Genetic Determination of Replication of Rice Hoja Blanca Virus Within Its Planthopper Vector, *Sogatodes oryzicola*

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ABSTRACT

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The inheritance of the ability of *Sogatodes oryzicola* to support replication of the rice hoja blanca virus (RHBV) was studied by following the segregation of progeny of crosses between insects of known pedigree and known ability to support virus replication and transmission. Virus transmission to plants required at least 20–25 days postacquisition incubation of RHBV in the insect. Enzyme-linked immunosorbent assay (ELISA) was developed to detect RHBV in vectors incubating, but not yet transmitting, the virus. A postacquisition incubation period in the insect of 12 days was required before the virus could be detected by ELISA ($ELISA^+$), indicating that the virus replicates within the vector. Potential vectors were insects that did not transmit RHBV after feeding acquisition, not yet completing the incubation period, and were differentiated from nonvectors as being capable of supporting RHBV replication ($ELISA^+$). Nonvectors were distinguished from vectors and potential vectors by their inability to transmit the virus to healthy plants and by their negative ELISA ($ELISA^-$) values following acquisition feeding and a 14-day incubation. Progenies of nonvector parents from lineages including at least one vector were allowed to acquire RHBV and then were assayed for postacquisition increase in virus titer. The progeny segregated in a manner consistent with a single recessive gene controlling planthopper ability to support virus replication (1:3, $ELISA^+$:$ELISA^-$). The $ELISA^+$ progeny could transmit RHBV after a normal incubation period. $ELISA^+ \times ELISA^+$ crosses of progeny from these crosses yielded all $ELISA^+$ progeny. Progeny of crosses of combinations of $ELISA^+$ and $ELISA^-$ segregated 1:1 or 0:1 ($ELISA^+$:$ELISA^-$). There was no evidence for sex linkage or determination of the ability to support RHBV replication; however, a strong maternal influence on progeny transmission ability was detected. Active female vectors transmitted RHBV transovarially to their progeny, regardless of the male parent and progeny genotype, and these could transmit the virus to plants. In progeny receiving the virus maternally, virus titers, as determined by ELISA, were lower and more variable in insects with a $ELISA^-$ male parent than in insects with two $ELISA^+$ parents. Individuals with an $ELISA^-$ male parent, and that had acquired the virus from the female parent, could lose the ability to transmit it to plants. It is concluded that the identified recessive gene controls the ability to support virus replication but not transmission ability per se. The implications of these findings are discussed in relation to the epidemiology of RHBV.

Rice hoja blanca (white leaf) virus (RHBV) transmitted by the planthopper *Sogatodes oryzicola* Muir has caused large yield losses in several rice (*Oryza sativa* L.) producing regions of Latin America. Casual observation of the periods between epidemics suggests that they follow a cyclical pattern (3). After two separate epidemics in the 1950s and 1960s (6,8), the disease virtually disappeared from most of this region for nearly 15 yr. In 1981, serious, widespread outbreaks recurred in Colombia, Ecuador, and Venezuela, causing losses of up to 100% (13,20). In some Andean valleys where rice is produced, shorter, cyclical fluctuations of RHBV severity occurred during the period when overall incidence was low.

The Rice Program at the Centro Internacional de Agricultura Tropical (CIAT) is developing RHBV-resistant lines destined for tropical America. Because even under natural epidemic conditions the proportion of vectors is low (<10%) (10,12,16), screening the thousands of lines per year required for this project demands that vector populations of *S. oryzicola* be mass reared (12). However, it has proved difficult to develop stable vector colonies. Typically, colonies initiated with vector adults decline to 10–20% vectors in only a few generations (12,14). To maintain a high proportion of vectors in a colony, a time-consuming, continuous crossing scheme must be maintained (5,12), complicating the creation of very large colonies.

RHBV is a member of the tenuivirus (rice stripe virus [RSV]) group and is related to RSV and maize stripe virus (MStpV) (9,17). Like other members of this group, it is probably propagative within its vector, *S. oryzicola*. Several characteristics of the relationship between RHBV and *S. oryzicola* resemble those for RSV (22) and support this assumption. The incubation period in the vector is relatively long after acquisition via feeding, and nymphs can acquire the virus maternally, via the egg, and can transmit virus shortly after hatching. Several successive generations of insects may receive the virus only through transovarian transmission, which may occur in nearly 100% of the progeny. Insects typically transmit the virus for their entire lives whether they acquire the virus maternally or by feeding

(5,7,10). The virus also has a deleterious effect on fecundity, nymph viability, and longevity, particularly when the virus is acquired transovarially (14,21). Virus particles are found within insect cells (19).

