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Occurrence of a Picorna-like Virus in Planthopper Species and Its Transmission in *Laodelphax striatellus*

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Himetobi P virus (HiPV) was detected in three planthopper species from the temperate monsoon region of Japan. High incidences of infection occurred in some cultures of *Laodelphax striatellus* (range: 0–100%), *Nilaparvata lugens* (0–88%), and *Sogatella furcifera* (0–58%). In the field HiPV was only detected, at low incidence, in *L. striatellus* (0–6%). The virus was not detected in three other homopterans: *Nephotettix cincticeps, Rhopalosiphum maidis,* and *R. padi,* which feed on the Poaceae. High concentrations of HiPV were detected in adults and nymphs of *L. striatellus.* The virus was not detected in eggs and was not transovarially transmitted. Maternal contamination and contamination from surrounding plant tissues may be a source of inoculum for hatching *L. striatellus* eggs. Frass contained high concentrations of HiPV and is a likely source of inoculum. HiPV did not multiply in rice or wheat nor was it translocated from inoculated leaves to other parts of the plant. Therefore host plants form, at best, a transient reservoir of inoculum. HiPV transmission is compared with that of rice stripe tenuivirus which infects both *L. striatellus* and its plant hosts rice and wheat.

KEY WORDS: ***Laodelphax striatellus; Nilaparvata lugens; Sogatella furcifera;*** **himetobi P virus; insect virus incidence; insect virus transmission; rice stripe tenuivirus.**

INTRODUCTION

An isometric virus was detected while attempting to purify rice stripe tenuivirus (RSV) from its planthopper vector: *Laodelphax striatellus.* An unusual feature of the virus is the presence of a genome length ds-RNA within the virions; otherwise it resembles insect picornaviruses. The virus has been named himetobi P virus (HiPV: Toriyama *et al.,* 1990) after the Japanese name for *L. striatellus.*

Other small RNA viruses have been purified from homopterans which feed on the Poaceae. Two of these, which were also isolated while studying plant virus–vector relationships, have been characterized. Aphid lethal paralysis virus (ALPV) has affinities with the picornaviruses and is transovarially transmitted (Hatfill *et al.,* 1990) in its aphid host *Rhopalosiphum padi.* The virions of *R. padi* α-like virus (RhPV) are only observed in the midgut and hindgut cells of infected aphids (Gildow and D'Arcy, 1990), and therefore, RhPV is not transovarially transmitted. Virus-free *R. padi* readily become infected when contaminated with these two viruses. An unusual feature of RhPV is its ability to use plants as passive reservoirs. Virus-free aphids become infected when fed on plants previously fed on by RhPV-infected aphids. However the virus does not multiply in plant tissue (Gildow and D'Arcy, 1990). The present study aims at determining the incidence of HiPV in Japanese homoptera and its transmission properties in *L. striatellus.*

MATERIALS AND METHODS

Insect Cultures

Insect colonies at Tsukuba were maintained on rice or wheat in growth cabinets kept on a 16-hr-day cycle at a constant temperature of 26°C. Colonies at other locations (Table 1) were maintained under similar conditions. The colonies were established from locally collected insects.

Field Collections

The plant- and leafhoppers were collected from fields at dusk using a sweep net. The aphids *Rhopalosiphum maidis* and *R. padi* were collected directly from cereals.

Sample Preparation

Individual and/or groups of eggs, nymphs, exoskeletons, and adult planthoppers were ground in 10 μl of PBS–Tween (140 mM NaCl, 1.4 mM KH_2PO_4, 8 mM Na_2HPO_4, 2.6 mM KCl, 3 mM Na_3N, 0.05% Tween 20, pH 7.4) containing 2% PVP and 0.2% bovine serum albumin at the bottom of microcentrifuge tubes. These extracts were diluted in 100 μl of buffer for testing by

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HiPV ELISA or in 200 μl for testing by both HiPV and RSV ELISA. Diluted extracts were centrifuged at 15,000 rpm/2 min immediately before use.

