Multiplication of Maize Stripe Virus in *Peregrinus maidis*

L. R. Nault and D. T. Gordon

Professor, Department of Entomology, and professor, Department of Plant Pathology, The Ohio State University (OSU), Ohio Agricultural Research and Development Center (OARDC), Wooster 44691.

Submitted as Journal Article 147-87 by the OSU-OARDC. Salaries and research support were provided by state and federal funds appropriated to the OSU-OARDC.

We gratefully acknowledge the technical assistance of Lakshman S. Negi, Marian Coffey, and Amy Juan Rubink in performing the enzyme-linked immunosorbent assays, W. E. Styer for the preparation of planthoppers and planthopper organs for assay, and N. H. Gordon for advice on the statistical analysis of the enzyme-linked immunosorbent assay data.

Accepted for publication 26 February 1988.

ABSTRACT

Nault, L. R., and Gordon, D. T. 1988. Multiplication of maize stripe virus in *Peregrinus maidis*. Phytopathology 78:991-995.

The enzyme-linked immunosorbent assay (ELISA) was used to demonstrate that the maize stripe virus (MStpV) multiplies in its delphacid planthopper vector, *Peregrinus maidis*. ELISAs were performed using an antiserum to purified MStpV. MStpV was detected by ELISA from a single planthopper immediately after a 2-day acquisition access period (AAP) and from increasing numbers of insects at 9, 16, and 23 days after the start of AAP. The values of the mean absorbance at 405 nm (A_{405nm}) for virus-exposed planthoppers increased significantly from 9 to 16 and 16 to 23 days after the start of AAP. Some but not all insects found to be positive for MStpV by ELISA transmitted the virus at 16 and 23 days after the start of AAP. MStpV was detected by ELISA from the following organs: midgut, hindgut, Malpighian tubules, brain, salivary glands, and hind femur muscle of both viruliferous males and females; ovaries, oviduct and spermatheca, bursa copulatrix, and single eggs of viruliferous females; and accessory gland and seminal vesicle of viruliferous males. In this last group, none of the 10 testes were found to be positive for MStpV by ELISA. In a time course experiment, MStpV was detected by ELISA from the midgut and ovaries but not the salivary glands at 0 and 2 days after a 7-day AAP. MStpV was detected from all three organs at 16 and 23 days after the start of AAP, and the mean A_{405nm} values significantly increased from 9 to 16 and 16 to 23 days after the start of AAP. MStpV was detected by ELISA from the salivary glands of all planthoppers that were inoculative in biossays and from some salivary glands of virus-exposed planthoppers that were not inoculative. The pattern of MStpV transmission for eight *P. maidis* was intermittent and infrequent except for one vector that transmitted virus to 18 of 22 test plants over a 7-wk period.

Additional keywords: propagative plant virus, tenuivirus.

A prolonged latent period and transovarial transmission of plant viruses by their insect vectors suggest that the virus multiplies in its vector. Although evidence of multiplication can be obtained by several techniques, in the past the most widely used and convincing technique has been serial passage of virus from insect to insect, either by needle injection or transovarial passage, until the dilution attained in the final inoculative insects exceeded the dilution end point of the initial inoculum (28,29). A second source of evidence widely cited that suggests multiplication is electron microscopic observation of virus particles and sites of viral assembly and/or accumulation in vector cells (24). The disadvantages of these methods are that they are either laborious and time consuming (serial passage) or provide only indirect evidence that multiplication occurred (electron microscopy). We have found that the enzyme-linked immunosorbent assay (ELISA) provides a rapid, easy-to-use, sensitive, and semiquantitative method for providing proof of plant virus multiplication in their vectors, and we and other investigators recently have reported use of ELISA to demonstrate propagation of maize rayado fino virus in its deltocephaline leafhopper vector (10,22,23) and maize mosaic virus (MMV) in its planthopper vector, *Peregrinus maidis* (Ashmead) (4).

The maize stripe virus (MStpV) (11,13) is a member of the newly described tenuivirus group (9). These viruses are transmitted by Gramineae-feeding, delphacid planthoppers in which they persist, have a minimum latent period of more than 1 wk, and are transovarially transmitted, suggesting that they multiply in their vectors. Proof of such multiplication has been presented for rice stripe virus (RSV) and is based on serial transovarial transmission by its vector, *Laodelphax striatellus* Fallen, for more than 40 generations (25) and on serial passage by injection of vectors resulting in a final dilution of 1.25×10^{-6} that exceeds the 10^{-2} to 10^{-3} dilution end point (21). Similarly, rice hoja blanca virus, another member of the group, appears to multiply in its vector, *Sogatodes orizicola* Muir, in which it was transovarially transmitted for 10 generations (6).