Within a given population of *S. oryzicola*, three types of insects, in terms of vectoring ability, may exist: vectors, which transmit the virus to plants; nontransmitting, potential vectors, which, after virus acquisition and incubation, eventually can transmit the virus to healthy plants or to progeny via the egg; and nonvectors, which cannot transmit the virus to a plant, even after acquisition feeding and a sufficient period for incubation. For rice dwarf virus, Fukushi (4) introduced the concept of genetically determined potential vectors to explain how some nontransmitting progeny from crosses between nonviruliferous females and male vectors could acquire rice dwarf virus from plants and transmit it, whereas others could not. That is, only some progeny (potential vectors) are capable of acquiring the virus, supporting replication, and eventually transmitting, and this capability was postulated to be under genetic control. Kisimoto (15) presented evidence to support genetic determination of transmission of RSV by its planthopper vector.

If vectoring of RHBV by *S. oryzicola* is genetically determined, selective breeding of the planthopper might permit the creation of stable vector colonies. However, no direct information is available regarding the inheritance of RHBV vectoring ability in *S. oryzicola*, primarily because of methodological problems in accurately characterizing the parents and progeny of crosses. The principal limitation in determining the inheritance pattern of RHBV acquisition and transmission ability is the long incubation period (20–25 days) in the insect after acquisition feeding. Because controlled acquisition feeding cannot be initiated conveniently before the second instar, the time required for completion of the incubation period may exceed normal insect life span, making it impossible to distinguish potential vector parents from nonvector parents using standard transmission tests. Similarly, potential vector progeny cannot be distinguished from nonvector progeny of crosses where the female parent was nonviruliferous. However, assuming that all tenuiviruses multiply within their planthopper vectors (18), pretransmission titers of RHBV could be detected by serology, as has been done for MStpV (18), to distinguish potential vectors from nonvectors.

In this paper, we report on the use of enzyme-linked immunosorbent assay (ELISA) to detect RHBV in potential vectors before completion of the incubation period. This method is used to study the manner in which the RHBV acquisition and vectoring ability of *S. oryzicola* is inherited, permitting a more precise methodology for creating highly active, stable vector colonies. Furthermore, an understanding of factors controlling levels of vectors and potential vectors in wild populations suggests explanations for the apparently cyclical nature of RHBV epidemics.

MATERIALS AND METHODS

Insect colonies. Individuals of *S. oryzicola* used to initiate this study were taken from a colony of approximately 30% vectors, maintained at CIAT. During the crossing and progeny evaluation procedure, individual insects were maintained on 10- to 25-day-old seedlings of the RHBV-susceptible rice cultivar Bluebonnet 50, inside butyl acetate tubes covered with fine nylon mesh (5,12).

Enzyme-linked immunosorbent assay. Antiserum (17) was used to implement the ELISA as described by Clark and Adams (2) with minor modifications. The immunoglobulin (IgG) fraction was precipitated with saturated ammonium sulfate and purified by passage through a DEAE-Sephacel column (Pharmacia, Uppsala, Sweden). Polystyrene Microelisa plates (Dynatech Corp., Alexandria, VA) were coated with IgG (1 mg/ml) in sodium carbonate buffer, pH 9.6, at 1 μg/ml. After an incubation period of 3–4 hr at 37 C, the test samples were added to the plates which were incubated overnight at 5 C. Alkaline phosphatase-labeled IgG conjugate was added to the wells, and the plates were incubated 3–5 hr at 37 C. Finally, 200 μl of substrate was added to each well at a concentration of 0.6 mg/ml in 10% diethanolamine buffer, pH 9.8, containing 0.02 M sodium azide. The reaction was stopped within 30 min by adding 50 μl of 3 M NaOH to each well. The resulting color reactions were assessed visually and spectrophotometrically (405 nm) by diluting each sample with distilled water to 1 ml. For the spectrophotometric analysis, values higher than the negative control plus three standard deviations of the mean were considered positive.

The planthoppers were homogenized for serological assay in 0.02 M phosphate-buffered saline containing 0.05 M Tween 20 and 2% polyvinyl pyrrolidone at the rate of 200 μl per sample (2). Insects not assayed immediately were stored dry at −10 C.

To establish that ELISA detects the virus in confirmed vectors, 300 planthoppers that were shown individually to have transmitted the virus to healthy plants were removed alive from rearing tubes and assayed individually. Another 50 confirmed vectors were assayed individually 24–48 hr after they died on the test plants to determine the effect of early insect decomposition on the ELISA values. Considering the known sensitivity of the ELISA test, vectors and presumed potential vectors allowed access to RHBV-infected plants were transferred onto virus-free rice plants for 2–5 days before the serological tests to "purge" the alimentary canal of ingested virus particles.

All insects were transferred live from plants to a freezer for dry storage at −10 C before ELISA for the remainder of the study. Hereafter, insects that had transmitted the virus will be referred to as vectors. Insects that did not transmit the virus to plants immediately after virus acquisiton feeding but did so after 20–25 days postacquisition incubation are considered to have been potential vectors. Insects that did not transmit the virus (that is, had not acquired the virus transovarially) but, after feeding acquisition and 12 days incubation, showed positive values in ELISA are referred to as ELISA$^+$. Those that did not transmit the virus and gave negative values in ELISA after acquisition feeding and 12 days postacquisition incubation (ELISA$^-$) are referred to as nonvectors.

