9. RECENT STUDIES ON RICE TUNGRO DISEASE AT IRRI

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Rice tungro disease includes a number of virus diseases with similar characteristics such as cella pance in Indonesia (Tantera, 1973); leaf yellowing in India (John, 1968); penyakit habang in Indonesia (Saito *et al.*, 1975; Tantera, 1973); penyakit merah in Malaysia (Ting and Paramsothy, 1970); and yellow-orange leaf in Thailand (Lamey, Surin, and Leeuwangh, 1967). The major symptoms of tungro are yellowing of leaves and stunting of rice plants. The disease is caused by a virus that is only known to be transmitted by leafhopper vectors. The five known vector species are *Nephotettix malayanus, N. nigropictus, N. parvus, N. virescens,* and *Recilia dorsalis* (Ling, 1972, 1973). *N. virescens* is the most important vector as far as the efficiency of virus transmission and the insect population in rice fields are concerned.

Tungro is prevalent across South and Southeast Asia. During the last 10 years, the disease has been epidemic (or epiphytotic), rather than endemic, because it has often struck suddenly and destroyed large areas of rice plants. Although the disease may often be found in an area for a few years after an outbreak, it has not been known to persist at severe or moderate levels in successive crops for any extended period. This was substantiated by the major tungro outbreak in Bangladesh in 1969 (Miah and Ahmad, 1974); in Uttar Pradesh and Bihar, India in 1969 (Anjaneyulu, 1974, Anonymous, 1969; John, 1970); in South Kalimantan, Indonesia in 1969 (Oka, 1971; Tantera, 1973) and South Sulawesi in 1972 (Tantera, 1973, 1974, van Halteren and Sama, 1973); in North Krian, Malaysia in 1969 (Lim, 1972); in Cotabato Philippines in 1970 and Central Luzon in 1971; and in Thailand in 1966 (Lamey *et al.*, 1967).

The irregularity of tungro outbreaks suggests that they are influenced and controlled by environmental and biological factors. This led to epidemiological studies to determine the effects of insect vector, virus source, host, and environment on disease incidence, as well as to the breeding or rice varieties resistant to the disease at the International Rice Research Institute. The recent findings are summarized as follows:

Effect of temperature on transmission

The effect of ambient temperature on the transmission of tungro virus by the adult green leafhopper (*N. virescens*) was studied under controlled conditions. The results (Ling and Tiongco, 1976) indicated that at temperatures ranging from 10 to 38°C, the insect can acquire the virus from diseased plants and become infective and can also inoculate rice seedlings that later become infected. Hence, the temperature in the tropical region may not be a factor restricting tungro virus transmission under natural conditions. However, the insect's efficiency in transmitting the virus tended to increase as the ambient temperature increased from 10 to 31°C.

Within the day-night temperatures range of 24–16°C and 30–22°C, the spread of tungro disease (measured by percentage of infected seedlings) increased as the day-night temperatures increased.

The life span of tungro-viruliferous adult insects increased as the temperature

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decreased from 34 to 13°C. The longest retention period at 13°C was 22 days after an acquisition feeding at room temperature, the longest period at 32°C was 6 days in tests of 6,895 insects. At 7°C, the insect lost less infectivity than at room temperatures.

Transitory leafhopper-transmitted virus

Like similar virus diseases, tungro does not persist in its leafhopper vectors (Ling, 1970, 1972). Ling (1966) suggested that "...tungro virus is nonpersistent in the rice leafhopper if 'short' refers to a duration of not longer than 1 week" because no characteristic of the virus-vector interaction other than the retention period precludes the grouping of tungro in the nonpersistent group.

Categorizing tungro virus as nonpersistent seems no longer appropriate beacuse new findings show that the retention period of *N. virescens* is longer than 3 weeks at 13°C. Therefore, Ling and Tiongco (1976) proposed a new term—"transitory"—for leafhopper-transmitted viruses that have the following characteristics of virus-vector interaction:

1) The virus does not persist in its leafhopper vector (the infectivity or percentage of infective insects decreases with time at hourly or daily intervals after acquisition feeding);
2) The retention period is generally less a week but depends on low temperatures to become longer;
3) There is no demonstrable latent period in the insect vector;
4) The infectivity is lost due to molting or transstadial blockage;
5) The insect needs reacquisition feeding to become reinfective.

Thus, we recommend that tungro be designated as a transitory leafhopper-transmitted virus. Also the virus-vector interaction of the rice tungro should be categorized as transitory instead of nonpersistent.

