Changes in Cultivar Reactions to Tungro Due to Changes in "Virulence" of the Leafhopper Vector

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ABSTRACT

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Populations of green leafhopper (GLH), *Nephotettix virescens*, were collected from five locations in the Philippines from August 1986 to January 1988 and reared on GLH-susceptible rice cultivar Taichung Native 1 (TN1). First or second generation adults that had fed on rice plants infected with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) were tested for their feeding behavior by the honeydew-based bromocresol-green test during a 1-day inoculation access feeding on seedlings of IR26 and IR36 which were moderately resistant to GLH; IR54, IR62, and IR64 which were resistant to GLH; and susceptible TN1. A colony maintained on TN1 in a greenhouse served as the control. Inoculated seedlings were indexed by latex serology. Third or fourth generations were tested for GLH preference, population increase, and nymphal mortality. Populations collected in 1987 from Bicol; Koronadal, South Cotabato; Maligaya, Nueva Ecija; Leyte; and Los Baños, Laguna, fed more from phloem and transmitted RTBV and RTSV together more efficiently on GLH-resistant test cultivars than did the greenhouse colony. On these cultivars, the field populations showed higher preference, less nymphal mortality, and greater population buildup than did the greenhouse colony, indicating higher virulence of the field populations to the resistant cultivars. GLH populations collected at Los Baños in 1986 fed largely from xylem and transmitted mainly RTBV alone on IR54, whereas those collected in 1987 and 1988 fed more from phloem and efficiently transmitted RTBV and RTSV together on IR54. At Los Baños, RTBV and RTSV incidences on IR54 and other related GLH-resistant cultivars were negligible in 1985 and 1986; the incidences were very high in 1987 and 1988. Apparently, IR54 and other related GLH-resistant cultivars were severely infected in 1987 due to a shift of GLH "virulence" to these cultivars. The "virulence" of a field population to the GLH-resistant cultivars was reduced drastically when the population was continuously reared on TN1 for seven or more generations.

Additional keywords: rice tungro disease, vector resistance.

Tungro (24) is one of the most important virus diseases of rice in South and Southeast Asia. It is a composite disease caused by rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (14,27). RTBV causes leaf yellowing and plant stunting. RTSV causes no clear symptoms except mild stunting and enhances the symptoms caused by RTBV infection (14). Both RTBV and RTSV are transmitted efficiently in a semipersistent manner by the rice green leafhopper (GLH) *Nephotettix virescens* (Distant) (3,11,12,15). Transmission of RTBV by GLH depends on the presence of RTSV, whereas RTSV is transmitted independently (3,12,14). On GLH-susceptible cultivars, tungro-viruliferous GLH generally transmits both viruses together or RTBV alone, but on GLH-resistant cultivars, GLH predominantly transmits RTBV alone (8,16,17). The GLH occasionally transmits RTSV alone (11,14–17). GLH mainly feeds in the xylem of resistant cultivars, and it feeds in the phloem and xylem of susceptible cultivars (1,20,21).

Several high-yielding cultivars that had little tungro have been bred and widely planted in the Philippines (23). However, some of these cultivars have succumbed to tungro after a few years of intensive cultivation (17). These cultivars appear to have resistance to the vector but not to the viruses (17,30). Reactions of these cultivars to tungro and GLH varied in the Philippines (31). Although experimental evidences are lacking, the increased incidence of tungro was attributed to the development of GLH populations capable of feeding and colonizing these cultivars. The term "virulence" used in this paper indicates the ability of leafhoppers to overcome mechanisms of resistance in plants, such as antibiosis and/or antixenosis (nonpreference) (6,7,9,10,29,31).

We compared GLH populations collected from five locations in the Philippines from 1986 to 1988 for their virulence to and tungro transmissibility on GLH-resistant cultivars and demonstrated that the increased incidence of tungro in IR50, IR54, and IR64 was due to a shift of GLH virulence to these cultivars.

MATERIALS AND METHODS

Viruses and insects. The tungro source was an isolate collected at Laguna, Philippines, and maintained on rice cultivar Taichung Native 1 (TN1) for 15 yr by means of successive transfers with GLH. About 50-day-old rice plants with tungro symptoms were tested individually by latex flocculation serology (26) to assure that source plants were infected with both RTBV and RTSV. Newly emerged GLH adults were allowed to feed on a source plant for 4 days. These viruliferous GLH were used for feeding behavior-transmission and preference-transmission tests.