Detecting increasing virus titer. The experiment schematically presented in Figure 1 was conducted to determine whether an increase in virus titer could be detected using ELISA before incubation was completed in the insect. Insects that did not transmit RHBV to the first set of test plants but did so before dying were considered to have been potential vectors. The percentage of these in the colony provided an estimate of the true potential vector composition of the colony. This could be compared with the estimate of potential vectors obtained by assuming that nontransmitting insects with positive ELISA values were potential vectors. To determine the minimum virus incubation period needed to detect potential vectors by ELISA, nymphs were permitted to acquire the virus and then were assayed after increasing incubation periods (Fig. 1). Insects that transmitted to Bluebonnet 50 plants during the first 10 days after removal from the colony probably had acquired the virus maternally and were not included in the analyses. The experiment was repeated three times. This procedure also was used to compare the reliability of ELISA and the transmission test in estimating the proportion of potential vectors in a given colony.

Crosses. The general scheme followed for crossing *S. oryzicola* and evaluating the progeny is presented in Figure 2. Several generations of controlled crosses were conducted to establish pedigree data and to avoid inbreeding effects (15), which were found to be severe even in the first full-sibling crosses. Crosses were made among progeny of various lineages that contained at least one proven vector in three generations and in all combinations of sex, vector, ELISA$^+$, and nonvector. Nonvector lines were obtained after several generations of selective nonvector × nonvector crosses as determined by ELISA values in progeny tests. In programming crosses to follow inheritance of virus acquisition, it was impossible to distinguish ELISA$^+$ from nonvector parents at the time the crosses were made. All nymphs (second or third instar) were fed first on healthy Bluebonnet 50 to separate those that transmitted and, therefore, had acquired the virus maternally from those that did not transmit initially

but did so later, having acquired the virus by feeding. After acquisition feeding, some progeny of nonvector × nonvector crosses were fed on healthy plants for up to 30 days to determine whether they could transmit the virus after this incubation period. This served as a check to confirm that some insects from nonvector × nonvector crosses, from which were obtained ELISA$^+$ individuals, could indeed transmit the virus.

Maternal effects. Experiments were conducted to compare the persistence of maternally acquired virus in the progeny of vector × nonvector and vector × vector crosses. Second instar nymphs from such crosses were placed individually on healthy rice plants. After 5 days, they were transferred to a second healthy plant, and after another 5 days, they were transferred to a third healthy plant. Five days later, the insects were tested by ELISA. Any virus in insects transmitting in the first two transfers must have come from the maternal parent because any virus acquired during

