

Transmission efficiency of three isolates of maize stripe tenuivirus in relation to virus titre in the planthopper vector

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Isolates of maize stripe tenuivirus (MStV) from Florida (US), Costa Rica (CR), and Nigeria, Africa (AF), were transmitted to maize plants by the planthopper *Peregrinus maidis* (from Hawaii) with respective frequencies of 0, 18, and 60% after a 1-day acquisition access period on diseased plants, and with frequencies of 18, 71 and 93%, respectively, after a 7-day access period. These isolates were transmitted transovarially to progeny planthoppers with respective frequencies of 21, 32, and 47%. The latent period in the vector, following oral acquisition of MStV, was significantly longer in the US isolate than in the AF- or CR isolates. ELISA tests of MStV-inoculative planthoppers indicated a significantly lower titre of MStV-US compared with MStV-CR or MStV-AF. These results suggest that, compared with the US isolate, the AF and CR isolates of MStV multiply faster and reach higher levels in, and are transmitted more efficiently by, *P. maidis* from Hawaii.

INTRODUCTION

Maize stripe tenuivirus (MStV) causes a severe disease of maize in Africa, Australia, Central America and the southern USA, and probably occurs in most tropical maize-growing regions worldwide (Gingery, 1985, 1988; Thottappilly *et al.*, 1993). MStV is transmitted in a persistent, propagative manner by the delphacid planthopper *Peregrinus maidis* (Ashmead) (Tsai & Zitter, 1982; Gingery, 1985, 1988; Nault & Ammar, 1989). It has been shown by enzyme-linked immunosorbent assay (ELISA) that MStV multiplies in its vector, and that it is transmitted transovarially from inoculative females to their progenies (Tsai & Zitter, 1982; Nault & Gordon, 1988). In the present study, the efficiency was compared of oral and transovarial transmission by *P. maidis* of three isolates of MStV obtained from Florida (USA), Costa Rica, and Nigeria (Africa). The titre of these three MStV isolates in the inoculative planthoppers was examined by ELISA, to test whether a dissemination barrier is responsible for the lower rate of transmission in some MStV isolates, as has previously been reported for a few other propagative plant and animal viruses in their vectors (Hardy, 1988; Ammar, 1994). A preliminary report on some of

this work has appeared earlier (Ammar *et al.*, 1990b).

MATERIALS AND METHODS

Planthopper culture and virus isolates

The culture of *P. maidis* used was originally obtained from Hawaii, and has been maintained on healthy maize (*Zea mays*) plants as described previously (Gingery *et al.*, 1979). All experiments were conducted in growth chambers at 25 ± 1 °C with a 14-h light period. Three isolates of MStV were used: the US isolate, originally obtained from Florida and characterized by Gingery *et al.* (1981); the African (AF) isolate, obtained from Nigeria; and the CR isolate, obtained from Costa Rica. These isolates were maintained in maize plants (cv. Aristogold Bantam Evergreen) by serial inoculation with *P. maidis*.

Transmission efficiency following oral acquisition of MStV

For oral acquisition of MStV, second or third instar nymphs of *P. maidis* were caged in groups on maize plants inoculated 3–4 weeks earlier. To ensure that all nymphs fed on infected tissue, the older and symptomless leaf blades and their sheaths were removed from the source plants.

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Nymphs were given either a 1-day or a 7-day acquisition access period and then tested for inoculativity (oral transmission of MSStV) by caging them singly for 4 weeks on healthy maize seedlings (one insect per seedling per week). For 1-day acquisition access periods, nymphs were held for 6 days on healthy plants before starting the inoculativity tests, in order to make them comparable with those given 7 day access periods. After removing insects from test plants weekly, the plants were sprayed with a systemic insecticide (resmethrin) and held for 3–4 weeks in an insect-containment glasshouse at 25–33 °C for symptom observation. The latent period was estimated for each insect as the number of weeks between acquisition and first MSStV transmission.