About 50 to 60 GLH were collected from each rice field at Albay and Camarines Sur, Bicol; Koronadal, South Cotabato; Leyte; Los Baños, Laguna; and Maligaya, Nueva Ecija, in the Philippines in 1987. Each GLH collection was reared separately on TN1 seedlings in a cage inside an insect-proof greenhouse. The populations were designated by the name of the location from which they were collected initially. First or second generation adults were tested for the feeding behavior and virus transmission on test seedlings. The third and fourth generations were used to test nymphal mortality, population buildup, and preference on the test seedlings. A GLH colony maintained on TN1 for several years in the greenhouse served as a control. Additional GLH collections were made at Koronadal, Maligaya, and Los Baños from 1986 to 1988. First or second generation adults also were tested for feeding behavior and virus transmission on the test seedlings. All the tests were conducted at room temperature (28 ± 2 C). After the tests, GLH were killed by insecticide sprays.

Plants. High-yielding cultivars IR26, IR36, IR54, IR62, and IR64 (13,17,23) with varying levels of GLH resistance and TN1 without GLH resistance were selected for the greenhouse tests.

Other high-yielding cultivars such as IR22, IR42, IR50, and IR60 (13,17,23) also were included in field experiments. IR26 has moderate resistance to GLH and resistance to RTSV infection (13) derived mainly from TKM6 (22). IR36 and IR42 have moderate resistance to GLH derived mainly from Ptb18 (22). IR50, IR54, and IR64 have GLH resistance derived mainly from Gam Pai 30-12-15 (19). IR60 and IR62 have GLH resistance derived mainly from Gam Pai 30-12-15 and Ptb33 (19). IR22 is susceptible to GLH.

Latex flocculation serology. About 20 days after inoculation, a portion 10–15 cm long was collected from the second youngest leaf of each test plant. All aboveground tissue was collected from plants with poor growth. Leaf and plant samples were extracted separately using a combined leaf and bud press (Erich Pollahne, Wennigsen, FRG), and extracts were diluted to 1/10 to 1/15 with 0.05 M Tris/Cl buffer (pH 7.2). Latex suspension (Bacto latex 0.81, Difco Laboratories, Detroit, MI) was sensitized with immunoglobulin to RTBV or RTSV as described by Omura et al (26). Antisera to RTBV and RTSV had titers of 1/2,560 and 1/640, respectively, in the ring interface precipitin test (4). Before use, a test was conducted to confirm that the latex preparation reacted specifically with extracts of plants infected with RTBV or RTSV. About 25 μl of sensitized latex suspension was mixed with an equal amount of leaf extract in a well of a used microtiter plate. The plate was shaken at 160 oscillations per minute for 45 min to 1 hr. Mixtures were observed under a light microscope at ×100. Clumping of latex particles indicated presence of the virus antigen in the extracts.

Tungro incidence in the field. Field trials were conducted in the wet season (June to November) during 1984 to 1988 at the Los Baños, Laguna, experimental farm. Similar trials also were conducted in the 1986 to 1987 dry season (November to June) at Koronadal, South Cotabato; Baybay, Leyte; Albay, Bicol; and Guimba, Nueva Ecija. Tungro disease occurred in the previous crop at all sites except in Guimba where high tungro incidence was reported in 1983 and 1984 (2). Three-week-old seedlings of test cultivars were transplanted in spacing of 20 × 20 cm at two to three seedlings per hill in plots of 2 × 2 m arranged in a randomized complete block design with four replications. About 60 days after transplanting, a single leaf was collected from each of 36 hills in a predetermined subplot of 1.2 × 1.2 m. Samples were mature leaves from each hill; leaves with tungro symptoms were selected if present. In 1988 at Los Baños, 49 hills were sampled randomly from each cultivar planted in demonstration plots of 4 × 4 m. Leaf samples were indexed by the latex test.

Feeding behavior and virus transmission. Viruliferous GLH females were confined for 1 day in a cage with a 7- to 10-day-old potted seedling of one of the six test cultivars. Honeydew

TABLE 1. Rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) incidence in nine cultivars at four Philippine locations 60 days after transplanting during the 1986 dry season

	RTBV and RTSV incidence (%)[v]												
	Guimba, Nueva Ecija[w]	Bicol[w]				Leyte[w]				Koronadal, South Cotabato[w]			
Cultivar	RTSV[x]	RTBV + RTSV	RTBV	RTSV	Total	RTBV + RTSV	RTBV	RTSV	Total	RTBV + RTSV	RTBV	RTSV	Total
IR22	11 ± 13[y]	31	12	8	51 ± 10	20	13	11	44 ± 6	50	8	29	87 ± 4
IR26	12 ± 15	0	1	1	2 ± 2	0	2	0	2 ± 1	0	34	0	34 ± 6
IR36	29 ± 3	3	1	18	22 ± 6	4	3	22	29 ± 16	19	12	19	50 ± 9
IR42	26 ± 38	...[z]	...	...	...	17	4	64	85 ± 8	25	14	20	59 ± 15
IR50	9 ± 10	...	...	...	...	2	2	28	32 ± 7	39	10	19	68 ± 9
IR54	4 ± 5	11	1	40	52 ± 16	4	2	48	54 ± 13	65	5	21	91 ± 6
IR60	26 ± 34	...	...	...	...	0	1	4	5 ± 1	1	3	11	15 ± 4
IR62	13 ± 17	...	...	...	...	0	0	4	4 ± 2	4	2	25	31 ± 13
IR64	6 ± 9	...	...	...	...	2	2	28	32 ± 7	31	15	22	68 ± 15