In this paper, we provide evidence using ELISA that MStpV multiplies in *P. maidis*, that the virus occurs in nearly all internal organs of infected males and females, and that in most insects the virus is detected by ELISA from the midgut before it is detected from the ovaries and salivary glands or before it is transmitted by vectors. Gingery (8) earlier reported a brief summary of our findings. Recently Falk et al (5) used ELISA to show that the percentage of *P. maidis* that was positive for MStpV increased with time over a portion of their test period after acquisition from plants, suggesting multiplication of the virus in its vector.

MATERIALS AND METHODS

The propagation of a Florida isolate of MStpV and Florida biotype of *P. maidis* has been described previously (11).

Planthopper transmission of MStpV. Depending on the number of viruliferous insects needed, varying numbers of third to fourth instar *P. maidis* nymphs were placed on MStpV-infected sweet corn, *Zea mays* L. 'Aristagold Bantam Evergreen,' that had been inoculated 2–3 wk previously. Planthoppers and source plants were confined in dacron-organdy-covered aluminum frame cages approximately 20 cm × 40 cm × 40 cm. Screen and tube cages used here have been illustrated (3).

After virus acquisition, MStpV-infected plants were replaced with healthy sweet corn plants that were changed every 2 or 3 days to prevent planthoppers from reacquiring virus from holding plants (Nault, *unpublished*). This change in holding plants was continued until planthoppers were collected for ELISA or bioassay.

In several experiments, insects were bioassayed for virus before they were collected for ELISA. Single planthoppers were confined in butyrate tube cages containing sweet corn test seedlings in the two- to three-leaf stage, maintained in a growth chamber at 26 C, a

14-hr day, and > 50% RH. After a 48-hr inoculation access period, insects were individually removed and prepared for ELISA, and assay plants were sprayed with resmethrin, a pyrethroid insecticide, and placed in a glasshouse to allow symptom development. Ratings for virus symptoms (diagnostic chlorotic stripes [8]) were made 2–3 wk later.

In the transmission-pattern experiment, single insects were confined to sweet corn plants in butyrate cages and serially transferred every 48 hr to new seedlings using a vacuum aspirator until they died or until 62 days had elapsed.

Preparation of *P. maidis* for ELISA. Whole insects or insect organs were homogenized with a 7-ml tissue homogenizer in 0.3 ml of PBS Tween buffer (PBS = 0.15 M sodium chloride + 0.02 M sodium phosphate + 0.02% sodium azide, pH 7.4; Tween 20 = 0.05% polyethylene sorbitan monolaurate). Organs were dissected from planthoppers partially embedded in paraffin and then rinsed twice in buffer before being homogenized for ELISA. Terminology used for internal organs of planthoppers is taken from Ammar (1). Planthopper homogenates were tested by ELISA for relative amounts of MStpV as previously described (11,14).

Enzyme-linked immunosorbent assay. Antiserum was collected from a rabbit immunized with purified MStpV (12). Immunoglobulins (Ig) were purified from this antiserum and conjugated with alkaline phosphatase, and the double-antibody sandwich ELISA was performed as described previously (14,20). The coating Ig concentration was 1 μg/ml and the secondary or detecting Ig dilution was 1:800. Absorbance (A_{405nm}) measurements of wells containing incubated substrate (*p*-nitrophenol phosphate) were made with a Gilford Stasar II Spectrophotometer (Gilford Instruments Laboratory Inc., Oberlin, OH) equipped with a rapid sampling system.