ELISA tests on inoculative planthoppers

Planthoppers that survived the 4-week inoculativity test period (by then 3- to 4-week-old adults) were individually frozen to await transmission results. Insects that transmitted MSStV in any of the four weekly tests were tested by ELISA to estimate the relative titre of MSStV.

Antigens were prepared by grinding individual planthoppers in a glass homogenizer in TBS-T (0.25 M Tris, 0.15 M NaCl plus 0.05% Tween-20, pH 8.0). Except for overnight incubation of antigens at 4 °C (D.T. Gordon, Ohio State University, Wooster, personal communication, 1992), F(ab')₂ ELISA was performed according to McDaniel & Gordon (1989) and utilized biotinylated Protein A (Amersham, Inc., Arlington Heights, IL, USA) (1:1500 in TBS-T), horseradish-peroxidase–streptavidin conjugate (Amersham) (1:1000 in TBS-T), and 0.1 M citric acid plus 1 mM 2,2'-azino-bis (3'-ethylbenzothiazoline sulphonic acid) diammonium salt, pH 4.2, as the enzyme substrate. The MSStV-US antiserum had been prepared previously (Gingery *et al.*, 1981) and was diluted to 1:400 in TBS-T. Colour development was monitored using a Model EL 309 Automated Microplate Reader (Bio-Tek Instruments, Inc., Winooski, VT, USA) and reactions were stopped by adding 30 µl of 5% sodium dodecyl sulphate.

To correlate the ELISA absorbance values (A_{405}) with the relative concentration of MSStV in the inoculative planthoppers, a dilution series of MSStV, purified according to Ammar *et al.* (1990a) from the three isolates AF, CR and US, was tested in the same ELISA plates as the planthopper samples. Control samples consisted of adult planthoppers of comparable age that

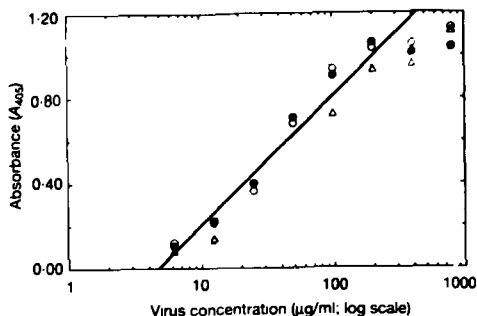


Fig. 1. Relationship between ELISA absorbance values and concentration of maize stripe tenui virus (MSStV) in purified preparations from the US (○), CR (●), and AF (△) isolates. Each point represents the mean of two replicates. Regression lines were fitted to the data up to a concentration of 400 µg/ml. There was no statistically significant difference between the three isolates. The mean regression line was absorbance = $-0.32 + 0.53 \log(\text{concentration})$.

had never been exposed to diseased plants. The results (Fig. 1) indicated that the best relationship between absorbance and antigen concentration (up to about 250 µg/ml) was

$$A_{405} = B_0 + B_1 \log_{10}(\text{concentration})$$

in which B_0 and B_1 are parameters. To estimate virus concentration in the individual planthoppers based on the absorbance readings, the equation was rearranged to

$$\text{concentration} = \text{antilog}[(A_{405} - B_0)/B_1]$$

These estimated concentrations only gave the relative values for virus in the insects, but were calculated to obtain a linear relationship for other statistical analyses (Fig. 1). Because absorbance values were generally less than 1.2 in subsequent insect tests, the linear relationship was satisfactory.

Efficiency of transovarial transmission of MSStV

Young female adults of *P. maidis* that had been exposed as nymphs to MSStV-infected plants (AF, CR or US isolates) for 1 or 7 days were paired with adult males that had not been exposed previously to any diseased plants. Eggs laid by inoculative females were carefully excised from maize leaves in which they had been oviposited, and placed on small pieces of healthy maize leaves on top of a moistened filter paper in a covered Petri dish, to avoid acquisition of MSStV by newly emerged nymphs from the plants on

which their inoculative mothers had previously fed. Hatched nymphs were collected every 24 h, then placed on maize seedlings to test their inoculativity singly for 3 weeks (one insect per seedling per week).