[v] RTBV + RTSV indicates plants doubly infected with RTBV and RTSV; RTBV indicates plants infected with RTBV alone; RTSV indicates plants infected with RTSV alone; the total is the number of plants infected with RTBV and RTSV either together or separately, ± standard deviation.
[w] A total of 144 plants was tested for each cultivar.
[x] No RTBV incidence was recorded.
[y] Mean total ± standard deviation.
[z] Not included in the trials.

TABLE 2. Rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) incidence in eight cultivars at the experimental farm in Los Baños, Laguna, in the wet season during 1984 to 1988

	RTBV and RTSV incidence (%)[w]																			
	1984[x]				1985[x]				1986[x]				1987[x]				1988[y]			
Cultivar	RTBV + RTSV	RTBV	RTSV	Total	RTBV + RTSV	RTBV	RTSV	Total	RTBV + RTSV	RTBV	RTSV	Total	RTBV + RTSV	RTBV	RTSV	Total	RTBV + RTSV	RTBV	RTSV	Total
IR26	...[z]	...	...	...	...	...	...	...	0	6	0	6 ± 5	0	10	0	10 ± 8	0	31	0	31 ± 15
IR36	6	9	26	41 ± 6	20	4	54	78 ± 26	4	1	29	34 ± 21	8	4	33	45 ± 6	38	6	36	80 ± 15
IR42	32	13	34	79 ± 4	14	0	69	83 ± 34	28	1	31	60 ± 23	45	2	37	84 ± 3	61	0	36	97 ± 25
IR50	0	1	0	1 ± 2	...	...	...	...	...	...	...	...	24	5	37	66 ± 12	39	3	28	70 ± 15
IR54	0	0	0	0 ± 0	0	0	2	2 ± 1	1	4	4	9 ± 8	24	2	51	77 ± 11	71	11	16	98 ± 27
IR60	0	0	0	0 ± 0	...	...	...	...	...	...	...	...	1	1	6	8 ± 5	0	3	14	17 ± 6
IR62	0	0	0	0 ± 0	...	...	...	...	...	...	...	...	0	0	3	3 ± 1	0	0	11	11 ± 6
TN1	...	...	...	...	65	13	15	93 ± 12	70	2	24	96 ± 5	80	7	11	98 ± 2	...	...	...	...

[w] Plants were indexed by the latex test. RTBV + RTSV indicates plants doubly infected with RTBV and RTSV; RTBV indicates plants infected with RTBV alone; RTSV indicates plants infected with RTSV alone; the total is the number of plants infected with RTBV and RTSV either together or separately, ± standard deviation.
[x] A total of 144 samples was collected from preset subplot (see text).
[y] A total of 196 samples was collected randomly from selected plants.
[z] Not included in the experiments.

excreted by GLH was collected on a bromocresol-green-treated filter paper disk placed around the base of the seedling (28). Blue spots (basic reaction) were assumed to indicate phloem feeding, and orange or brown spots (acidic reaction) were assumed to indicate xylem feeding (1,28). Honeydew excreted by GLH was quantified by measuring the size of acidic and basic spots on the disks. Seedlings were indexed by the latex test. Tests were staggered using at least 20 GLH for each combination of cultivar and GLH population.

Preference and virus transmission. Seven-day-old seedlings of the six test cultivars were transplanted in randomized rows in each 28 × 36 cm plastic tray at 10 seedlings per row. Viruliferous GLH adults (two adults per seedling) were introduced into a cage with the tray. Numbers of GLH on seedlings of each cultivar were counted 6, 9, and 12 hr after insect release. After the last counting, seedlings were sprayed with an insecticide and grown in an insect-proof greenhouse. The average of the three counts indicated the level of GLH preference on each cultivar. Seedlings were indexed by the latex test. Each GLH population had a least two replications.

Test tube inoculation. In January and February 1988, GLH adults collected directly from tungro-affected fields at Los Baños were allowed a 3-day acquisition feeding on source plants infected with both RTBV and RTSV. Then they were confined individually for 1 day in test tubes with 7-day-old seedlings of the six test cultivars. Seedlings were transplanted in pots, grown in the greenhouse, and indexed by the latex test.