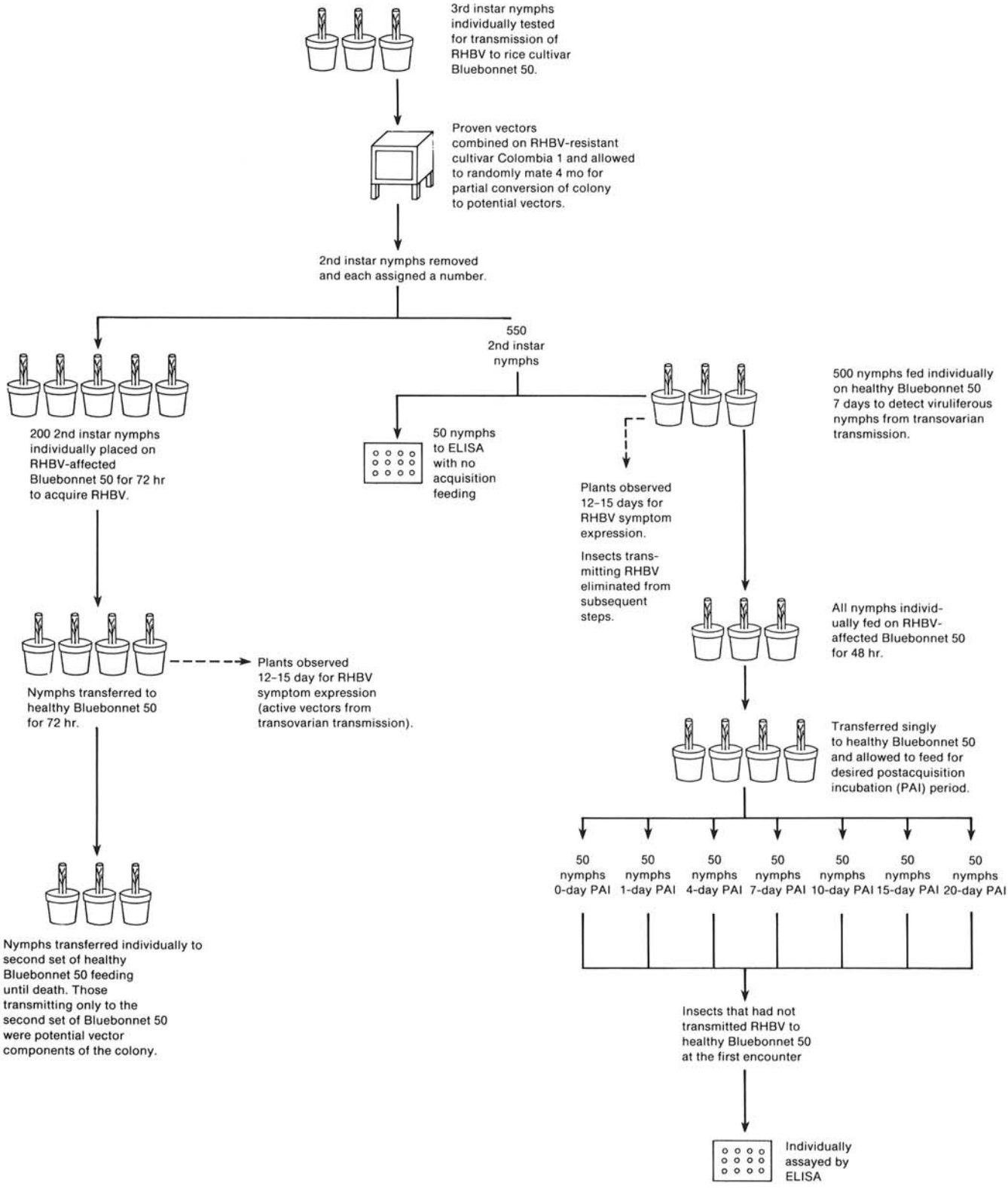

Fig. 1. Procedure followed to determine whether an increase in virus titre in insects could be detected using enzyme-linked immunosorbent assay (ELISA) before insects became vectors. RHBV = rice hoja blanca virus.

the first feeding after hatching could not have completed its incubation. Segregation patterns (ELISA$^+$:nonvector) were compared among progeny from parents with and without maternally acquired virus.

RESULTS

Enzyme-linked immunosorbent assay. Purified RHBV serially diluted with buffer solution was detected by ELISA at