The above five features of virus-vector interaction can be demonstrated by transmission experiments. If results of a leafhopper-borne virus are contrary to these five virus-vector characteristics, it would remain classified as a persistent leafhopper-borne virus.

Thus, the leafhopper-transmitted viruses can be categorized as transitory and persistent rather than nonpersistent, semipersistent, and persistent, as the aphid-borne viruses are categorized.

Infective capacity

The infective capacity is a newly proposed term for the number of plants or seedlings that an insect can infect in 1 day or in any given period of time—a quantitative aspect of insect transmission. The infective capacity of *N. virescens* to transmit rice tungro virus was determined by transferring individual viruliferous adult insects to rice seedlings consecutively for 10 hours at intervals of 5, 15, 30, and 60 minutes.

As the inoculation access time (or the transfer interval) of an infective insect lengthens, the percentage of infected seedlings increases, but the possible number of seedlings that an infective insect can inoculate in a given time lowers. The infective capacity is determined, therefore, by both the percentage of infected seedlings and the number of inoculated seedlings. Neither the shortest inoculation access time that gives the largest number of inoculated seedlings nor the longest inoculation access time that gives the highest percentage of infected seedlings would maximize the infective capacity of an insect. Based on the results from exposing 10,000 seedlings to 200 viruliferous insects, the maximum infective capacity of *N. virescenes* in transmitting tungro virus was found at the transfer interval (or the inoculation access time) of 30 minutes. The maximum infective capacity was 30 infected seedlings/insect/day, assuming that the insect's infectivity in the first 10-hour period did not change for the remainder of the day. We suspect that a more efficient insect may infect a higher number of seedlings under more favorable conditions (Ling, 1976a).

In fact, Ling and Tiongco (1976) obtained the maximum infective capacity of 40 infected seedlings/insect/day for *N. virescens* in transmitting tungro virus. But the maximum infective capacity that they found is much lower than the theoretical one of 288 infected seedlings/insect/day when calculated on the basis of the shortest inoculation access time (5 minutes) for positive transmission of the tungro virus by *N. virescens*. The reason is that the insect does not infect a seedling in every inoculation time, particularly during short access times.

A cage method for studying experimental epidemiology

Epidemiological studies can be categorized as statistical and experimental or analytical and synthetic. The statistical approach includes collecting, collating, and analyzing observations on disease incidence and information about existing factors that are related to the disease under natural field conditions. In the experimental approach, the effect of individual or combined factors on the disease incidence are studied in the greenhouse or in the field under controlled conditions.

A cage method (Ling, 1976b) was developed to simulate field experiment of tungro epidemiology because in a cage we can manipulate all factors essential for spreading the disease such as insect vector, virus source, and test seedlings. The investigator can vary individual or combined factors among the cages to determine their effects on percentage of infection of the test seedlings. Further, the cage can be kept under various environmental conditions to investigate their effects on disease incidence.

Experimental epidemiology of rice tungro disease

We studied the effects of several factors on tungro incidence in terms of percentage of infected seedlings of the variety Taichung Native 1 (TN1) by the cage method in a greenhouse. We introduced 180 virus-free *N. virescens* into cages, each of which contained 300 test seedlings in 12 pots, as well as four pots of diseased plants as virus sources, and confined them for 7 days. The basic composition of each of the three factors—number of insects, number of pots of diseased plants, and duration of confinement—was constant in all experiments except for those in which we varied each individual factor to determine its effect on tungro incidence (Ling, 1976c, 1976d).

The percentage of infected seedings (Y) increased as the number of adult insects (x) increased in the cage by Y=100–100 $(1+ax+bx^2)$, where a and b values varied according to the mortality of the insects during the test period.

When the insects were confined for a number of days (x) in the cage the percentage of infected seedlings (Y) increased as the duration of confinement lengthened by $Y=100 \ (1-e^{-0.14}x)$, where $e=2.718$.

Based on the percentage of infected seedlings adult insects appeared to be about three times more efficient than nymphal insects in spreading tungro disease.

Watering the seedlings in the cages during the test period caused the insects to move, increasing the percentage of infected seedlings. The ratio of infected seedlings with watering to the percentage without watering was 1.20:1 for adults and 2.65:1 for nymphs.

The percentage of infected seedlings (Y) increased as the percentage of pots of diseased plants (x) that acted as virus sources in the cages increased by $Y=a+bx$

for the test amount of virus sources (6.25 to 50 percent) and by $Y=ax+b\sqrt{x}$ when zero infection was included for no diseased plants and 100 percent infection for all diseased plants in the cages. The a and b values varied according to the growth stage of the insect.

