Rice and Weed Hosts of Rice Tungro-Associated Viruses and Leafhopper Vectors

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ABSTRACT

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Transmission of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) to rice and weeds by *Nephotettix virescens, N. nigropictus, N. malayanus*, and *Recilia dorsalis* was compared. *N. virescens* was the most efficient vector of both viruses to rice and retained them for 4 days after an acquisition access period on infected rice. *N. nigropictus* was less efficient but retained both viruses for 5 days. *N. malayanus* and *R. dorsalis* were the least efficient and retained both viruses for only 1 day. Young seedlings or rhizomes of *Eleusine indica, Echinochloa crusgalli, E. glabrescens, E. colona, Leptochloa chinensis, Leersia hexandra, Panicum repens*, and *Cyperus rotundus* were infected with RTBV and RTSV, either together or separately, when exposed to the four leafhopper species that had fed on doubly infected rice plants. *N. nigropictus* transmitted both viruses from doubly infected *E. crusgalli, P. repens*, or *C. rotundus* plants to rice seedlings, whereas other leafhopper species failed to transmit the viruses from these infected weed plants to rice seedlings. On rice, *N. virescens* fed mainly from the phloem and showed high adult longevity, nymph survival, and population growth, indicating a high level of its biological relationship. *N. virescens* fed from the xylem on all the weed hosts, showing a low level of its biological relationship with the weeds. *N. nigropictus* showed its relationship with *E. crusgalli, E. glabrescens, E. colona, L. hexandra*, and *C. rotundus*. *N. malayanus* showed its relationship only with *L. hexandra*, and *R. dorsalis*, with rice. These results indicate that *N. nigropictus* has a potential for dispersing RTBV and RTSV in weeds in idle fields.

Tungro (14,22) is the most important virus disease of rice (*Oryza sativa* L.) and has caused devastating epidemics in South and Southeast Asia. The virus agent(s) are transmitted in a semipersistent or transitory manner (13) by the leafhoppers *Nephotettix virescens* (Distant) (22,) *N. nigropictus* (Stål) (14), *N. cincticeps* Uhler (7), *N. malayanus* Ishihara & Kawase (14), *N. parvus* Ishihara & Kawase (14), and *Recilia dorsalis* (Motschulsky) (14). In South and Southeast Asia, *N. virescens, N. nigropictus*, and *R. dorsalis* are common in rice fields, and *N. virescens* mainly disperses the virus agents in rice fields. *N. malayanus* is commonly found on *Leersia hexandra* Sw., which grows alongside rice fields. The population of *N. parvus* is very low in the Philippines. Efficiency of tungro transmission by leafhoppers of each species differs remarkably among locations where transmission tests have been conducted (14,24). The role of weed hosts and leafhopper species other than *N. virescens* in epidemics of tungro has remained obscure. Host ranges of the virus agent(s) have been studied in several countries (1,3,11,14,15,17,21,25), with conflicting results. Wild rices (*Oryza* spp.), which can be infected with tungro by artificial inoculation (1,3,11,14,21), are absent or uncommon in the Philippines.

Tungro is a composite disease associated with rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (8,19,23). In rice plants, RTSV causes very mild stunting and enhances the symptoms (leaf yellowing and plant stunting) caused by RTBV (8). *N. virescens* that had fed on plants infected with both viruses transmit RTBV and RTSV either together or separately (8,9). Leafhopper transmission of RTBV depends on the presence of RTSV, but leafhopper transmission of RTSV is independent (8,9). Therefore, RTSV occurs and spreads as an independent pathogen (4). The transmission profiles of the two viruses vary according to vector species (7,9). The profiles also vary according to host plants, apparently because of their resistance to the vector leafhoppers and the viruses (7,10). Serological detection of RTBV and RTSV in infected plant sap made possible precise analysis of their vector transmission and epidemiology (5,18,19).

We compared four leafhopper species for their: 1) transmission efficiencies of RTBV and RTSV in combination or separately on rice and on weeds that are common in rice fields in the Philippines and 2) behavior on rice and the weeds.

MATERIALS AND METHODS

Virus. The tungro source was an isolate collected at Laguna, Philippines, and has been maintained on the rice cultivar Taichung Native 1 (TN1) by successive transfer with *N. virescens*. RTSV was isolated from a tungro-infected plant and maintained similarly (6,8).