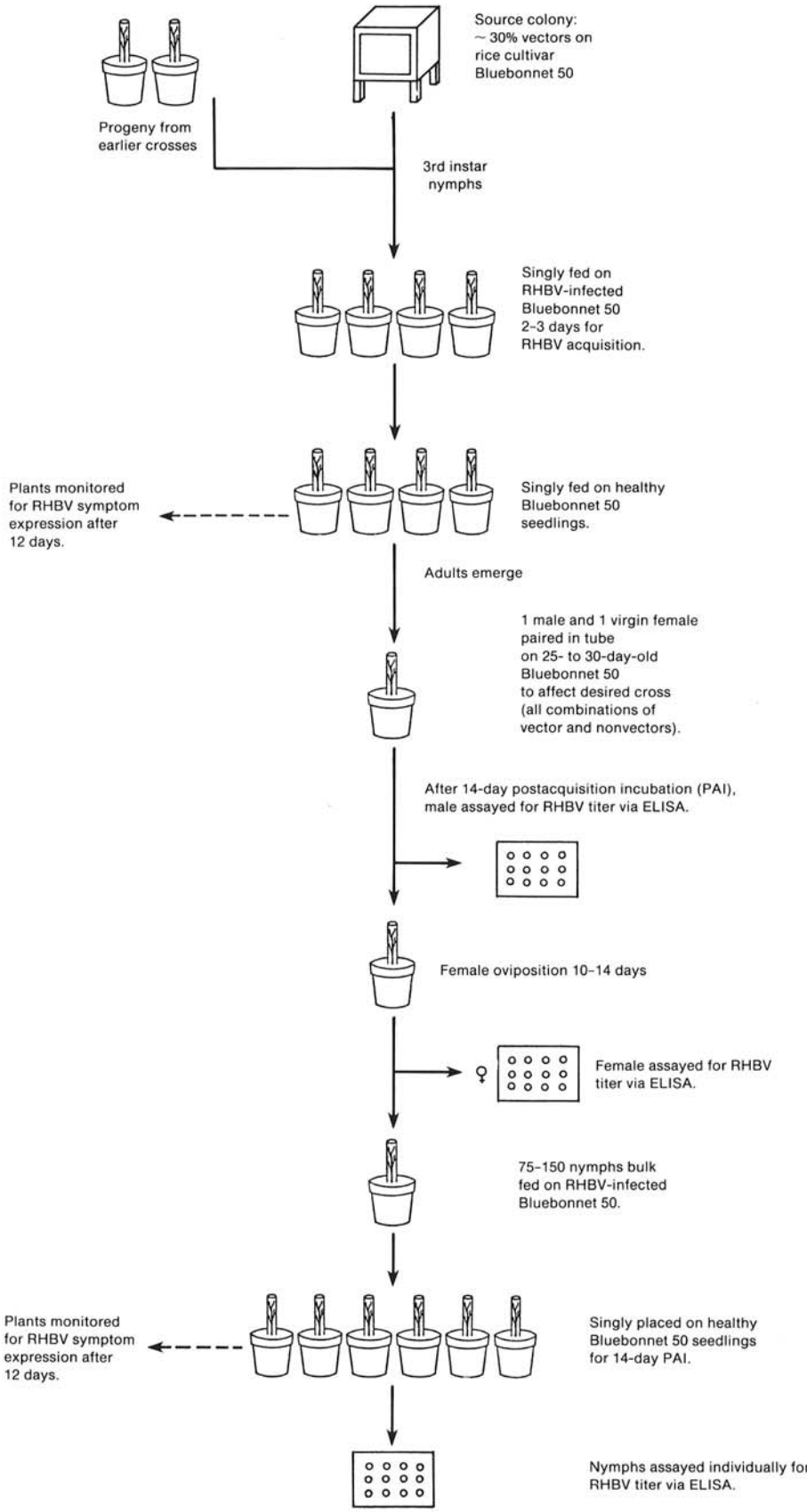

Fig. 2. General crossing and evaluation scheme followed to establish the inheritance pattern of the ability of *Sogatodes oryzicola* to acquire and support rice hoja blanca virus (RHBV) replication, as determined by a positive enzyme-linked immunosorbent assay (ELISA) reaction.

concentrations of approximately 0.1 μg/ml. Assuming a similar level of sensitivity for the ELISA using extracts from viruliferous planthoppers of *S. oryzicola*, 10–50 μg of viral antigen was detected in individual insects. Of the 300 insect vectors removed alive from their tubes, 97.3% were ELISA$^+$. Of the 50 vectors removed dead, only 56% were ELISA$^+$. Nontransmitting insects either with no acquisition feeding or tested immediately after acquisition feeding assayed as ELISA$^-$. No difference in ELISA values was detected in nontransmitting insects assayed immediately after acquisition feeding or after purge feeding.

Detecting increasing virus titer. The results of the experiment using ELISA to detect nontransmitting insects supporting virus replication are shown in Table 1. After an incubation period of up to 9 days, there was no significant increase in the numbers of ELISA$^+$ insects. The few insects that were ELISA$^+$ upon removal from the colony but that did not transmit will be referred to as "nontransmitting hosts." After an incubation period of 12 days, the proportion of viruliferous insects increased to about 24%, none of which had transmitted the virus previously. None of the insects allowed to incubate the virus for 20 days before ELISA transmitted RHBV as expected, given the reported incubation periods. The proportion of insects with detectable virus did not change after a 12-day incubation period, and this proportion was very close to the estimate of 26% potential vectors obtained by the direct transmission test, indicating that potential vectors in the colony sample were being detected. Thus, ELISA detects RHBV in nontransmitting, presumably incubating, potential vectors, and not all nontransmitting insects show detectable virus titer after acquisition feeding. Only nontransmitting insects that are also ELISA$^-$ 12 days after virus acquisition will be referred to as nonvectors.

TABLE 1. Influence of incubation period of rice hoja blanca virus (RHBV) in *Sogatodes oryzicola* on enzyme-linked immunosorbent assay (ELISA) values used to estimate the proportion of potential vectors within a colony

Days post access to RHBV-infected plants	Number of insects tested[w]	Percent insects positive by ELISA[x]
No acquisition feeding	50	4.0 a
0	91	4.4 a
1	137	4.4 a
4	135	5.9 a
7	131	11.4 ab
10	130	24.6 b
15	112	24.1 b
20	116	24.5 b
Control[y]	150	...[z]

[w]Includes only nontransmitting insects from a colony with high transmission potential. Sum of three replications.
[x]Values followed by same letter do not differ significantly at $P = 0.05$.
[y]Control = nonvectors taken from the colony, allowed to acquire RHBV by feeding for 24–48 hr, then fed individually on susceptible cultivar Bluebonnet 50 until death. 26% transmitted RHBV cultivar.
[z]Not done.

Crosses. Parents and progeny from crosses between nontransmitting insects were characterized by ELISA and transmission assays. Nonvector × nonvector crosses where pedigrees of each parent included at least one proven vector in the previous three generations consistently yielded ELISA$^+$ progeny. These segregated in 1:3 and 0:1 ELISA$^+$:nonvector ratios (Table 2). Some of the progeny of the nonvector × nonvector crosses that were permitted to feed for 30 days did transmit the virus. All such insects were ELISA$^+$.

Progeny from the segregating crosses in Table 2 were crossed as shown in Table 3. Nonvector × nonvector crosses segregated consistent with either 1:3 or 0:1 (ELISA$^+$:nonvector) ratios. Crosses between ELISA$^+$ and nonvector segregated consistent with 1:1 or 0:1 (ELISA$^+$:nonvector) ratios. Segregation of crosses between ELISA$^+$ progeny from nonvector parents was consistent with a 1:0 (ELISA$^+$:nonvector) ratio.

Maternal effects. The results of the experiment to assess the persistence of maternally acquired virus are presented in Table 4. Only 3% of nymphs from ELISA$^+$ × ELISA$^+$ crosses (parents descended from a nonvector × nonvector cross) lost the ability to transmit the virus, and all retained a detectable virus titer 20 days postemergence. In the progeny from crosses between an ELISA$^+$ vector female, with both parents ELISA$^+$, and a nonvector male, transmission ability dropped 16%, and the virus titer in some insects (6%) declined to below detectable levels.