Test for ELISA sensitivity. The test was done to determine the least amount of MStpV antigen detectable by ELISA and involved preparation of a dilution series of purified MStpV (provided by R. E. Gingery, USDA, Department of Plant Pathology, Ohio Agricultural Research and Development Center, Wooster) with PBS Tween buffer as the diluent. Ten two-fold or 2.5-fold dilutions ranging from 366 to 0.366 ng/ml were tested in eight to 10 replications per dilution by ELISA. Significant differences were obtained for MStpV protein concentrations of adjacent pairs of dilutions from 366 and 183 to 9 and 3.7 ng/ml. Thus the lowest detectable MStpV antigen concentration was 3.7 ng/ml and the saturation point was 366 ng/ml. Concentrations between these two values were used to demonstrate the relationship of A_{405nm} to antigen concentration. The model $A_{405nm} = \alpha(C)^B$ epsilon (i.e., log A_{405nm} was linearly related to log C) was fit to the data, where C = the concentration (ng/ml), B = the regression parameters, and epsilon = the random error variable.

Analysis of ELISA data. Reactions were scored positive for MStpV when the absorbance at 405 nm (A_{405nm}) was greater than the detection threshold value defined as the A_{405nm} mean plus five times the population standard deviation for the unexposed *P. maidis* (negative) controls. In addition, contents of wells scored positive by absorbance values had to show a yellow color.

To determine the lower limit for detection of MStpV antigen, mean A_{405nm} values for each dilution of purified antigen were compared statistically with means for the adjacent more-diluted and less-diluted preparations. A one-tailed *t* test at a level of α = 0.05 was used to determine significant differences between the paired means.

Tests of absorbance (A_{405nm}) for contents of internal versus external wells of microtiter plates. In view of reports that A_{405nm} values for the contents of external wells of microtiter plates showed differences from those for the contents of internal wells of the same plate in ELISA of other viruses, we tested this possibility for MStpV ELISA. The mean A_{405nm} for peripheral wells containing purified MStpV was 1.042 (n = 36; standard deviation [SD] = 0.076) and for internal wells mean A_{405nm} was 0.964 (n = 34; SD = 0.034). Based on the detection threshold value, which was the mean A_{405nm} value plus five times the population standard deviation for the internal wells, five of the peripheral wells were positive; that is, they yielded A_{405nm} values greater than this detection threshold value. The test of significance for the difference between the mean A_{405nm} value for the peripheral wells and that for the internal wells gave a *t* value of 5.52, P = 0.01. For the data reported in Table 1, only internal wells were used except for the 16th and 23rd days of replication when approximately equal numbers of samples of MStpV-exposed and unexposed *P. maidis* were placed in peripheral and internal wells.

Tests for detection of MStpV in individual and multiple *P. maidis*. To establish whether our ELISA for MStpV could detect virus from individual *P. maidis*, we assayed individual planthoppers and groups of five planthoppers exposed and not exposed to MStpV 14 days after the start of a 2-day acquisition access period (AAP) by ELISA. For individually assayed *P. maidis*, the mean A_{405nm} for wells that contained extracts from exposed insects was 0.287 (n = 10; SD = 0.079) and the mean for the unexposed insects was 0.082 (n = 10; SD = 0.010). Based on the detection threshold value used in this study, all 10 of the individual, exposed *P. maidis* were positive. A test for significance of the difference of the mean A_{405nm} values for the exposed and unexposed groups (*t* test) gave t = 8.167, P = 0.001. For virus-exposed and unexposed *P. maidis* assayed in groups of five, the mean A_{405nm} values were 0.646 (n = 5; SD = 0.247) and 0.099 (n = 5; SD = 0.005), respectively. Based on our detection threshold value, all five of the groups exposed to MStpV were positive for the virus and the test of significance of the difference of the mean A_{405nm} values for the two groups gave t = 4.94, P = 0.01.

RESULTS

Time course of MStpV detection in *P. maidis*. In one set of experiments, planthoppers were given a 2-day AAP to infected plants. Subsequently, planthoppers were assayed immediately for MStpV or placed on holding plants and tested by ELISA 9, 16, or 23 days after the start of AAP. Forty-eight hours before their sacrifice for ELISA, planthoppers were bioassayed for MStpV. Virus was detected in one of 198 planthoppers by ELISA immediately after the 48-hr AAP (Table 1). After the 2-day exposure to infected plants, the number of planthoppers positive for MStpV by ELISA increased at 9, 16, and 23 days after the start of AAP. The mean absorbance (ELISA) values for exposed planthoppers (data pooled for negative and positive insects) increased significantly at 16 and 23 days after the start of AAP (Table 1). None of the unexposed planthoppers assayed at 2, 16, or

TABLE 1. Detection of maize stripe virus (MStpV) in *Peregrinus maidis* by enzyme-linked immunosorbent assay (ELISA) and transmission by planthoppers at weekly intervals following a 2-day acquisition access period (AAP) from virus-infected plants