Insects. Leafhoppers were collected at Laguna, Philippines. The *N. virescens, N. nigropictus*, and *R. dorsalis* colonies were reared on TN1, and the *N. malayanus* colony was reared on cuttings of virus-free *L. hexandra*. From time to time, infectivity of the leafhopper colonies was tested on TN1 seedlings to confirm absence of RTBV and RTSV.

Plants. Weed species selected were: *Eleusine indica* (L.) Gaertn., *Echinochloa glabrescens* Munro ex Hook.f, *E. crusgalli* (L.) P. Beauv. var. *hispidula* (Retz.), *E. colona* (L.) Link, *Paspalum distichum* L., *Leersia hexandra, Leptochloa chinensis* (L.) Nees, *Ischaemum indicum* (Houtt.) Merr., *Panicum repens* L., *Cynodon dactylon* (L.) Pers., *Rottboellia exaltata* L.f., *Cyperus rotundus* L., *C. iria* L., *C. difformis* L., and *Monochoria vaginalis* (Burm.f.) Presl. These weeds are common in rice fields in the Philippines and many have been listed as potential hosts of the tungro viruses (1,3,11,14,15, 17,21,25,26). Weed seeds were soaked in water for 24 hr, kept at 35 C for 48 hr, and then sown in pots. Plants of the perennials *Leersia hexandra, Panicum repens, Paspalum distichum, Cynodon dactylon, Ischaemum indicum*, and *Cyperus rotundus* were collected from idle fields where rice was not grown. After these weeds were tested by enzyme-linked immunosorbent assay (ELISA) (4,5), virus-free plants were selected and grown in cages. Young rhizomes of the perennial weed plants were transplanted in clay pots. Seedlings and rhizomes were grown in cages. Rice seedlings were inoculated at 7 days after sowing, and weed seedlings and rhizomes were used at 15 days after sowing or transplanting.

ELISA. ELISA followed the standard procedure described earlier (4,5). Antisera to RTBV and RTSV had titers by the ring interface precipitin test of 1/2,560 and 1/640, respectively (6). Leaf

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samples were separately homogenized with 0.01 M phosphate buffer (pH 7.4) containing 0.14 M NaCl, 0.05% Tween 20, and 0.02% NaN3 in a combined leaf and bud press (Erich Pollahne, Wennigsen, Germany). For each sample, two wells—one for RTBV and one for RTSV—were used. ELISA reactions were evaluated by measuring absorbance at 405 nm using ELISA reader MR59O (Dynatech, Alexandria, VA). Rice leaf samples that gave absorbance values higher than three times the mean absorbance of healthy leaf samples were considered infected. Weed samples with absorbance values over 1.0 and higher than five times the mean absorbance of healthy leaf samples were considered infected.

Inoculation. Young adults of the four leafhopper species were given an acquisition access period (AAP) of 1 or 2 days on 2-mo-old TN1 plants infected with either RTBV and RTSV together or RTSV alone in a cage. Each of the leafhoppers was confined with a TN1 seedling in a test tube for 1 day. For serial daily inoculation, TN1 seedlings were replaced every day for 4 days. Inoculated seedlings were transplanted in pots and grown in an insect-proof cage. For inoculation of weeds, each potted weed seedling or rhizome was confined in a cage with 10 virus-exposed leafhoppers for 1 day. Uninoculated rice seedlings served as controls. Weed seedlings or rhizomes confined with virus-free leafhoppers also served as controls. Inoculated plants were tested for the presence of RTBV and RTSV by ELISA at 15 days after inoculation for rice and at 15, 30, and 45 days for weeds.

Virus recovery from infected weeds. Adults of the four leafhopper species were given a 1-day AAP on each weed plant infected with either RTBV and RTSV together or RTSV alone. Immediately after the AAP, five leafhoppers were given a 1-day inoculation access period (IAP) on a 7-day-old TN1 seedling in a test tube. For recovery of RTBV from weed plants infected only with RTBV, leafhoppers that had fed for 12 hr on RTSV-infected TN1 plants were given a 12-hr AAP on the RTBV-infected plants. Immediately after the second AAP, five leafhoppers were given a 1-day IAP on a 7-day-old TN1 seedling in a test tube. Inoculated seedlings were transplanted in pots and grown in cages. TN1 seedlings that were confined in test tubes with nonviruliferous leafhoppers served as controls. Seedlings were indexed by ELISA 1 mo after inoculation.