ELISA values of female progeny from ELISA$^+$ × ELISA$^+$ crosses and from crosses between transmitting insects that each had one nonvector parent were different. Only 2% of the progeny of the first type of cross were nonvector and more than 80%

TABLE 2. Segregation of enzyme-linked immunosorbent assay (ELISA) positive and nonvector progeny from nonvector × nonvector crosses

Parents[a]		Crosses	Progeny[a] Female ELISA$^+$	Female NV	Male ELISA$^+$	Male NV	Total ELISA$^+$	Total NV	Probability[b]
NV	NV	3	15	44	16	50	31	94	0.95
NV	NV	2	0	37	0	29	0	66	0.001

[a]ELISA$^+$ = positive ELISA reaction after 12 days postacquisition incubation; NV = nontransmitting insects and insects with negative ELISA values after 12 days postacquisition incubation.
[b]Probability of obtaining, by chance, the observed deviation from a 1:3 ratio.

TABLE 3. Segregation of enzyme-linked immunosorbent assay (ELISA) positive and nonvector progeny from crosses of parents originating from segregating nonvector × nonvector crosses, shown in Table 2

Parents[a] Female	Parents[a] Male	Crosses	Progeny Female ELISA$^+$	Female NV	Male ELISA$^+$	Male NV	Total ELISA$^+$	Total NV	Probability (ELISA$^+$:NV)[b] 1:1	9:7	1:3	1:15
NV	NV	12	2	153	2	151	4	304	0.001	0.001	0.001	0.001
NV	NV	16	91	237	68	255	159	492	0.001	0.001	0.75	0.001
NV	ELISA$^+$	2	1	45	2	71	3	116	0.001	0.001	0.001	0.1
NV	ELISA$^+$	13	137	110	127	129	264	239	0.5	0.1	0.001	0.001
ELISA$^+$	NV	1	10	7	9	8	19	15	0.5	0.2	0.001	0.001
ELISA$^+$	ELISA$^+$	4	117	3	95	1	212	4	0.001	0.001	0.001	0.001

[a]ELISA$^+$ = positive ELISA reaction 12 days postacquisition incubation; NV = nontransmitting insect with negative ELISA reaction after 12 days postacquisiton incubation.
[b]Probability of obtaining, by chance, the observed deviation from the indicated ratios.

gave strong positive values (Fig. 3). Progeny from the second type of cross gave a broad distribution of ELISA values.

Segregation patterns (vector:nonvector) of progeny from crosses where parental biology was influenced by transovarially acquired virus were different from those where no maternal virus was involved (Table 5). Nonvector × nonvector crosses, where both parents had only one vector parent, gave vector:nonvector ratios of 1:3. Where the female vector parent had ELISA$^+$ parents, but the vector male had only an ELISA$^+$ female parent, the segregation pattern was 1:1. Where a vector female was crossed with a nonvector male, with one vector parent, nearly all progeny were vectors.

TABLE 4. Persistence of rice hoja blanca virus (RHBV) in nymphs of *Sogatodes oryzicola* receiving the virus transovarially from vector × nonvector crosses in which the female parent transmitted before mating

	Number of insects transmitting RHBV[b]			
Parents[a]	First feeding[c]	Second feeding[c,d]	Third feeding[c,d]	Positive ELISA reaction[c,d]
V × NV	271	248	227	254
V × V	63	61	61	63

[a]V = transmitting insect positive for enzyme-linked immunosorbent assay (ELISA) after 12 days postacquisition incubation and from NV × NV lineage.
[b]Five days on rice cultivar Bluebonnet 50 for each feeding period.
[c]Second instar nymphs at first feeding; includes only insects surviving three transfers.
[d]Determined by testing those insects that transmitted at the first feeding.

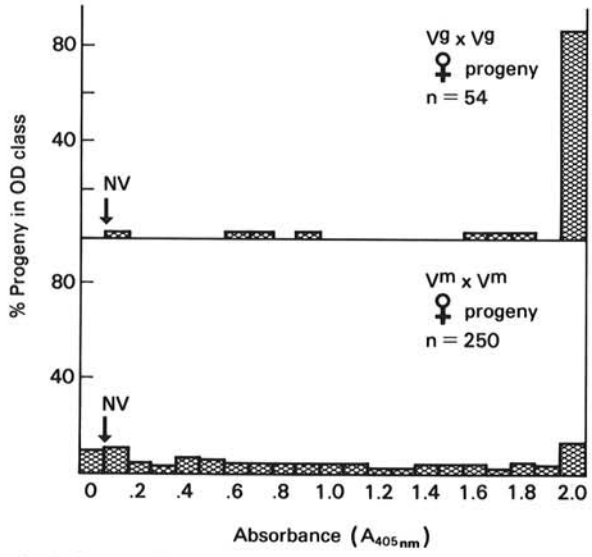

Fig. 3. Optical density (OD) (A_{405nm}) of enzyme-linked immunosorbent assay (ELISA) reaction from V^g and V^h crosses of *Sogatodes oryzicola*. V^g = transmitting insect from a nonvector (NV) × NV cross. V^m = transmitting insect from an ELISA$^+$ × NV cross.