		ELISA		
Days after start of AAP[a]	n	Mean absorbance (A_{405nm})[b]	Percent positive[c]	Percent transmission[d]
2	198	0.122 A	0.5	···
9	193	0.221 A	9.3	0.0
16	186	0.553 B	18.8	4.3
23	147	0.753 C	23.1	8.8

[a] Experiments were replicated four times (three times at 23 days after the start of AAP) with up to 50 insects assayed per test period after the start of AAP per replicate. A similar number of unexposed control *P. maidis* were used, and all tested negative for MStpV by ELISA.

[b] Values are the means of the individual A_{405nm} values for all (positive and negative) MStpV-exposed *P. maidis*. Means were tested for separation by *t* tests (least significant difference), Duncan's multiple range test, and Tukey's studentized range (HSD) test, respectively, and each test yielded the same pattern of means separation. Means followed by the same letter are not significantly different, $P \leq 0.05$.

[c] The detection threshold values for the MStpV-positive *P. maidis* were calculated as five times the standard deviation plus the mean A_{405nm} for the unexposed *P. maidis* included for each replicate assay. The percentage was calculated as the number of MStpV-positive *P. maidis* per the total number of MStpV-exposed planthoppers assayed.

[d] Forty-eight hours before insects were sacrificed for ELISA, they were singly placed on sweet corn plants to test for MStpV transmission.

23 days after the start of AAP tested positive for MStpV by ELISA. Five adjacent wells of the unexposed *P. maidis* control from the fourth test at 9 days after the start of AAP were positive for MStpV. We believe that wells containing these controls had become contaminated from other adjacent wells that had contained MStpV from infected maize leaf extracts; these had served as positive virus controls for the test. The data for these five controls in question were removed from those used to calculate the mean and standard deviation for the unexposed control. In the bioassays, no planthoppers transmitted MStpV at 9 days after the start of AAP, but some were inoculative at 16 and 23 days after the start of AAP (Table 1). Virus was confirmed for all 70 inoculative planthopper vectors by ELISA, but an additional 65 insects positive for virus by ELISA failed to transmit virus.

Detection of MStpV in organs of *P. maidis*. In a second series of experiments to determine the organs that contained detectable MStpV, planthoppers were collected from a holding cage where they had fed continuously on infected plants for 3 or more weeks. Virus was detected by ELISA in extracts of the midgut, hindgut, Malpighian tubules, brain, salivary glands, and hind femur muscle of both sexes (Table 2). For females, MStpV also was detected from the ovaries, oviduct and spermatheca, bursa copulatrix, and single eggs. For males, MStpV also was detected from the accessory glands and seminal vesicles, but not from the testes. The testes were the only organs from which virus was not detected by ELISA. None of the extracts of guts from the 28 unexposed planthoppers tested positive for MStpV.

Time course of MStpV detection in the midgut, ovaries, and salivary glands of *P. maidis*. In a third series of experiments, the midgut, ovaries, and salivary glands were assayed for MStpV at 7, 9, 16, and 23 days after the start of a 7-day AAP. Virus was detected from the midguts and ovaries, but not from the salivary glands, from planthoppers on the seventh and ninth day (Table 3). Increases in the number of positive midguts and ovaries were noted for 16 and 23 days after the start of acquisition. Virus was detected for the first time in the salivary glands at 16 days after the start of acquisition. The mean absorbance values for exposed planthopper data (pooled for negative and positive) increased significantly at 16 and 23 days after the start of acquisition in all three organs. Only 4 of 30 and 3 of 30 insects transmitted MStpV before the 16- and 23-day ELISA, respectively, and later tested positive for MStpV by ELISA. Among the 110 insects tested in this series of experiments, 24 of 31 whose salivary glands tested positive for MStpV failed to transmit virus. None of the 40 midguts, 39 ovaries, or 40 salivary glands from the unexposed planthoppers tested positive for MStpV by ELISA.

Comparison of ELISA and infectivity assay for detection of MStpV from *P. maidis*. In a final series of experiments to determine why ELISA consistently detected virus more readily than the bioassay, planthoppers were transferred serially after the AAP every 48 hr until the insect died or until 62 days had elapsed. The transmission pattern for seven of the eight planthoppers (Table 4) was infrequent and intermittent, whereas the eighth planthopper (number 3 in Table 4) transmitted MStpV regularly. If we assume that maximum transmission by planthoppers would be to all plants exposed from the first inoculated (the minimum latent period) until death or until 62 days had elapsed, then these eight planthoppers inoculated only 36.9% of 131 exposed test plants.