Nymphal mortality and population buildup. For nymphal mortality, two or three 7-day-old test seedlings were placed in a test tube with a small amount of tap water and infested with five second or third instar nymphs. Chlorotic seedlings were replaced with fresh ones. Four days later, surviving nymphs were counted. Each cultivar had 20 replications.

For population buildup, 7-day-old test seedlings were singly transplanted in clay pots. Thirty days after transplanting, each plant was infested with one pair of newly emerged male and female adults. Twenty-one days later, the living progeny were counted. Eight pairs were tested for each combination of cultivar and GLH population.

RESULTS

RTBV and RTSV incidences in 1986 and 1987 at four locations. In the 1986-1987 dry season, tungro disease occurred in the experiment sites in Bicol, Leyte, and Koronadal, South Cotabato, but not in Guimba, Nueva Ecija, where only RTSV incidence was recorded (Table 1). In Guimba, RTSV incidence in IR54 and related cultivars IR50 and IR64 was lower than that in IR36 and GLH-susceptible cultivar IR22. In Bicol, overall RTBV and RTSV incidence in IR54 was as high as that in IR22, whereas the incidence was lower in IR36. In Leyte and Koronadal, overall RTBV and RTSV incidence was high in IR50, IR54, and IR64 as well as in IR22 and IR36. IR26 had low overall incidences in all locations, and the incidence was mostly for RTBV alone. In Bicol, Leyte, and Koronadal, cultivars that had high overall incidences generally had higher levels of infection with RTBV and RTSV together. These results indicate that cultivar reactions to tungro in the field varied among the four locations.

RTBV and RTSV incidences from 1984 to 1988 at Los Baños, Laguna. From 1984 to 1986, overall RTBV and RTSV incidences were low in IR54 (Table 2). In 1984, the incidences also were low in IR50, IR60, and IR62. In 1987 and 1988, however, the overall incidence was high in IR50 and IR54, but it was low in IR60 and IR62. IR26 had infections with RTBV alone in these trials. IR36, IR42, and TN1 had consistently high overall incidences of RTBV and RTSV. These results indicate a change in field reactions of IR50 and IR54 to tungro in 1987.

Variability of leafhoppers from five locations. *Feeding behavior and tungro transmission.* In the feeding behavior-transmission test, GLH populations collected in 1987 at five Philippine