DISCUSSION

RHBV can be detected in its insect vector by ELISA. Virus titer in nymphs that had not received virus maternally reached ELISA-detectable levels approximately 9–12 days after acquisition feeding. Insects that can or cannot support RHBV replication can be identified more quickly (12 days postacquisition incubation) using ELISA than via the direct feeding transmission test (approximately 25 days postacquisition incubation + 6–10 days incubation in plant). A sharp increase in the number of insects with detectable RHBV is strong evidence for replication of the virus within the insect vector (11). This happened after an initial 9 days of incubation, a period that is similar to that reported for MStpV in its vector, *Peregrinus maidis* Ashmead (18). RHBV, like other tenuiviruses (for example, MStpV and RSV) has a long postacquisition incubation period, trans-stadial persistence, transovarian transmission, and a deleterious influence of the virus on insect viability and fecundity (14). These are all properties associated with virus replication within the insect.

The concept of potential vectors as introduced by Fukushi (4) was found to be valid for RHBV-*S. oryzicola* in that only some insects are capable of acquiring RHBV and supporting viral replication whereas others are not. It is not certain if all insects that support virus replication are capable of transmitting the virus to rice and/or their progeny (transovarian transmission). For MStpV, many viruliferous planthoppers are incapable of transmitting the virus (18); however, in this study, as a rule, transmission rate of ELISA$^+$ insects was very high (Table 4). Nonetheless, a few insects that apparently could not transmit to plants did have detectable and high RHBV titers (Table 1). This may indicate that the ability of an insect to transmit RHBV is independent of its ability to support virus replication. In this study, successful acquisition could be determined only indirectly from successful virus replication; therefore, it was impossible to treat the two phenomena independently. Based on the very low frequency of nontransmitting hosts, most ELISA$^+$ individuals in this study probably were potential vectors.

The segregation pattern (1:3, ELISA$^+$:nonvector) of the progeny of crosses between nonvector parents suggests that the ability to acquire and support replication of RHBV is mediated by a single recessive gene and is not sex linked or sex determined. This is confirmed by the crosses among the F_1 progeny of crosses where some individuals were ELISA$^+$. Progeny of ELISA$^+$ × ELISA$^+$ were virtually 100% ELISA$^+$, whereas crosses among ELISA$^+$ and nonvector individuals segregated either 1:1 or 0:1 (ELISA$^+$:nonvector). In such crosses, the nonvector parent was either heterozygous for the allele that does not permit acquisition and replication (yielding 1:1, ELISA$^+$:nonvector) or homozygous (yielding 0:1, ELISA$^+$:nonvector). In nonvector × nonvector crosses, segregation ratios of 0:1, ELISA$^+$:nonvector, correspond to crosses where at least one parent was homozygous for the gene that does not permit acquisition and replication. Nonvector × nonvector crosses with progeny ratios of 1:3

TABLE 5. Segregation of vector-nonvector progeny of cross between genetically or maternally determined vectors and heterozygous nonvectors

		Progeny[a]							
		Female		Male		Total		Probability[b]	
Parents[a]	Crosses	V	NV	V	NV	V	NV	1:1	1:3
NV^h × V^m	6	32	81	25	87	57	168	0.001	0.9–0.95
V^g × V^m	2	15	18	14	22	29	40	0.01–0.25	0.005
V^m × NV^h	7	100	4	81	5	181	9	0.001	0.001

[a]V = second instar nymph transmitted rice hoja blanca virus to a healthy plant and yielded positive enzyme-linked immunosorbent assay (ELISA) reaction; NV = nontransmitting insect with negative ELISA reaction. V^m = transmitting vector receiving virus maternally and from an ELISA$^+$ × NV cross; Vg = transmitting insect with positive ELISA reaction from an NV × NV cross that segregated 1:3 ELISA$^+$:NV; NV^h × nontransmitting insect with negative ELISA reaction from an NV × V^g cross.
[b]Probability of obtaining by chance the observed deviation from the indicated ratios.

(ELISA$^+$:nonvector) correspond to those between two heterozygous parents.

The heritable nature of potential vectors, as proposed by Fukushi (4) and Kisimoto and Watson (16), is confirmed for this system. For the sake of discussion, the dominant allele that does not permit acquisition and replication will be referred to as "*R*," and the recessive allele that does permit acquisition and replication will be referred to as "*r*." Using this notation, ELISA$^+$ progeny of a nonviruliferous female (that is, where transovarian transmission has not occurred) are *rr*, and ELISA$^-$ progeny are either *Rr* or *RR*. The occasional ELISA$^-$ that occurs when ratios of 1:0 ELISA$^+$:ELISA$^-$ are expected probably results because the nymph does not feed on virus-infected tissue during acquisition. The rare ELISA$^+$, where none are expected, may reflect incomplete dominance of *R*. Such genetic control has been reported for other insect-virus systems (1).