Frass was collected from disposable centrifuge tubes in which groups of planthoppers had been caged for 16 hr. Frass droplets were centrifuged to the bottom of the tubes after carefully shaking 100 μl of buffer around the walls of each tube. Control tubes contained 50 dead planthoppers and were treated as above to check for surface contaminating virus.

To study the presence of virus in plant tissues, wheat and rice plants were washed thoroughly to remove cadavers, frass and exoskeletons before grinding in buffer.

Enzyme-Linked Immunosorbent Assays (ELISA)

A New Zealand white rabbit was given a series of injections containing purified HiPV. IgG was purified from a serum sample (reciprocal titer: 4096) collected 12 days after the third injection. An aliquot was conjugated with alkaline phosphatase according to the method of Clark and Adams (1977). IgG from a rice stripe virus antiserum (reciprocal titer:1024) was purified and conjugated in the same way.

An HiPV dilution series of 100, 50, 10, and 1 ng/ml of purified virus was included as a control for each set of HiPV tests. Healthy and RSV-T (Toriyama and Watanabe, 1989) -infected leaf tissue were used as controls in the RSV tests.

The ELISA methods were essentially those for the double antibody sandwich technique described by Clark and Adams (1977) with the following variations. Working volume for each step was 100 μl in Nunc Maxisorb plates. HiPV and RSV plates were coated with 0.5 and 1 μg/ml IgG, respectively, while both conjugates were added to wells at 1/1000 dilution and incubated at 4°C for 16 hr. Insect and plant extracts were incubated in coated wells at 30°C for 4 hr.

The A_{405} was read after incubating the substrate, *p*-nitrophenol phosphate (0.5 mg/ml) in the wells for 60 min. Reactions having an absorbance value greater than twice the maximum negative control value were regarded as positive.

Surface Sterilization of Eggs

Groups of four eggs were excised from leaves, washed in 10-μl drops of 2.5% sodium hypochlorite for 3 min and then rinsed three times in distilled water before being hatched on young rice seedlings in water agar petri dishes or test tubes.

RESULTS

The HiPV ELISA had a detection limit of 10 ng/ml. Freezing purified virus raised the detection limit to 300 ng/ml. HiPV could readily be detected in insect extracts which showed no decline in detection limit (1/300) after 10 days at 4°C.

The virus was detected in cultures of the three planthopper species (*L. striatellus, Nilaparvata lugens, Sogatella furcifera*) but not in the small numbers of field-collected specimens of the latter two species. HiPV was detected at two of four field sites and infected 4% (range: 0–6%) of *L. striatellus* (Table 1). In contrast, the incidence of HiPV in infected *L. striatellus* cultures (3/5) was high (79, 100, 100%); infected *N. lugens* (7/10) and *S. furcifera* (1/3) cultures had incidences of 88% and 58%, respectively. Detection in most of the planthopper cultures tested suggests that the virus is wide-

TABLE 1
Homoptera Tested for the Presence of Himetobi P Virus

Species	Source	Numbers tested	infected
Laodelphax striatellus	Culture collections		
	Kyoto	67	0
	Niigata	12	12
	Shiraoka	20	0
	Tsukuba	73	73
	Yokohama	29	23
	Field collections		
	Kohnosu, rice	65	4
	Nagoya, rice	14	0
	Niigata, rice	7	0
	Tsukuba, rice	124	4
Nephotettix cincticeps	Culture collections		
	Oomagari	16	0
	Tsukuba	10	0
	Yokohama	20	0
	Field collections		
	Kohnosu, rice	33	0
	Niigata, rice	12	0
	Tsukuba, rice	4	0
Nilaparvata lugens	Culture collections		
	Kyoto	69	55
	Oomagari	25	22
	Saga No. 1	18	3
	No. 2	19	0
	No. 3	20	0
	Shiraoka	20	0
	Tsukuba No. 1	30	1
	No. 2	45	1
	No. 3	24	13
	Yokohama	34	2
	Field collections		
	Kohnosu, rice	9	0
	Tsukuba, rice	21	0
Rhopalosiphum maidis	Tsukuba barley fields	70	0
Rhopalosiphum padi	Tsukuba wheat fields	64	0
Sogatella furcifera	Culture collections		
	Oomagari	20	0
	Shiraoka	20	0
	Yokohama	19	11
	Field collections		
	Kohnosu, rice	59	0
	Nagoya, rice	9	0
	Tsukuba, rice	19	0
	Niigata, rice	9	0