Relation of leafhoppers to plants. Adult longevity, population growth, nymph survival, and feeding behavior of the four leafhopper species were determined on rice and potential weed hosts. Adult longevity was determined by confining 10 adults in a cage with a potted 20-day-old seedling or young rhizome until all leafhoppers died. For population growth, one each of newly emerged male and female leafhoppers were confined in a cage with a potted 20-day-old seedling or young rhizome for 10 days. The number of nymphs hatched was counted. Nymph mortality was determined by confining 10 second- or third-instar nymphs in a cage with a 20-day-old seedling or young rhizome for 3 days, then counting the number of surviving leafhoppers. For adult longevity, population growth, and nymph mortality, four plants were tested for each combination of weed and leafhopper species.

Feeding behavior of leafhoppers was determined by measuring areas of acidic and basic honeydew spots on a filter paper disk treated with bromcresol green and placed at the base of a 20-day-old seedling or young rhizome after 1-day confinement of a leafhopper adult in a cage (20). Blue spots (basic honeydew) were assumed to indicate phloem feeding and orange or brown spots (acidic honeydew), to indicate xylem feeding (20). Honeydew excreted by each leafhopper was quantified by measuring the size of acidic and basic honeydew spots on the disk. For feeding behavior, eight plants were tested for each combination.

RESULTS

Transmission to rice. Adults of the four leafhopper species that had fed on source plants with both RTBV and RTSV transmitted the viruses either together or separately to TN1 seedlings (Fig. 1). They also transmitted RTSV after feeding on source plants infected only by RTSV (Fig. 2). *N. virescens* was the most efficient vector from either source. In the serial daily transfers, *N. virescens* lost infectivity for both RTBV and RTSV in 4 days (Figs. 1 and 2). *N. nigropictus* lost infectivity for RTBV and RTSV in 5 days, whereas *N. malayanus* and *R. dorsalis* lost infectivity in 1 day.

Virus transmission to weeds. Inoculated weeds were tested three times at 15, 30, and 45 days after inoculation for the presence of RTBV and RTSV. Plants that gave a positive ELISA even in one test were considered infected. Of 15 weed species inoculated with either a mixture of RTBV and RTSV or RTSV alone, eight were infected with RTBV and/or RTSV (Tables 1 and 2). Uninfected species were *Paspalum distichum, Ischaemum indicum, Cynodon dactylon, Rottboellia exaltata, Cyperus iria, C. difformis*, and *Monochoria vaginalis*. However, not all leafhoppers transmitted the viruses to all the susceptible species. ELISA values obtained from each infected plant at 15, 30, and 45 days after inoculation often varied remarkably. Often, plants were ELISA-positive in one or two tests but ELISA-negative in other tests. ELISA values of all infected weeds were lower than those of rice plants infected with the same virus (Table 3). With the exception of *Echinochloa colona*, none of the infected weed plants showed symptoms. *E. colona* plants infected with both RTBV and RTSV showed reduced growth and excessive tillering.

Virus recovery from infected weeds. *N. nigropictus* acquired RTBV and RTSV from doubly infected plants of *Echinochloa crusgalli, Panicum repens*,

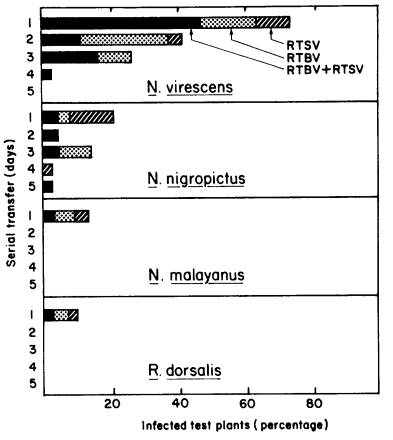

Fig. 1. Percentage of adult leafhoppers of *Nephotettix virescens, N. nigropictus, N. malayanus*, and *Recilia dorsalis* that transmitted rice tungro bacilliform virus (RTBV) or rice tungro spherical virus (RTSV), or both, in five daily serial transfers on seedlings of rice cultivar Taichung Native 1 (TN1) after a 1-day acquisition access period on TN1 plants infected with both viruses.