Where the female parents were transmitting vectors before oviposition, the ratios of progeny were skewed, such that virtually all progeny transmitted in the second or third instar, even though the male parent was heterozygous, giving an expected ratio of 1:1 (Table 5). Crossing a female ELISA$^+$ progeny of an ELISA$^-$ × ELISA$^-$ cross (that is, a female incubating the virus during oviposition, but not yet transmitting, and subsequently shown to be ELISA$^+$, or *rr*) with a viruliferous male from a vector × nonvector cross yields segregation patterns consistent with 1:1 vector:nonvector (Table 5). That is, although phenotypically the cross was ELISA$^+$ × vector, and a 1:0 ELISA$^+$:ELISA$^-$ ratio was expected, genotypically the cross was *rr* × *Rr* and yielded a 1:1 ratio because the female parent did not transmit virus to the progeny via the egg. Similarly, nontransmitting female progeny (*Rr*) of a nonvector × vector cross, when crossed with transmitting males (*Rr*) from a vector × nonvector cross, yield progeny that segregate 1:3 vector:nonvector (Table 5). That is, the male, although viruliferous and capable of transmitting the virus (at least in the nymphal stages), was heterozygous. Comparing the ELISA values of insects that were viruliferous progeny of *rr* × *rr* crosses with those that were viruliferous progeny of *rr* × *Rr* or *rr* × *RR* crosses shows that the former have nearly uniform high values whereas the latter are distributed across the range (Fig. 3). It is not certain whether *Rr* nymphs can support replication of maternally acquired virus.

The segregation of the progeny of the ELISA$^+$ vector females from nonvector × nonvector crosses presented in Tables 3 and 5 was not affected by transovarial transmission. The nymphs were not placed on diseased plants until the third instar, and the resulting adult females were removed for ELISA after oviposition but before the virus incubation period had been completed.

The rather direct genetic control of the ability of an insect to acquire and support virus replication has some intriguing implications for interpreting the cyclical nature of rice hoja blanca epidemics. Jennings and Pineda (14) showed, and we have confirmed (*unpublished*), that the virus has a deleterious influence on vector fecundity and viability. In other words, the insect is suffering from a viral disease. This is supported by the increase in virus titer over time in insects that support virus replication. Within the context of an insect disease, the alleles related to virus replication within the insect may be viewed as conferring resistance and susceptibility to the virus. Thus *R* is an insect gene for resistance to the RHBV virus. Susceptible insects, which support virus replication, lack the resistance gene and are *rr*. Given the generally low frequency of vectors, even during an epidemic, virus resistance is the rule in populations of *S. oryzicola*.

For an epidemic of RHBV in rice to occur, a necessary requirement is an increase in vectors in the insect population (over the usually very low percentage of vectors). That is, an RHBV epidemic in rice must be preceded or accompanied by an RHBV epidemic in the population of *S. oryzicola*. At least two conditions must be met for this. First, adequate susceptibility (potential vectors) must be present in the insect population and there must be a source of virus (as yet undetermined) that then can move through the insect and rice population. However, as the virus moves through the insect population, the viruliferous insects suffer a competitive disadvantage because of the negative effect of the virus on their fecundity. There is then a shift to insects incapable of supporting viral replication (*RR* or *Rr*), and the epidemic dies out as insect susceptibility declines in the population. However, the allele for susceptibility, *r*, remains in the population as *Rr*. In the absence of the virus, the frequency of uninfected *rr* individuals, which presumably are at least as fit as *Rr* and *RR*, will increase in the population through random mating among *Rr* individuals and recombination. As the *rr* level reaches some critical and unknown threshold, the stage is set for another RHBV rice epidemic.

Although the ability of *S. oryzicola* to acquire RHBV via feeding is under simple recessive genetic control, nymphs that acquire the virus maternally can transmit RHBV to plants and, to some extent, to their progeny, regardless of their ability to acquire the virus via feeding and permit replication. This maternal influence can substantially modify vector:nonvector ratios and probably prolong epidemics to some degree.

The masking effect of transovarian transmission on acquisition ability clarifies a number of points. The decline in transmission percentage of colonies initiated only from proven vectors probably results because many of the insects actually are genotypically *Rr*, transmitting maternal virus. This, with incomplete transovarial transmission, may explain as well the occurrence of between 25 and 50% nonvector progeny from vector × vector crosses reported by Everett and Lamey (3), which suggested to them that the character is dominant.

Considering that nontransmitting hosts are encountered occasionally in planthoppers colonies, it is likely that the systemic virus-vector relationship is very complex. Factors mediating virus replication, circulation, feeding transmission, and transovarian transmission may well be under independent genetic control. Nonetheless, the ability to distinguish potential vectors from nonvectors and understanding that maternally acquired virus may mask the genotype of an individual related to supporting virus replication have permitted the establishment of stable vector colonies required to implement a large-scale field screening method (23). New RHBV-resistant rice cultivars identified from these screenings were released in Colombia in 1989.

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