Seedling infection was influenced by the amount of virus sources surrounding the distance between the test seedlings and the diseased plants. Based on the distance that *N. cincticeps* travels (Miyashita *et al.*, 1964) and the retention period of tungro virus, we estimated that a diseased plant at a distance greater than 250 m could no longer be a direct source of tungro virus for a healthy plant under natural conditions in the tropical region. However, we suspect that this distance may be longer, particularly if the insect is carried by strong winds.

When diseased plants of rice varieties TN1 or IR22 were used as virus sources, the percentages of infected seedlings were significantly higher than when diseased plants of the varieties C4-63G or IR20 were used. Consequently, we concluded that diseased plants of different varieties were not identical in their "quality" as sources of tungro virus. By "quality" we mean the percentage of insects that become infective after acquisition feeding on diseased plants or seedling infection as result of using diseased plants of various types as virus source.

Movement of viruliferous insects

We observed the hourly movement of 1,330 tungro-viruliferous *N. virescens* adults from 0800 to 1700 hours after the insects were individually confined in screened cages with seedlings of IR8, which is resistant to the green leafhopper, or of TN1, which is susceptible. We studied only three types of insect movement that were related to the spread of tungro—seedling-to-seedling, off-seedling, and back-to-seedling.

More insects moved and each individual insect moved more times on the IR8 seedlings than on the TN1 seedlings in the test period (Ling and Carbonell, 1976). Consequently, more IR8 seedlings were visited by viruliferous insects, but the visiting duration per seedling and the interval between two movements of each insect were shorter on IR8 than on TN1 seedlings.

We found that seedlings of either IR8 or TN1 that became infected had been visited significantly longer by viruliferous insects than those that did not become infected. These results suggest that a rice variety's field resistance (Robinson, 1969) to tungro may be related to the shorter duration that viruliferous insects visit each seedling. Visiting duration may be shorter because the insects move more often due to their nonpreference for the particular rice variety, in addition to the variety's antibiosis to the insects.

References

1. ANJANEYULU, A. (1974). Epidemiological studies of rice tungro virus disease in India. Paper presented at the International Rice Research Conference, Los Baños, Philippines, April 22–26. p. 12 (mimeo).
2. Anonymous (1969). Surveillance of tungro virus in India, 3, 1–6. *In* Progress Report of the All-India Coordinate Rice Improvement Project, Indian Council of Agricultural Research, New Delhi, India
3. JOHN, V. T. (1968). Identification and characterization of tungro, a virus disease of rice in India. *Plant Dis. Reptr.* **52**, 871–875.
4. ———— (1970). Yellowing disease of paddy. *Indian Farming* **20**(3), 27–30.
5. LAMEY, H. A., SURIN, P. & LEEUWANGH, J. (1967). Transmission experiments on the tungro virus in Thailand. *Int. Rice Comm. Newslett.* **16**(4), 15–19.
6. LAMEY, H. A., SURIN, P., DISTHAPORN, S. & WATHANAKUL, L. (1967). The

epiphytotic of yellow orange leaf disease of rice in 1966 in Thailand. *FAO Plant Prot. Bull.* **15**, 67–69.