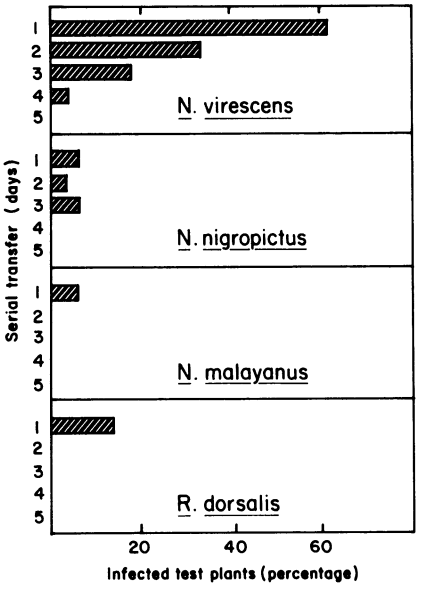

Fig. 2. Percentage of adult leafhoppers of *Nephotettix virescens, N. nigropictus, N. malayanus*, and *Recilia dorsalis* that transmitted rice tungro spherical virus (RTSV) in five daily serial transfers on seedlings of rice cultivar Taichung Native 1 (TN1) after a 1-day acquisition access feeding on RTSV-infected TN1 plants.

and *Cyperus rotundus*. RTBV and RTSV were transmitted from *E. crusgalli* to two of 21 TN1 seedlings tested. Both viruses were transmitted to only three of 26 seedlings when *C. rotundus* was the source plant. RTBV and RTSV were transmitted from *P. repens* to one TN1 seedling, and RTSV alone to two seedlings, of 16 tested. Leafhoppers other than *N. nigropictus* failed to acquire the viruses from all doubly infected weeds. All four leafhopper species failed to recover RTBV and RTSV from all weed species infected with either RTBV or RTSV alone.

Relation of leafhoppers to weeds and rice. Adult longevity, nymph survival, and population growth for *N. virescens* were high on TN1 rice but low on all weeds (Fig. 3). *N. nigropictus* had high adult longevity and population growth on rice, *E. crusgalli, E. glabrescens, E. colona*, and *Leersia hexandra*. Survival of *N. nigropictus* nymphs was high on these weeds. *N. malayanus* had high adult longevity, nymph survival, and population growth only on *L. hexandra*, and *R. dorsalis* had high adult longevity and population growth only on rice.

Feeding behavior of each leafhopper species differed among plant species tested (Fig. 3). *N. virescens* excreted mainly basic honeydew on TN1 rice, indicating higher phloem feeding, and mainly acidic honeydew on all weeds, indicating xylem feeding. *N. nigropictus* excreted both basic and acidic honeydew on rice, *E. crusgalli, E. glabrescens, E. colona, L. hexandra*, and *C. rotundus* but mainly acidic honeydew on other weed species. *N. malayanus* excreted both basic and acidic honeydew on *L. hexandra* but mainly acidic honeydew on other weeds and rice. *R. dorsalis* excreted mainly basic honeydew on rice but mainly acidic honeydew on weeds.

Table 1. Transmission of rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) to eight species of weeds by adults of *Nephotettix virescens, N. nigropictus, N. malayanus*, and *Recilia dorsalis*

Weeds / Vectors	Number of plants infected[a] RTBV + RTSV	RTBV	RTSV
Eleusine indica			
N. virescens	1	1	0
N. nigropictus	0	1	0
N. malayanus	0	2	0
R. dorsalis	0	0	0
Echinochloa crusgalli			
N. virescens	0	0	0
N. nigropictus	0	2	3
N. malayanus	0	0	0
R. dorsalis	0	0	0
E. glabrescens			
N. virescens	0	0	2
N. nigropictus	0	2	2
N. malayanus	7	0	3
R. dorsalis	0	1	0
E. colona			
N. virescens	0	0	0
N. nigropictus	0	3	1
N. malayanus	3	0	1
R. dorsalis	1	0	4
Leptochloa chinensis			
N. virescens	2	0	0
N. nigropictus	0	0	1
N. malayanus	1	0	1
R. dorsalis	0	1	0
Leersia hexandra			
N. virescens	0	0	1
N. nigropictus	0	0	0
N. malayanus	0	0	1
R. dorsalis	0	1	0
Panicum repens			
N. virescens	1	1	1
N. nigropictus	0	0	0
N. malayanus	4	0	4
R. dorsalis	0	0	0
Cyperus rotundus			
N. virescens	1	0	1
N. nigropictus	1	2	2
N. malayanus	0	0	0
R. dorsalis	0	0	0

[a]Twelve plants were separately exposed for 1 day to 10 leafhoppers that had fed on RTBV- and RTSV-infected rice plants.