DISCUSSION

Our ELISA results support the hypothesis that MStpV multiplies in *P. maidis*, as indicated by detection of MStpV from only a single planthopper immediately after the 2-day AAP followed by detection in increasing numbers from 9, 16, to 23 days after the start of AAP and by the increase of mean absorbance values at 16 and 23 days after the start of AAP. Previously, two rhabdoviruses, MMV and the vertebrate infecting vesicular

TABLE 2. Detection of maize stripe (MStpV) in various organs of female and male *Peregrinus maidis* by enzyme-linked immunosorbent assay (ELISA)[a]

Insect organ	Female	Male
Midgut	10/14[b] (2.30 ± 0.58)[c]	5/5 (1.51 ± 0.89)
Hindgut	5/8 (0.90 ± 0.76)	2/5 (0.60 ± 0.10)
Malpighian tubules	3/5 (0.64 ± 0.36)	2/6 (0.61 ± 0.26)
Brain	6/6 (1.69 ± 1.25)	4/5 (2.18 ± 0.54)
Salivary glands	7/7 (1.27 ± 0.83)	4/4 (0.83 ± 0.58)
Hind femur muscle	4/4 (1.84 ± 1.26)	3/8 (2.34 ± 0.72)
Accessory gland	...	10/10 (2.93 ± 0.03)
Testes	...	0/10
Seminal vesicle	...	5/6 (1.24 ± 0.44)
Ovary	8/8 (2.20 ± 0.02)	...
Oviduct and spermatheca	6/6 (2.27 ± 1.04)	...
Eggs (single)	3/9[d] (0.75 ± 0.16)	...
Eggs (groups of 5)	6/9[d] (1.46 ± 0.11)	...
Bursa copulatrix	6/6 (2.27 ± 1.04)	...

[a] Organs from up to 14 female and 10 male *P. maidis* that had fed for a minimum of 3 wk on MStpV-infected maize were assayed. The midguts and hindguts from 28 unexposed control *P. maidis* were assayed by ELISA and all were negative for MStpV. The detection threshold value (0.40) for scoring a planthopper organ positive for MStpV was the mean absorbance at 405 nm for the unexposed control *P. maidis* (0.10) plus five times the standard deviation of the mean (0.06).

[b] The numerator is the number of MStpV-positive organs, and the denominator is the number of organs tested by ELISA.

[c] The numbers in parentheses are the mean absorbance at 405 nm for the MStpV-positive (numerator) organs and the standard deviation of the mean.

[d] The numerator is the number of females from which single eggs or groups of five eggs tested positive for MStpV, and the denominator is the number of females whose eggs were tested by ELISA.

TABLE 3. Detection of maize stripe virus (MStpV) in the midgut (MG), ovaries (O), and salivary glands (SG) of *Peregrinus maidis* by enzyme-linked immunosorbent assay (ELISA) after a 7-day acquisition access period (AAP) on MStpV-infected plant and thereafter at various intervals while held on healthy plants[a]

Days after start of AAP	*n*			ELISA					
				Means of absorbance of A_{405nm}[b]			Percent positive[c]		
	MG	O	SG	MG	O	SG	MG	O	SG
7	29(30)	29(28)	30(30)	0.233 A(0.073)	0.122 A(0.078)	0.078 A(0.078)	37.9	6.9	0
9	19(20)	20(20)	20(20)	0.166 A(0.057)	0.074 A(0.056)	0.066 A(0.058)	36.8	5.0	0
16	30(28)	30(27)	30(28)	0.928 B(0.059)	0.999 B(0.058)	0.526 B(0.061)	70.0	56.7	43.3
23	30(28)	30(28)	30(30)	1.389 C(0.076)	1.496 C(0.075)	1.102 C(0.074)	66.7	63.3	60.0

[a] Experiment was replicated three times (two times at 9 days after the start of AAP) with a set of organs from 9 to 10 MStpV-exposed insects for each test period (days after the start of AAP) per replicate.

