultivar to *N. virescens* and the level of resistance to the virus. Percentage of infection in generation 0 (unselected) was 0% in 'Pankhari 203' and 'TAPL 796' and ranged from 35 to 65% in the other three test cultivars. In all cultivars except 'Pankhari 203,' there was an increase in the percentage of tungro virus-infected plants with selection time. It appears that the resistance of 'TAPL 796' to *N. virescens* in generation 0 protected it from tungro infection, and as the resistance decreased with selection time, tungro infection subsequently increased. 'Pankhari 203,' however, apparently has resistance both to the vector and to the virus; when the resistance to the vector is lost, it continues to remain free of virus because of its resistance to the pathogen.

It is not known how the rates of response to selection in the laboratory for a given cultivar compares with the response rate to selection under field conditions. It should be noted that, although the response to selection was rapid in this study, it is not expected to occur at such a rapid rate under field conditions because of less selection pressure (i.e., alternative host plants such as weeds and susceptible rice cultivars). There is, however, evidence that after several years of commercial production in the Philippines, *N. virescens* virulence increased on the extensively grown rice cultivars 'IR 36' and 'IR 42' (Rapusas & Heinrichs 1986).

*N. virescens* is a major threat to rice production in Asia because of its efficiency as a tungro virus vector. Because tungro virus-resistant rice cultivars have not been identified, the major emphasis in breeding for tungro control has been on resistance to the vector, *N. virescens*. However, it is important to breed rice cultivars for resistance to both *N. virescens* and tungro virus so that if the insect...
resistance is lost because of an increase in the level of vector virulence, the virus resistance will still be able to protect the plant from infection. For this purpose, the genetic evaluation program of the International Rice Research Institute has increased its screening activities to identify tungro-resistant cultivars suitable for use in the breeding program.

References Cited


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