Table 2. Transmission of rice tungro spherical virus (RTSV) to eight species of weeds by adults of *Nephotettix virescens, N. nigropictus, N. malayanus*, and *Recilia dorsalis*[a]

Weeds	*N. virescens*	*N. nigropictus*	*N. malayanus*	*R. dorsalis*
Eleusine indica	0	0	0	0
Echinochloa crusgalli	0	2	0	0
E. glabrescens	0	2	0	0
E. colona	0	2	0	0
Leptochloa chinensis	3	4	0	0
Leersia hexandra	0	0	0	0
Panicum repens	1	0	0	0
Cyperus rotundus	4	3	0	0

[a]Twelve plants were separately exposed to 10 leafhoppers that had fed on RTSV-infected rice plants.

DISCUSSION

In these experiments, *N. virescens, N. nigropictus, N. malayanus*, and *R. dorsalis* transmitted RTBV and RTSV from rice to rice and from rice to several weed species. With the exception of *E. colona* plants infected with both RTBV and RTSV, none of the weeds infected with the viruses, either together or separately, developed symptoms. Only *N. nigropictus* was able to transmit the viruses from doubly infected weeds to rice seedlings. In earlier investigations, many weeds, including wild rices, were tested for susceptibility to infection with "tungro virus" using *N. virescens* (1,3,11,15,17,21,25,26), and many of the weeds were reported to be susceptible to the agents. The tungro virus agents caused symptoms on some weeds in some studies but did not cause symptoms on the same weeds in other studies. Leafhopper recovery tests of the tungro viruses from infected weed plants also had conflicting results. Some tests indicated positive recovery from infected weeds but others indicated no recovery from the same weeds. In a recent trial (1), only one weed that was artificially infected with both RTBV and RTSV showed symptoms. *Monochoria vaginalis, Cynodon dactylon*, and *Cyperus difformis* were infected with either RTBV or RTSV in that trial (1) but not in the present trial. These conflicting results might be due to the strict threshold level for positive reaction in the present trial and, also, may indicate the presence of RTBV and/or RTSV isolates that cause different symptoms in infected weeds. The presence of symptomatic strains of "tungro virus" have been reported from India and also from the Philippines

(2,14,16). Which of the two tungro-associated viruses is the real cause of the differences in symptomatology is not known, however. The discrepancy in results could also be due to lack of a reliable diagnostic method in the earlier experiments. Precise studies based on a reliable indexing method are necessary to understand epidemiology of tungro.

In the present experiments, *N. nigropictus*, which transmitted RTBV and RTSV from rice to some weeds and from some weeds to rice, had higher adult longevity, nymph survival, and reproductive potential and greater phloem feeding on a number of the potential weed hosts for the viruses. Apparently, *N. nigropictus* is able to disperse RTBV and RTSV in weed hosts in idle fields once the viruses are introduced from a rice field. In the present experiments, *N. virescens* and *R. dorsalis* also showed their ability to serve as carriers of RTBV and RTSV and to introduce them into idle fields. As the transmission efficiency of *N. nigropictus* for RTBV and RTSV was low on the weed hosts, a high leafhopper population is required for the viruses to predominate in the environment. Similarly, *N. malayanus* is likely to have a potential to disperse RTBV and RTSV in *Leersia hexandra*. *N. nigropictus* populations are generally high in idle fields and *N. malayanus* populations are high in fields inhabited by *L. hexandra*.

The four leafhopper species transmitted RTBV and RTSV together or separately to rice after feeding on rice plants infected with both viruses. The four species also transmitted RTSV from RTSV source plants. In earlier reports from Indonesia (9) and Japan (7), *N. nigropictus* that had fed on doubly infected plants transmitted either RTBV or RTSV with very low efficiencies. These conflicting results can be explained by the presence of leafhopper populations whose transmission efficiencies of the viruses on rice differed, as indicated earlier (14,24). Transmission efficiency of rice waika virus, which is identical or closely related to RTSV (7,23), also differs according to the *N. cincticeps* colonies used (12).

Relative areas of acidic or basic honeydew spots excreted by leafhoppers and virus transmission efficiency of the leafhoppers on each host were not correlated among the four leafhopper species used in our experiments. The low percentage of infection with RTBV and/or RTSV on weeds is probably due to their resistance to infection, not to their resistance to leafhopper feeding.