[b] The A_{405nm} values are the means of the individual A_{405nm} values for all MStpV-exposed and unexposed (values in parentheses) *P. maidis*. Means of MStpV-exposed *P. maidis* separated by Student-Newman-Keuls procedure. Means in a column followed by the same letter are not significantly different, $P < 0.05$.

[c] The detection threshold values for the MStpV-positive *P. maidis* were calculated as five times the standard deviation plus the mean A_{405nm} for the unexposed *P. maidis* included for each replicate assay. The percentage was calculated as the number of MStpV-positive *P. maidis* per the total number of MStpV-exposed planthoppers assayed.

TABLE 4. Transmission patterns of maize stripe virus (MStpV) by a single *Peregrinus maidis*

Insect No., wing type[b], and sex	Days after the start of acquisition[a]																								
	10	12	14	16	18	20	22	24	26	28	30	32	34	36	38	40	42	44	46	48	50	52	54	56	58	60	62
1. M ♂											+	+			+									D			
2. B ♂																+			+								+
3. B ♀			+	+	+	+	+	+	+	+	+	+	+		+	+		+	+		+	+		+	D		
4. B ♂		+														+										D	
5. B ♀											+	+	+					D									
6. B ♀	+	+			+							D															
7. B ♀					+	+	+				+															D	
8. B ♂						+	+		+	+	+	+	+				+			+	+			+			D

[a] Planthoppers were given a 48-hr acquisition access period, held individually on holding plants 8 days, then serially transferred every 2 days to a new test plant. Insects 1–4 were maintained at 25 C, and insects 5–8 were maintained at 20 C. + = test plant showing MStpV symptoms; D = insect died.
[b] M = macropterous; B = brachypterous.

stomatitis virus, had been shown to multiply in *P. maidis* (2,4,17,19).

The ELISA evidence also supports the hypothesis that MStpV multiplies in all the organs tested (Table 2) with the possible exception of the testes. Alternatively, it is plausible that MStpV multiplies only in the midgut, the organ where virus was first detected, and subsequently accumulates without multiplication in other organs. However, we cannot conceive of a mechanism that would allow accumulation within these organs without multiplication. Our results are similar to those for wound tumor virus (26) wherein the virus was detected in the midgut before other internal organs of its leafhopper vector and to those for the aster yellows mycoplasmalike organism which showed a similar sequence of infection (27).

Among the tenuiviruses, RSV has been detected in the brain, salivary glands, intestines, Malpighian tubules, and ovarioles (ovaries) of its vector, *L. striatellus*, by immunofluorescent staining (18). RSV also was detected in the nurse cells, oocytes, follicular cells, and mycetocytes; these results are consistent with the report of transovarial passage of the virus. We found MStpV in eggs, which also is consistent with the transovarial transmission of MStpV (11). Detection of MStpV in the accessory gland of the male where the seminal fluids are stored suggests that MStpV could be transmitted venereally from viruliferous males to virus-free females. However, bioassays of more than 50 females inseminated by MStpV-infected males were negative for virus (Nault, *unpublished*). To date there is no known instance of transovarially transmissible viruses being sexually transmitted from males to females (16).

In all tests, we detected MStpV in planthoppers more frequently by ELISA than by bioassay. This was not unexpected when assays were conducted shortly after acquisition because virus was detected frequently only in the midgut, rarely in the ovaries where it must be for transovarial transmission, and not in the salivary glands where it must be for inoculation of maize plants by feeding. However, in our study, presence of virus in the salivary gland of *P. maidis* as detected by ELISA did not ensure that the vector would transmit the virus (Table 3). To illustrate, at 16 days after the start of AAP, virus was detected in the salivary glands of 13 planthoppers, yet only four transmitted virus; at 23 days after the start of AAP, 18 planthoppers had salivary glands positive for MStpV by ELISA, yet only three transmitted the virus. Our study of MStpV transmission patterns also demonstrated that MStpV is not consistently transmitted by viruliferous planthoppers (Table 4). Although all eight planthoppers in this study were viruliferous and inoculative, as few as one or at most five planthoppers transmitted virus during any 48-hr bioassay period. The infrequent and intermittent transmission pattern reported here and previously (12) for our Floridia isolate of MStpV contrasts to the more regular transmission pattern reported for an Australian MStpV isolate (15). The latter isolate was transmittted to 83.4% of test plants by *P. maidis*, whereas our MStpV isolate was transmitted to only 36.9%. We postulate that failure of viruliferous planthoppers to transmit MStpV may be due to insufficient multiplication and secretion of virus particles from the salivary glands into the salivary ducts and hence the saliva or to the failure of the planthopper to salivate in phloem cells. Our data suggest that not all viruliferous *P. maidis*, including insects with virus in the salivary glands, are able to transmit virus. The fact that virus can be associated with the salivary glands of viruliferous but noninoculative insects was shown earlier by Gildow and Rochow (7) who found that an isolate of barley yellow dwarf virus is associated extracellularly in the basal lamina of salivary glands of a nonvector aphid species.