spread in the temperate monsoon region of Japan. The virus was not detected in field or culture collections of *N. cincticeps, R. maidis, or R. padi.*

All tested adult planthoppers from the main (Tsukuba) culture were infected with HiPV and 94% of adults became infected with RSV during acquisition feeds; there were no differences between the incidences of either virus in males or females (Table 2).

HiPV could not be detected in single or batches of 10–40 eggs, whereas transovarially transmitted RSV was detected in 50% of single eggs from infected females. Only 36% of nymphs became infected when eggs were dissected out of leaves and transferred to seedlings not previously contaminated with planthoppers. When eggs were surface sterilized before transfer the resulting nymphs remained uninfected (Table 3).

HiPV was detected in frass from groups of 50 planthoppers. Although only ca. 1 μl of frass was washed from each tube high concentrations ($A_{405} > 2.000$), comparable to adult extracts ($A_{405} > 1.300$), were detected. HiPV was not detected in exoskeleton extracts ($A_{405} < 0.018$) or in washings from the control tubes which contained dead planthoppers for 16 hr ($A_{405} < 0.038$).

After 2 day RSV-transmission feeds both HiPV and RSV were detected in 1/6 rice seedlings. However 10 days later higher concentrations of RSV were detected in all seedlings (6/6) but HiPV was not detected (Table 4). When the tests were repeated in wheat and inoculated leaves were separated from leaves which expanded after the transmission feeds, similar concentrations of RSV were detected in both, whereas HiPV was only detected in inoculated leaves (Table 4). The limited distribution and declining concentration of HiPV (in contrast to RSV) suggests that it does not multiply in rice or wheat.

DISCUSSION

The width of insect picornavirus host ranges is variable. Bee picornaviruses have very narrow host ranges and only acute bee paralysis virus's host range extends beyond the honey bee to the bumble bee (Bailey, 1976). In contrast, cricket paralysis virus (CrPV) infects species of Diptera, Heteroptera, Lepidoptera, and Orthoptera (Scotti *et al.*, 1981). Between these two extremes, we found that HiPV infected the three planthopper species but not the leafhopper *N. cincticeps* kept in contaminated facilities, nor the aphid *R. padi* when it was exposed to the virus in mixed culture with *L. striatellus*. Further field collections of *N. lugens* and *S. furcifera* will be necessary to see if these species actually become infected under field conditions or whether they only become infected in overcrowded cultures in close proximity to infected *L. striatellus* cultures.

The occurrence of HiPV in 61% of planthopper cultures was high. Scotti *et al.* (1981) reported that in a survey of *Drosophila* cultures from nine countries, 47% were infected with small isometric viruses but that only 17% were infected with *Drosphila* C picornavirus (DCV). D'Arcy *et al.* (1981) found that RhPV occurred in 25% of North American *Rhopalosiphum* and *Schiza-*

TABLE 2

Infection of Adult *Laodelphax striatellus* and Lack of Infection of *Rhopalosiphum padi,* with Himetobi P (HiPV) and Rice Stripe (RSV) Viruses

	No. tested	No. infected	
		HiPV	RSV
L. striatellus			
Female	17	17	0[a]
	19	19	18
Male	9	9	0[a]
	17	17	16
R. padi[b]	50	0	0

[a] Controls, fed on seedlings not infected with RSV.
[b] *R. padi* fed among *L. striatellus* on RSV-infected plants.