Table 3. Absorbance at 405 nm in enzyme-linked immunosorbent assay of extracts of weeds and rice plants considered infected with rice tungro bacilliform virus (RTBV) and/or rice tungro spherical virus (RTSV) after inoculation by *Nephotettix virescens, N. nigropictus, N. malayanus,* or *Recilia dorsalis* exposed to both viruses or to RTSV alone

	Absorbance at 405 nm			
	RTBV		RTSV	
Plant species	Infected plants	Uninoculated plants	Infected plants	Uninoculated plants
Eleusine indica	0.10–0.18	0.01–0.02	0.13	0.01–0.02
Echinochloa crusgalli	0.16–1.05	0.00–0.01	0.10–0.23	0.00–0.01
E. glabrescens	0.11–0.50	0.01–0.02	0.11–0.45	0.01–0.02
E. colona	0.10–0.20	0.00–0.01	0.12–0.74	0.01–0.03
Leptochloa chinensis	0.11–0.20	0.00–0.03	0.14–0.20	0.00–0.02
Leersia hexandra	0.32	0.00–0.01	0.12–0.22	0.00–0.01
Panicum repens	0.10–0.34	0.01–0.03	0.10–1.31	0.01–0.03
Cyperus rotundus	0.15–0.95	0.01–0.05	0.15–1.18	0.02–0.05
Oryza sativa (cv. TN1)	0.70–1.50	0.00–0.01	0.50–1.00	0.00–0.01

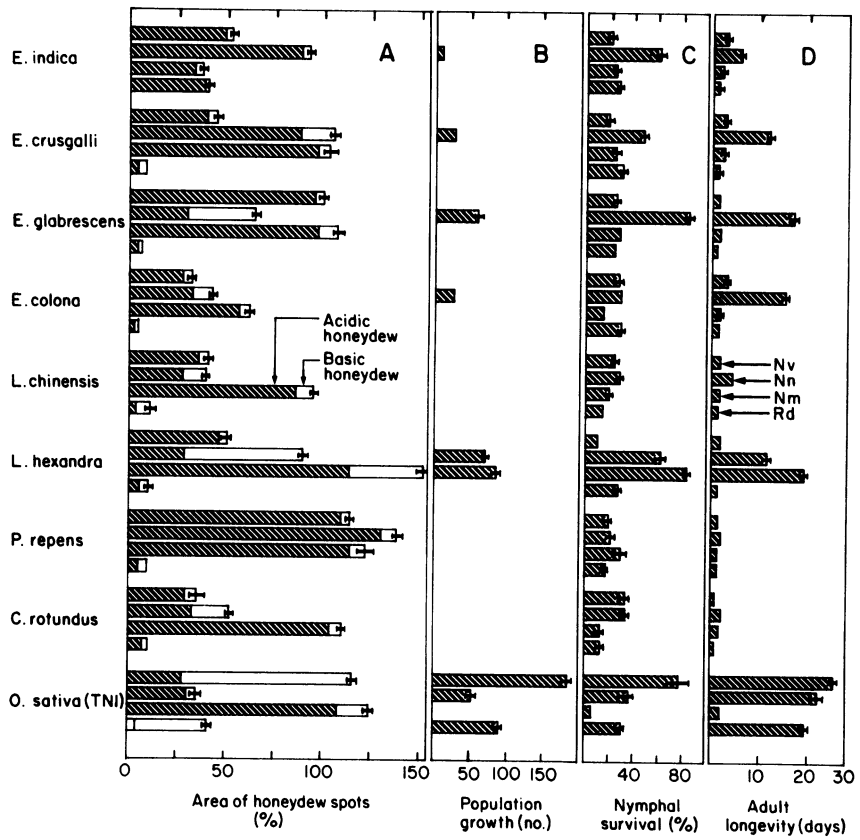

Fig. 3. Population characteristics and feeding behavior of *Nephotettix virescens* (Nv), *N. nigropictus* (Nn), *N. malayanus* (Nm), and *Recilia dorsalis* (Rd) on young seedlings or rhizomes of eight weeds (*Eleusine indica, Echinochloa crusgalli, E. glabrescens, E. colona, Leptochloa chinensis, Leersia hexandra, Panicum repens*, and *Cyperus rotundus*) and rice cultivar Taichung Native 1 (TN1). **(A)** Areas of basic and acidic honeydew spots secreted by single adult leafhoppers during 1 day of feeding on each seedling or cuttings, **(B)** population growth from one adult breeding pair after 10 days, **(C)** percentage of nymphs surviving after 3 days, and **(D)** adult longevity in days. Horizontal lines indicate standard deviation of total areas of basic and acidic honeydew spots, population growth, nymph survival, and adult longevity.

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