Our results that show an increase in the percentage of MStpV-positive *P. maidis* over time are similar to findings recently reported by Falk et al (5). Also using ELISA to test for MStpV-postive *P. maidis*, their data showed an increase in the percentage of positive *P. maidis* from the seventh day after the start of AAP (which was 5 days) to the ninth or 11th day depending on the experiment. Thereafter, they showed no consistent increase. Our data showed an increase in percentage of MStpV-positive *P. maidis* with time from 0 to 5% after a 2-day AAP to 23.1% at 21 days after AAP. We found that both exposed inoculative and noninoculative *P. maidis* were positive for MStpV, a finding consistent with multiplication of other viruses or mollicutes in their vectors when monitored by ELISA as well as by infectivity assay (10,14). In contrast, Falk et al (5) reported that only inoculative *P. maidis* were positive by ELISA. In addition to an increase in percentage of MStpV-positive *P. maidis*, we demonstrated an increase in MStpV for the population of MStpV-exposed *P. maidis*. Falk et al (5) did not report attempts to measure change in the amount of MStpV-capsid protein for their test period of 18 days after the start of AAP. Furthermore, they did not attempt to demonstrate an increase in the percentage of MStpV-positive organs or an increase in the amount of MStpV-capsid protein within organs over time as we did. Thus, our findings greatly expand the evidence reported by Falk et al (5) and obtained using ELISA for the hypothesis that MStpV multiplies in *P. maidis*.

The ability to detect MStpV in individual *P. maidis* by ELISA would make this technique valuable for epidemiological studies such as we have reported involving ELISA detection of maize rayado fino virus and corn stunt spiroplasma in field-collected *Dalbulus* species (14). However, although results of the present study indicate that ELISA for MStpV from *P. maidis* may correctly estimate the number of viruliferous individuals present in a population of field-collected planthoppers, ELISA data do not accurately predict transmission efficiency of vectors.

LITERATURE CITED

1. Ammar, E. D. 1985. Internal morphology and ultrastructure of leafhoppers and planthoppers. Pages 127-162 in: The Leafhoppers and Planthoppers. L. R. Nault and J. G. Rodriguez, eds. John Wiley & Sons, New York. 500 pp.
2. Ammar, E. D., and Nault, L. R. 1985. Assembly and accumulation sites of maize mosaic virus in its planthopper vector. Intervirology 24:33-41.
3. D'Arcy, C. J., and Nault, L. R. 1982. Insect transmission of plant