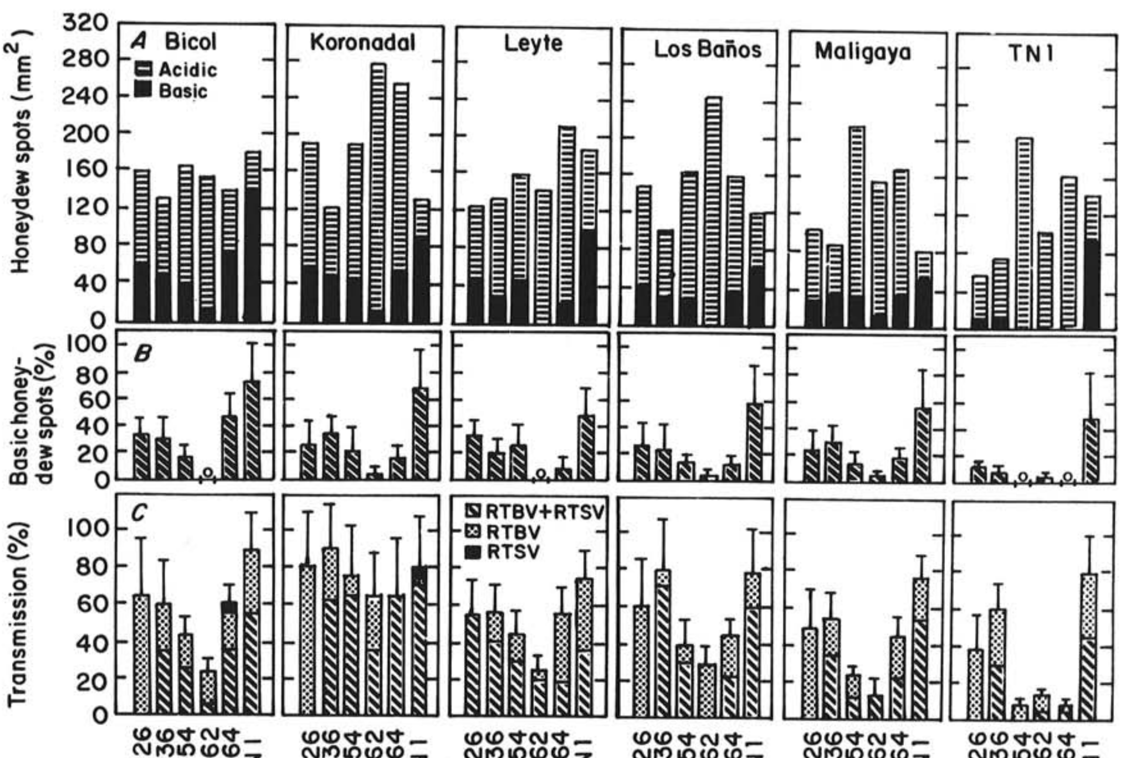

Fig. 1. A, Area of acidic and basic honeydew spots, **B,** area of basic honeydew spots as a percentage of total spots, and **C,** percent transmission of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) either together or separately, on seedlings of six rice cultivars in a 22-hr inoculation feeding by tungro-viruliferous green leafhopper adult females of populations collected from Bicol; Koronadal, South Cotabato; Leyte; Los Baños, Laguna; Maligaya, Nueva Ecija; and a greenhouse colony from rice cultivar Taichung Native 1 (TN1). Field populations were maintained on TN1, and the first or second generations were used. The vertical lines indicate standard deviation of percent area of basic honeydew spots and total percent transmission of RTBV and/or RTSV.

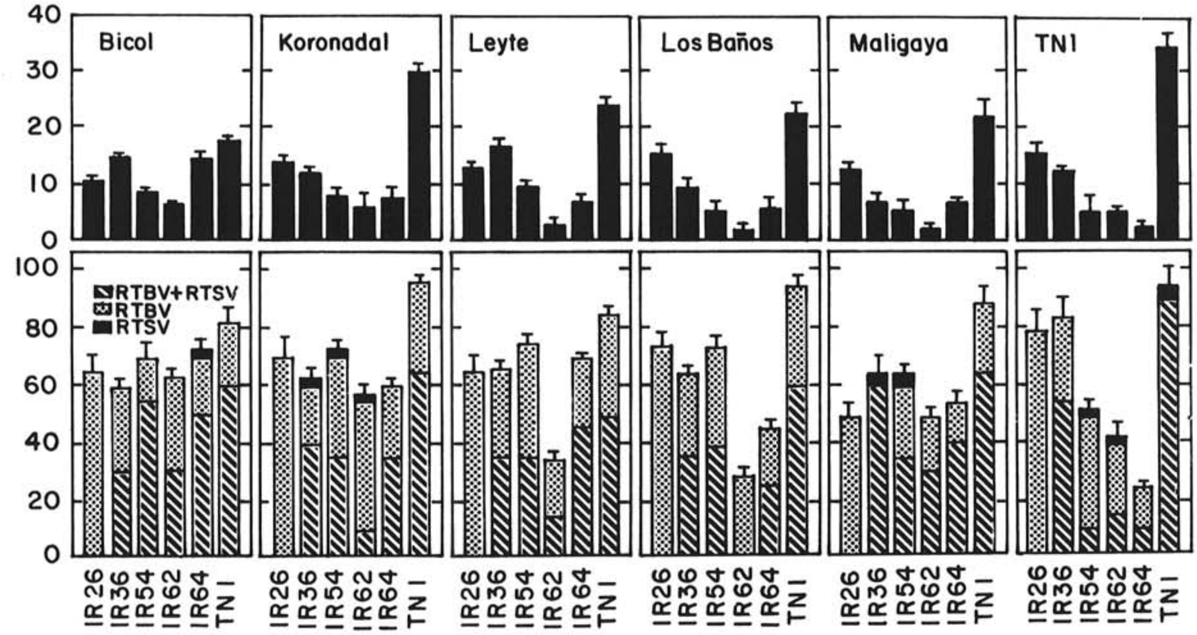

Fig. 2. Average number of tungro-viruliferous green leafhopper adults of populations collected from Bicol; Koronadal, South Cotabato; Leyte; Los Baños, Laguna; and Maligaya, Nueva Ecija; and a greenhouse colony from rice cultivar Taichung Native 1 (TN1) that were present on 10 seedlings of six rice cultivars 6, 9, and 12 hr after infestation and percent seedling infection with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) either together or separately during infestation. Field populations were maintained on TN1, and the third or fourth generations were used. The vertical lines indicate standard deviation of leafhopper number and total percent seedling infection with RTBV and/or RTSV.

locations transmitted RTBV and RTSV to the test cultivars differently. The field populations excreted more basic honeydew on IR36, IR54, and IR64 than on IR62. On IR36, IR54, and IR64, the five field populations transmitted RTBV and RTSV together or RTBV alone (Fig. 1). On IR62, Los Baños and Maligaya, Nueva Ecija, populations transmitted RTBV alone, whereas populations from Bicol, Koronadal, and Leyte transmitted RTBV and RTSV together or RTBV alone. On all test cultivars except IR26, Koronadal and Leyte populations efficiently transmitted RTBV and RTSV together. The greenhouse TN1 colony excreted mainly acidic honeydew alone and transmitted the viruses at low efficiency on IR54, IR62, and IR64.