Data analysis

Analysis of variance (ANOVA) was used to determine the effects of virus isolate and acquisition access period (1 or 7 days) on the latent period of MStV in the vector, and to evaluate the effects of isolate, access period and planthopper sex on the relative concentration of MStV in transmitting insects. The general linear model (GLM) procedure of the Statistical Analysis System (SAS; Cory, NC, USA) was used for ANOVA. The least significant difference (LSD) was calculated in order to compare means in each experiment.

Regression analysis was performed in order to estimate the parameters of the relationship between virus concentration and ELISA absorbance values. Chi-square analysis was performed to evaluate the frequency of planthoppers that transmitted MStV and to evaluate the effects of isolate, acquisition access period or sex on this parameter.

RESULTS

Transmission efficiency following oral acquisition of MStV

Following a 1-day or a 7-day acquisition access period on MStV-diseased plants by *P. maidis*, the

transmission efficiency (percentage of inoculative planthoppers) was significantly higher ($P < 0.01$) for the AF and CR isolates than for the US isolate in three experiments, and it was also higher ($P < 0.01-0.05$) for the AF isolate than for the CR isolate in two out of three experiments (Table 1). With the 7-day acquisition access period, the transmission efficiency of the US isolate was highly variable, ranging from 0 to 36%, whereas that of the other two isolates was much less variable (Table 1). Generally, the transmission efficiency of MStV was not affected by the planthopper sex ($P < 0.20$) (data not shown).

A 7-day acquisition access period resulted in a significantly higher transmission efficiency for all three isolates than a 1-day acquisition access period ($P < 0.01$; Table 1), based on a Chi-square test between experiments. The latent period of MStV in the vector, particularly following a 7-day access period, was significantly shorter ($P < 0.01$) for the AF isolate than for the US or CR isolates, and it was also shorter for the CR isolate compared to the US isolate (Table 1).

MStV titre in inoculative planthoppers

The estimated mean concentrations of the three isolates of MStV in inoculative planthoppers, according to ELISA absorbance values 4 weeks after a 1-day or 7-day acquisition access period on diseased plants, are presented in Table 2. Analysis of variance on these data indicated that

Table 1. Efficiency of transmission of maize stripe tenuivirus (MStV) isolates by *Peregrinus maidis*, following 1-day or 7-day acquisition access periods (AAP) on diseased plants during the early nymphal stage

Experiment no. ^a	AAP (days)	MStV isolate	Percentage of insects transmitting MStV ^b	Latent period (weeks) ^b
1	1	US	0 a	
		CR	18 b	1.44 a
		AF	60 c	1.37 a
2	7	US	0 a	—
		CR	74 b	1.58 a
		AF	96 b	1.20 b
3	7	US	36 a	2.59 a
		CR	68 b	1.88 b
		AF	90 c	1.38 c

^a In each experiment, 50 planthoppers per virus isolate were tested for MStV transmission for 4 weeks following virus acquisition.

^b Percentages in the same experiment that are marked with different letters are significantly different ($P < 0.01-0.05$).

^c Indicates that no transmission occurred.

Table 2. Mean relative concentrations of maize stripe tenuivirus (MStV), according to ELISA absorbance values, in inoculative (orally transmitting) planthoppers 4 weeks following 1-day or 7-day acquisition access periods (AAP) on maize plants infected with three MStV isolates

MStV isolate	Relative concentration of MStV (μg/ml)				Overall mean ^a
	1-day AAP		7-day AAP		
	males	females	males	females	
US	— ^b	—	53.46	59.15	56.31 a
CR	91.37	225.97	124.62	260.67	179.05 b
AF	53.07	207.12	112.31	423.11	244.47 b

^a Different letters indicate statistically significant differences based on LSD.