viruses and mycoplasmalike and rickettsialike organisms. Plant Dis. 66:99-104.
4. Falk, B. W., and Tsai, J. H. 1985. Serological detection and evidence for multiplication of maize mosaic virus in the planthopper, *Peregrinus maidis*. Phytopathology 75:852-855.
5. Falk, B. W., Tsai, J. H., and Lommel, S. A. 1987. Differences in levels of detection of maize stripe virus capsid and major non-capsid proteins in plant and insect hosts. J. Gen. Virol. 68:1801-1811.
6. Galvez, E., G. E. 1967. Frecuencia de *Sogata orizicola* Muir y *S. cubana* Crawf. en campos de arroz y Echinochloa en Colombia. Agric. Trop. 23:384.
7. Gildow, F., and Rochow, W. F. 1980. Role of accessory salivary glands in aphid transmission of barley yellow dwarf virus. Virology 104:97-108.
8. Gingery, R. E. 1983. Maize stripe virus. Pages 69-74 in: Proc. Int. Maize Virus Disease Colloq. Workshop, Wooster, OH. D. T. Gordon, J. K. Knoke, L. R. Nault, and R. M. Ritter, eds. Ohio Agric. Res. Dev. Cent., Wooster. 261 pp.
9. Gingery, R. E. 1988. The rice stripe virus group. In: The Plant Viruses. R. G. Milne, ed. Plenum Publishing Corp., New York 4:297-329.
10. Gingery, R. E., Gordon, D. T., and Nault, L. R. 1982. Purification and properties of an isolate of maize rayado fino virus from the United States. Phytopathology 72:1313-1318.
11. Gingery, R. E., Nault, L. R., and Bradfute, O. E. 1981. Maize stripe virus: characteristics of a member of a new virus class. Virology 112:99-108.
12. Gingery, R. E., Nault, L. R., Tsai, J. H., and Lastra, R. J. 1979. Occurrence of maize stripe virus in the United States and Venezuela. Plant Dis. Rep. 63:341-343.
13. Gingery, R. E., Nault, L. R., and Yamashita, S. 1983. Relationship between maize stripe virus and rice stripe virus. J. Gen. Virol. 64:1765-1770.
14. Gordon, D. T., Nault, L. R., Gordon, N. H., and Heady, S. H. 1985. Serological detection of corn stunt spiroplasma and maize rayado fino virus in field-collected *Dalbulus* spp. from Mexico. Plant Dis. 69:108-111.
15. Greber, R. S. 1980. Maize stripe disease in Australia. Aust. J. Agric. Res. 32:27-36.
16. Harris, K. F. 1979. Leafhoppers and aphids as biological vectors: Vector virus relationships. Pages 217-308 in: Leafhopper Vectors and Plant Plant Disease Agents. K. Maramorosch and K. F. Harris, eds. Academic Press, New York. 654 pp.
17. Herold, F., and Munz, K. 1965. Electron microscopic demonstration of virus like particles in *Peregrinus maidis* following acquisition of maize mosaic virus. Virology 25:412-417.
18. Kitani, K., Kiso, A., and Yamamoto, T. 1968. Immunofluorescent studies on the localization of rice stripe virus antigens in the internal organs of viruliferous insect vector, *Laodelphax striatellus* Fallen. Bull. Shikoku Agric. Exp. Stn. 18:117-138.
19. Lastra, J. R., and Esparza, J. 1976. Multiplication of vesicular stomatitis virus in the leafhopper *Peregrinus maidis* (Ashm.), a vector of a plant rhabdovirus. J. Gen. Virol. 32:139-142.
20. Nault, L. R., Gordon, D. T., Gingery, R. E., Bradfute, O. E., and Castillo Loayza, J. 1979. Identification of maize viruses and mollicutes and their potential insect vectors in Peru. Phytopathology 69:824-828.
21. Okuyama, S., Yora, K., and Asuyama, H. 1968. Multiplication of the rice stripe virus in its vector, *Laodelphax striatellus* Fallen. Ann. Phytopathol. Soc. Jpn. 34:255-264.
22. Rivera, C., and Gamez, R. 1986. Multiplication of maize rayado fino virus in the leafhopper vector *Dalbulus maidis*. Intervirology 25:76-82.
23. Rivera, C., Kozuka, Y., and Gamez, R. 1981. Rayado fino virus: Detection in salivary glands and increase in titre in *Dalbulus maidis*. Turrialba 31:78-80.
24. Shikata, E. 1979. Cytopathological changes in leafhopper vectors of plant viruses. Pages 309-325 in: Leafhopper Vectors and Plant Disease Agents. K. Maramorosch and K. F. Harris, eds. Academic Press, New York. 654 pp.
25. Shinkai, A. 1962. Studies on insect transmissions of rice virus diseases in Japan. Bull. Nat. Inst. Agric. Sci. Ser. C 14:1-122.
26. Sinha, R. C. 1965. Sequential infection and distribution of wound-tumor virus in the internal organs of a vector after ingestion of a virus. Virology 26:673-686.
27. Sinha, R. C., and Chiykowski, L. N. 1967. Initial and subsequent sites of aster yellows virus infection in a leafhopper vector. Virology 33:702-708.
28. Smith, K. M. 1967. Insect Virology. Academic Press, New York. 256 pp.
29. Whitcomb, R. F. 1972. Transmission of viruses and mycoplasma by the Auchenorrhynchous Homoptera. Pages 168-203 in: Principles and Techniques in Plant Virology. C. I. Kado and H. O. Agriwal, eds. Van Nostrand-Reinhold, New York. 688 pp.