Preference and virus transmission. All populations tested preferred TN1 (Fig. 2). Next to TN1, Koronadal, Leyte, Los Baños, and Maligaya populations preferred IR26 or IR36, whereas the Bicol population preferred IR36 and IR64. The percentage overall seedling infection with RTBV and RTSV in each cultivar in this test was higher than those in the feeding behavior and transmission test (Fig. 1). Percentage of seedling infection with both RTBV and RTSV by the five field populations was high on IR36, IR64, and TN1. On IR62, the Bicol population gave infection higher than 30% with both RTBV and RTSV, whereas the Los Baños population gave infection only with RTBV. The greenhouse TN1 colony gave a low percentage of infection with both RTBV and RTSV on IR54, IR62, and IR64.

Population buildup. The size of progenies in GLH-resistant cultivars produced by one pair of Bicol, Koronadal, and Leyte populations was generally greater than that of Los Baños and Maligaya populations (Fig. 3). The greenhouse TN1 colony produced the smallest number of progeny on seedlings of all cultivars except TN1. The progeny increase of all populations was small on IR62.

Nymphal mortality. On all cultivars except TN1, all field populations tested generally had lower nymphal mortality than did the greenhouse TN1 colony (Fig. 3). On IR64, nymphal mortality of the field populations was especially low. On IR62, the mortality was high for all populations.

Changes in virulence and transmission efficiencies of leafhopper populations (1986–1988). GLH populations collected at Koronadal in August 1986 and September 1987 (data not shown) were comparable in feeding behavior and tungro transmission

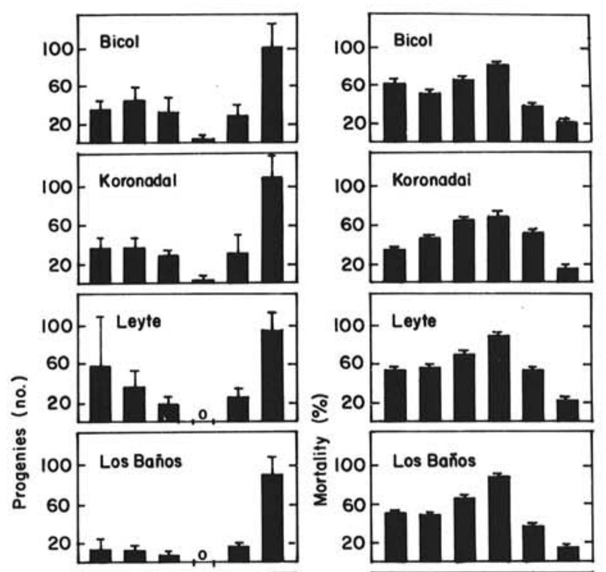

Fig. 3. Number of progeny produced by one pair of adults and percent nymphal mortality on seedlings of six rice cultivars of green leafhopper populations collected from Bicol; Koronadal, South Cotabato; Leyte; Los Baños, Laguna; and Maligaya, Nueva Ecija; and a greenhouse colony from rice cultivar Taichung Native 1 (TN1). Field populations were maintained on TN1, and the third or fourth generations were used. The vertical lines indicate standard deviation.

efficiency to the populations collected at the same location in April 1987 (Fig. 1). Likewise, GLH populations collected at Maligaya in January 1986 and September 1987 (data not shown) also were similar to the populations collected in June 1987 at the same site (Fig. 1).

Remarkable differences in feeding behavior and transmission efficiency were observed between Los Baños populations collected in 1986 and those taken in 1987 or 1988 (Fig. 4). On IR54, the GLH of the first three collections excreted mostly acidic honeydew and transmitted mainly RTBV alone. The GLH of the later three collections excreted more basic honeydew and efficiently transmitted RTBV and RTSV together on IR54. On IR64, the GLH of the later three collections also efficiently transmitted both viruses together. On IR62, the later four collections transmitted the viruses either together or separately more efficiently than did the earlier two collections.

GLH adults collected at Los Baños in January and February 1988 efficiently transmitted RTBV and RTSV together on IR50, IR54, IR60, and IR64 when tested directly without rearing on TN1 for transmission efficiency after an acquisition access feeding on tungro-infected TN1 plants (data not shown). On the six test cultivars, percent transmission of RTBV and/or RTSV by GLH was comparable to the percentages obtained by the 1987 or 1988 Los Baños collections after rearing one to two generations on TN1. These results indicate that GLH populations did not change their transmission efficiency after they were reared on TN1 for one or two generations.