^b Indicates that no transmission occurred.

virus titre was significantly affected by isolate ($P < 0.05$), with a higher concentration for the AF and CR isolates compared to that for the US isolate. No significant difference was found between the AF and CR isolates. A highly significant difference in virus titre was found between males and females ($P < 0.01$). However, as indicated by a significant interaction ($P < 0.05$), differences between the sexes depended on the virus isolate; females apparently contained much more virus than males for the AF and CR isolates, but not for the US isolate. Furthermore, for the AF and CR isolates, higher virus titres were found in planthoppers that had a 7-day access period, compared to those that had a 1-day access period (Table 2), but the overall effect of acquisition access period on virus titre, taking into account all isolates, was not significant.

Efficiency of transovarial transmission

The fraction of planthoppers that acquired MStV transovarially and transmitted it orally within 3 weeks of hatching from eggs was significantly higher for the AF isolate than for the US ($P < 0.01$) or the CR isolate ($P < 0.05$); the latter two isolates did not differ significantly (Table 3). The sex of the progeny did not significantly affect transovarial transmission ($P < 0.20$).

DISCUSSION

This study provides the first direct comparison of a vector's ability to transmit different geographical isolates of a tenuivirus (Gingery, 1988). *P. maidis* from Hawaii transmitted MStV-AF much more efficiently and with a shorter latent period than MStV-US; the transmission efficiency of MStV-CR was of intermediate status. Because the difference between the AF and US isolates was observed for both oral and transovarial transmission, neither differences in (oral) acquisition efficiency nor different rates of virus translocation across the insect midgut barrier seem likely explanations. The latter barrier was reported with Iranian maize mosaic rhabdovirus in *P. maidis* (Nault & Ammar, 1989; Ammar, 1994), and with tomato spotted wilt virus in its thrips vector (Ullman *et al.*, 1992). Because the AF and CR isolates of MStV reached higher titres in *P. maidis* than did the US isolate, it is probable that another dissemination barrier might be responsible for the lower transmission efficiency and lower titre of the US isolate in this vector. Dissemination barriers for propagative plant and animal viruses in their vectors include the midgut-escape and salivary gland-infection

Table 3. Transovarial transmission of maize stripe tenuivirus (MStV) isolates detected by oral transmission tests on *Peregrinus maidis* nymphs^a

MStV isolate	Number of nymphs tested	Percentage of nymphs transmitting MStV ^b
US	47	21 a
CR	102	32 a
AF	91	47 b

^a *P. maidis* nymphs, obtained from MStV-inoculative females, were tested singly for oral transmission for 3 weeks following hatching from the eggs.

^b Different letters indicate significant differences, according to a Chi-square test.

barriers, as well as the ability of a particular virus or virus strain to multiply efficiently in these and probably other tissues of the vector (Hardy, 1988; Ammar, 1994). In addition to the dissemination barrier suggested in the present work, a previous serological study of MStV-US indicated that some non-inoculative *P. maidis* contained virus in the salivary glands (Nault & Gordon, 1988), which suggested a salivary gland-escape barrier for MStV-US in these planthoppers.

Increasing the acquisition access period on diseased plants from 1 to 7 days increased the proportion of planthoppers that transmitted MStV. This is typical of several propagative viruses (Nault & Ammar, 1989), and may be a dosage effect (Falk & Tsai, 1985). However, longer access periods on MStV-diseased plants did not significantly affect the virus titre in inoculative planthoppers. This is likely to be due to MStV multiplication in *P. maidis* (Nault & Gordon, 1988). In other words, longer acquisition access periods increased the probability of MStV acquisition, but once acquired, virus titres reached comparable levels in transmitting individuals. However, the final virus concentration in vectors was dependent on the MStV isolate. The higher MStV titre in females compared to males was probably due to the fact that females weighed about 2.6 times more than males of comparable age (unpublished data).

Since *P. maidis* from Hawaii transmitted MStV-US with much lower efficiency than it transmitted MStV-AF or MStV-CR, it would be interesting to compare the transmission efficiency of the latter two isolates by native and non-native populations of their planthopper vector. Differences in transmission efficiency of various geographical isolates of a particular virus by native and non-native populations of the vector are important epidemiologically, e.g. for making decisions on quarantine regulations, as well as experimentally when comparing vector efficiency results of the same virus in different regions.

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