7. LIM, G. S. (1972). Studies on penyakit merah disease of rice III. Factors contributing to an epidemic in North Krian, Malaysia. *Malaysian Agr. J.* 48, 278–294.
8. LING, K. C. (1966). Nonpersistence of the tungro virus of rice in its leafhopper vector, *Nephotettix impicticeps*. *Phytopathology* **56**, 1252–1256.
9. ———— (1970). Ability of *Nephotettix apicalis* to transmit the rice tungro virus. *J. Econ. Entomol.* **63**, 583–586.
10. ———— (1972). Rice virus diseases. International Rice Research Institute, Los Baños, Philippines. 142 p.
11. ———— (1973). Synonymies of insect vectors of rice viruses. International Rice Research Institute, Los Baños, Philippines. 29 p.
12. ———— (1976a). The capacity of *Nephotettix virescens* to infect rice seedlings with tungro. *Philippine Phytopathology* **10**. (in press)
13. ———— (1976b). A cage method for studying experimental epidemiology of rice tungro disease. *Philippine Phytopathology* **10**. (in press)
14. ———— (1976c). Experimental epidemiology of rice tungro disease I. Effect of some factors of vector (*Nephotettix virescens*) on disease incidence. Submitted for publication in the Philippine Phytopathology.
15. ———— (1976d). Experimental epidemiology of rice tungro disease II. Effect of virus source on disease incidence. Submitted for publication in the Philippine Phytopathology.
16. LING, K. C. & CARBONELL, M. P. (1976). Movement of individual viruliferous *Nephotettix virescens* in cages and tungro infection of rice seedlings. Submitted for publication in the Philippine Phytopathology.
17. LING, K. C. & TIONGCO, E. R. (1976). Effect of temperature on the transmission of rice tungro virus by *Nephotettix virescens*. Submitted for publication in the Philippine Phytopathology.
18. MIAH, S. A. & AHMAD, M. S. (1974). Sources of resistance and breeding for resistance to tungro and bacterial blight in Bangladesh. Paper presented at the International Rice Research Conference, Los Baños, Philippines, April 22–26. 15 p. (mimeo.)
19. MIYASHITA, K., ITO, Y., YASUO, S., YAMAGUCHI, T. & ISHII, M. (1964). Studies of the dispersal of plant- and leafhoppers II. Dispersals of *Delphacodes striatella* Fallen, *Nephotettix cincticeps* Uhler, and *Deltocephalis dorsalis* Motschulusky in nursery and paddy field. *Jap. J. Ecol.* **14**, 233–241.
20. OKA, I. N. (1971). On an outbreak of a rice disease showing tungro symptoms in South Sumatra and Lampung provinces. Min. Agr. Centr. Res. Inst. Agr., Bogor, Indonesia. 4 p. (mimeo.)
21. ROBINSON, R. A. (1969). Disease resistance terminology. *Rev. Appl. Mycol.* 48, 593–606.
22. SAITO, Y., ROECHAN, M., TANTERA, D. M. & IWAKI, M. (1975). Small bacilliform particles associated with penyakit habang (tungro-like) disease of rice in Indonesia. *Phytopathology* **65**, 793–796.
23. TANTERA, D. M. (1973). Studies on rice virus/mycoplasma disease in 1972. Paper presented at Staff Meeting, Centr. Res. Inst. Agr., Bogor, Indonesia, July 25–26. 25 p. (mimeo.)
24. ———— (1974). Field screening for tungro and grassy stunt in Indonesia, 1972–1974. Paper presented at the International Rice Research Conference, Los Baños, Philippines, April 22–26. 12 p. (mimeo.)
25. TING, W. P. & PARAMSOTHY, S. (1970). Studies on penyakit merah disease of rice I. Virus-vector interaction. *Malaysian Agr. J.* 47, 290–298.
26. VAN HALTERERN, P. & SAMA, S. (1973). The tungro disease in South Sulawesi. Paper presented at Staff Meeting, Centr. Res. Inst. Agr., Bogor, Indonesia, July 25–28. 51 p. (mimeo.)

Discussion

D. A. Benigno, Philippines: When leafhoppers are placed under higher temperature what is their frequencies of feeding compared with those kept at lower temperature?

Answer: We don't have such data, because we don't know how to define "feeding".

W. P. Ting, Malaysia: Your consideration of tungro virus as transitory/leafhopper—transmitted virus is based on your recent studies on effect of temperature on retention. It would appear to me that we may have to re-examine the viruses classified earlier. Some persistent viruses may not be so when exposed to higher temperature.

Answer: We classified tungro as a transitory leafhopper-transmitted virus, because the retention period of 3 weeks is too long for nonpersistent.

Y. Nagai, Japan: What is the difference in meaning of the two words, transitory and nonpersistent? Which word has longer retention period in the strict sense?

Answer: "Transitory" and "nonpersistent" may have the same meaning as far as the virus-vector interaction is concerned. However, nonpersistent refers to a short retention period. Transitory refers to a short retention period but the period can be long.

I. N. Oka, Indonesia: If you found that temperature range may not be the key factors in causing the spread of tungro disease in the tropics, what other factors do you think it will cause?

Answer: Incidence of a virus disease is a result of spreading of the disease. The incidence of a virus disease is a function of virus source (quantity and quality), host (susceptible and plant age), environment (biological and nonbiological), and insect vector (population and activity). Any of these factors would affect the incidence of the disease.

M.D. Mishra, India: Dr. Ling, the hoppers became sluggish at cold temperature. In this case, what will affect the ability of the vectors to transmit the disease?

Answer: It is general that the living organism becomes less active at a low temperature. However, at present time, we don't know what criteria should be used to indicate the activity of an insect.