Stability of virulence and transmission efficiency in a field population. The Koronadal populations collected in August 1986 were continuously reared on TN1 plants. Every two or three generations, GLH females were subjected to the feeding mode and transmission test.

The second generation was similar to the original GLH populations in feeding behavior and tungro transmission efficiency on test cultivars (Figs. 1 and 5). The seventh generation adults excreted less basic honeydew on IR26, IR36, and IR54. They transmitted the viruses less efficiently on IR26 and IR54 (Fig. 5). The 14th and 22nd generations were almost comparable to the greenhouse TN1 colony in feeding behavior and transmission efficiency on test cultivars. On IR54, the area of basic honeydew spots was about 15% of total area of honeydew spots by the second generation females and 1.3% by the seventh generation females.

DISCUSSION

Because of the complex interactions between the tungro-associated viruses and plants or vectors, information on variability in cultivar reaction to tungro is limited, and the variability in GLH virulence in relation to tungro incidence is not well documented. In these experiments, changes in tungro incidence on cultivars due to a shift of GLH virulence to the cultivars were demonstrated by testing the efficiency of GLH populations (collected from 1986 to 1988 from five locations) in transmitting tungro-associated viruses and their feeding behavior on test cultivars. The feeding behavior-transmission test and the serological indexing of inoculated seedlings efficiently illustrated the complex interactions among viruses, vector, and cultivars. The feeding-transmission test could differentiate populations based on their virulence to some GLH-resistant cultivars.

In the feeding behavior-transmission test, GLH populations collected in 1987 at five Philippine locations transmitted RTBV and RTSV to the test cultivars differently. On GLH-susceptible IR22 and TN1, all populations transmitted RTBV and RTSV more efficiently, fed more from phloem, showed greater preference, and had greater population buildup and less nymphal mortality than on the other cultivars. On cultivars where nymphal mortality was greater, GLH transmitted predominantly RTBV alone, fed less from the phloem, showed less preference, and had a smaller population buildup. These five features have been used

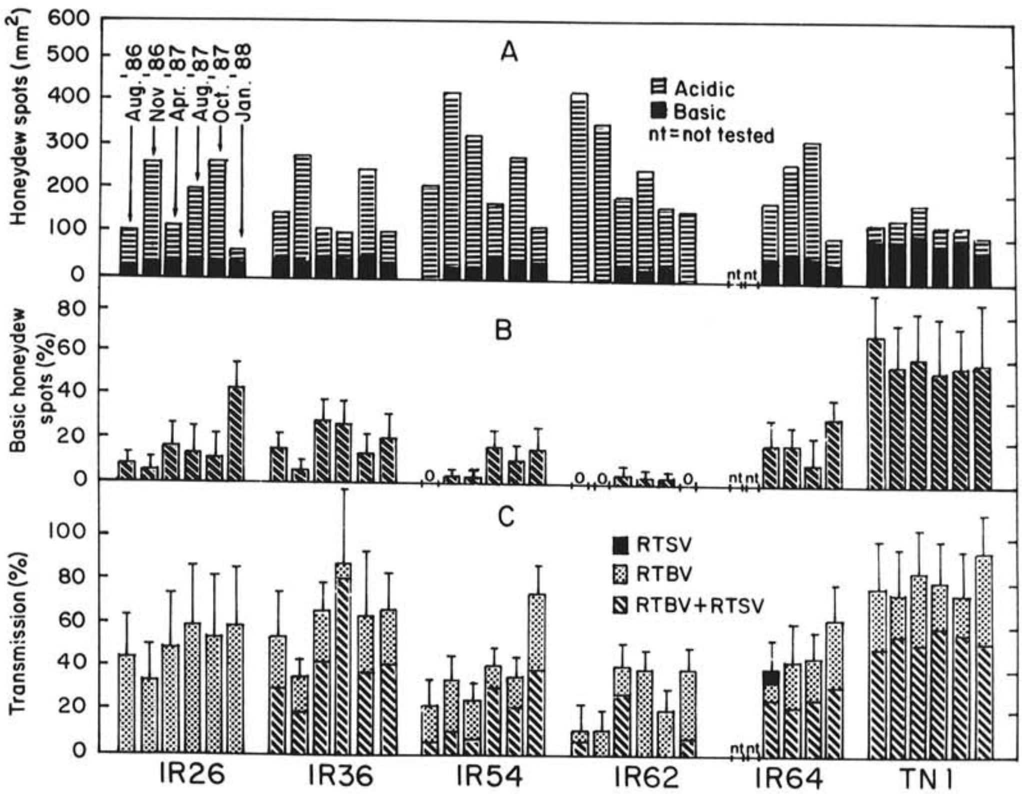

Fig. 4. A, Area of acidic and basic honeydew spots, **B,** area of basic honeydew spots as a percentage of total spots, and **C,** percent transmission of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), on seedlings of six rice cultivars in a 22-hr inoculation feeding by tungro-viruliferous green leafhopper adult females of populations collected at Los Baños in August 1986, November 1986, April 1987, August 1987, October 1987, and January 1988. Populations were maintained on rice cultivar Taichung Native 1 (TN1), and the first or second generations were used. The vertical lines indicate standard deviation of percent area of basic honeydew spots and total percent transmission of RTBV and/or RTSV.