TABLE 3

Detection of HiPV in Nymphs of *L. striatellus* Grown from Different Egg Treatments

Source of nymphs	HiPV	
	No. tested	No. infected
Main culture	35	29
Eggs removed to uncontaminated seedlings[a]	25	9
Surface sterilized eggs removed to uncontaminated seedlings	31	0

[a] Rice seedlings raised in an insect-free glasshouse.

TABLE 4

Detection of Himetobi P Virus (HiPV) and Rice Stripe Virus (RSV) in Rice and Wheat Seedlings

	HiPV[a]	RSV[a]
Control rice	0.082[b]	0.014[b]
End of 2 day feed	(5) 0.097 ± 0.024	(5) 0.005 ± 0.004
	(1)[c] 0.444 ± 0.023	(1)[c] 0.177 ± 0.019
10 days after hoppers removed	0.101 ± 0.018	0.430 ± 0.169
Control wheat	0.017[b]	0.009[b]
End of 2 day feed	0.129 ± 0.007	0.004 ± 0.003
10 days after hoppers removed: inoculated leaves	0.100 ± 0.003	0.560 ± 0.034
Leaves expanded after hoppers removed	0.012 ± 0.006	0.585 ± 0.024

[a] Four samples from each of six replicate plants were used for each treatment—A_{405} ± SE.
[b] Maximum control value; treatment values exceeding twice this value were regarded as positive (see Materials and Methods).
[c] Not the same plant.

phis cultures. Williamson *et al.* (1988) found that 23% and 78% of *R. padi* in their cultures were infected with ALPV and RhPV, respectively.

The field occurrence of small insect viruses is quite variable. Allen and Ball (1990) did not detect *Sitobion avenae* virus (SAV) among 23 samples from 12 sites in southeast England while Reinganum *et al.* (1981) found CrPV at 43% of 232 sites during a survey of cricket diseases in the Western District of Victoria, Australia. Like the present study, the latter of these two studies concluded that virus incidence at each of these sites was low.

HiPV was not transovarially transmitted (in contrast to RSV) through *L. striatellus*. Other insect picornaviruses vary in their transovarial transmissibility: CrPV and DCV are not while ALPV (Hatfill *et al.*, 1990) and *Drosophila* P,A and iota viruses are transovarially transmitted through their hosts (Scotti *et al.*, 1981).

Maternal contamination of the egg surface, as described by Reinganum *et al.* (1970) for CrPV and DCV, accounted for 36% of nymphs becoming infected (Table 3). Another potential source of HiPV infection is from the plant host. Host plants are sources of inoculum for a number of insect viruses. Ofori and Francki (1985) reported that maize was a source of infection of leafhopper A reovirus and Gildow and D'Arcy (1988) found that RhPV infected *R. padi* when ingested from barley or oats. Leaves fed on by planthoppers may be transient reservoirs of HiPV. However, in view of the low concentrations and limited distribution, this source is probably not as important as contamination with inoculum from the outside of the leaf surface.

The high concentration of virus detected in frass makes it a potential source of inoculum. Microscopic droplets were observed sticking to leaves and the bodies of planthoppers (and *R. padi*). Allen and Ball (1990) found that SAV could be detected in honeydew from infected aphids and that healthy *S. avenae* were infected when they came in contact with the contaminated honeydew. It has not been reported whether honeydew is a source of inoculum for ALPV or RhPV but Williamson *et al.* (1988) report that *R. padi* was infected when exposed to aphid extracts on the leaf surface.

All the planthopper hosts of HiPV are important pests of cereals and vectors of plant viruses (Greber, 1984). *L. striatellus* is a vector of rice stripe, rice black streak dwarf and northern cereal mosaic viruses. *N. lugens* transmits rice ragged stunt and rice grassy stunt viruses while *S. furcifera* is the vector of pangola stunt virus. All of these viruses multiply in their planthopper vector and therefore it would be useful to know whether HiPV affects the replication and transmission of any of these economically important viruses.

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