to compare virulence of leafhoppers and planthoppers to cultivars (6,7,9,10,29,31). Correlations between degree of xylem and phloem feedings by GLH and percent transmission with RTBV alone or both RTBV and RTSV in each cultivar were significant among cultivars but were not within individuals in most combinations of populations and cultivars.

On IR54, Los Baños populations collected in 1986 fed primarily from the xylem and transmitted mostly RTBV alone. The populations collected after August 1987 fed more from the phloem and efficiently transmitted both RTBV and RTSV on IR54. In the field, tungro incidence on IR54 and other IR cultivars with resistance mainly from Gam Pai 30-12-15 was very low in this and other trials (5,13) from 1984 to 1986 in Guimba, Nueva Ecija and in Los Baños. The incidence was high in 1987 and 1988 at Los Baños. The field response of IR54, IR64, and other related IR cultivars to tungro changed at Los Baños in early 1987. In Nueva Ecija, these cultivars also changed their field responses to tungro during the same period but less drastically. These changes probably are not due to a change in the virulence of the viruses but to a shift of GLH virulence to these cultivars.

Similar changes in field response to tungro of GLH-resistant cultivars have been reported in Thailand (18) and Indonesia (25). In Thailand, RD1 and RD3 had little tungro when released in 1969 but had severe tungro in 1974 (18). In Indonesia, IR36 and IR42 had severe tungro infection from 1982 to 1985 after a few years of intensive cultivation (33). IR50 and IR54, introduced to replace IR36 and IR42, also had severe tungro infection after a few years of cultivation (33). In the Philippines, IR5 and IR8 had limited tungro infection when released in 1966-1967 but had severe tungro infection in 1970-1972 in central Luzon and south Cotabato (32). IR36 and IR42, which were released in 1976-1977, had severe tungro infection in the southern Philippines in 1979 and in central Luzon and Bicol in 1983-1984 (13,17). IR50 and IR54, which were released in 1979-1980 had high tungro incidence in the southern Philippines in 1986 (17). This high tungro incidence on GLH-resistant cultivars which formerly had little tungro also might be due to shifts in GLH virulence to the cultivars.

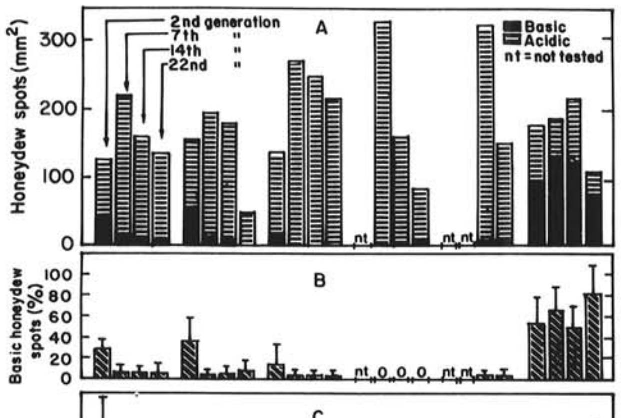

Fig. 5. A, Area of acidic and basic honeydew spots, **B,** area of basic honeydew spots as a percentage of total spots, and **C,** percent transmission of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV), on seedlings of six rice cultivars in a 22-hr inoculation feeding by tungro-viruliferous green leafhopper adult females of a population collected at Koronadal in August 1986 after being maintained on rice cultivar Taichung Native 1 (TN1) for 2, 7, 14, or 22 generations. The vertical lines indicate standard deviation of percent area of basic honeydew spots and total percent transmission of RTBV and/or RTSV.

In these experiments, field populations had reduced virulence to IR54 when continuously reared on susceptible cultivar TN1. In more recent studies, field populations retained their virulence to the resistant test cultivars when maintained on GLH-resistant or moderately resistant cultivars (G. Dahal and H. Hibino, *unpublished data*). High levels of virulence of field populations can be explained by the fact that GLH-resistant, high-yielding cultivars have been grown intensively in the Philippines. Sequential release of GLH-resistant cultivars may further elevate virulence of GLH to a wide range of resistant cultivars. These results indicate that GLH virulence patterns to resistant cultivars depends on which rice cultivars predominate in the area.

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