Differences in Levels of Detection for the Maize Stripe Virus Capsid and Major Non-capsid Proteins in Plant and Insect Hosts

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SUMMARY

Antisera to the M₄ 32000 (32K) capsid and M₅ 16500 (16.5K) major non-capsid proteins were used for immunological analyses of extracts from maize stripe virus (MStpV)-infected maize (Zea mays) plants and from inoculative Peregrinus maidis, the MStpV plant hopper vector. The 32K protein was easily detected in extracts of both MStpV-infected plants and inoculative P. maidis by ELISA and by immunological analysis of Western blots. In a time course study, no 32K protein was detected in P. maidis until 8 days after the beginning of a 5 day acquisition access period on MStpV-infected plants. The percentage of MStpV-positive P. maidis increased with time indicating multiplication of MStpV in P. maidis. The 32K protein was detected only in individual P. maidis that also transmitted MStpV to plant hosts. The 16.5K protein was also detected in MStpV-infected plant hosts but not in extracts of groups or of individual MStpV-inoculative P. maidis. In vitro translation of MStpV virion RNAs in rabbit reticulocyte lysates showed that both the 32K and 16.5K proteins were present in the translation products. The ready detection of the MStpV-coded 32K protein in both plant and insects and detection of the 16·5K protein in only plant hosts is discussed.

INTRODUCTION

Maize stripe virus (MStpV) infects several plant species of the Poaceae and is transmitted by the maize plant hopper, Peregrinus maidis Ashmead. MStpV has several properties in common with a group of other plant hopper-borne viruses, including rice hoja blanca virus (RHBV) (Morales & Niessen, 1983), rice stripe virus (RSV) (Toriyama, 1982; Gingery et al., 1983), European wheat striate mosaic virus (Ammar et al., 1985) and rice grassy stunt virus (Hibino et al., 1985). These viruses have been tentatively grouped in the RSV group (Gingery, 1987).

Based on their virus-vector relationships all members of the RSV group are believed to infect their plant hopper vectors. All exhibit a 10 to 20 day incubation period in their vectors between virus acquisition and subsequent transmission, and transovarial transmission by viruliferous females to a high percentage of their progeny has been shown for MStpV and RHBV (Tsai & Zitter, 1982; Morales & Niessen, 1983). Also, once an individual insect (e.g. P. maidis) begins to transmit a given virus (e.g. MStpV), it retains the ability to transmit for the remainder of its life. Taken together, these data suggest that these viruses have persistent-propagative relationships with their insect vectors. However, proof for MStpV multiplication in P. maidis has not yet been demonstrated.

Two virus-specific proteins, the virion capsid (M₄, 32000, 32K) and a major non-structural protein (16·5K), have been detected in MStpV-infected plants (Gingery et al., 1981; Falk & Tsai, 1983; Ammar et al., 1985). The 16·5K protein appears to be a major component of MStpV-specific inclusion bodies in infected maize (Zea mays L.) (Ammar et al., 1985), and the protein accumulates to very high concentrations in MStpV-infected Z. mays as well as other plant hosts (Gingery et al., 1981; Falk & Tsai, 1983). Although the 16·5K protein can be readily detected in
MStpV-infected plants, we were unable to detect it previously in MStpV-inoculative P. maidis (Falk & Tsai, 1983). That the 16.5K protein is also virus-coded has been questioned (Gordon, 1984; Jones et al., 1984).

We now provide serological data which support the contention that MStpV replicates in its vector, P. maidis. Also, we show that both the 16.5K and 32K proteins are detectable in MStpV RNA-directed in vitro translation products and are therefore encoded by the viral genome. However, only the 32K protein is readily detectable in extracts of MStpV-inoculative (infected) P. maidis.

METHODS

**Virus and vector maintenance.** The MStpV and P. maidis isolates were the Florida, U.S.A. isolates used previously (Falk & Tsai, 1983, 1984; Tsai & Zitter, 1982). Z. mays L. was used as the maintenance host for both MStpV and P. maidis as described (Tsai & Zitter, 1982). For experiments analysing the MStpV-specific proteins, P. maidis were given a 48 to 120 h acquisition access period (AAP) on MStpV-infected Z. mays. P. maidis were then transferred to healthy Z. mays every 3 to 4 days and were harvested at 25 days post-AAP. MStpV-infected Z. mays was harvested 10 to 20 days after inoculation, when plants showed symptoms typical of MStpV infection.

To assay P. maidis at intervals after AAP on MStpV-infected Z. mays, 500 P. maidis were given a 5 day AAP on MStpV-infected Z. mays. Insects were then transferred to healthy plants every 2 to 3 days. Twelve to 15 P. maidis were collected on each sampling date (day 0, before AAP; day 5 at end of AAP, and daily from day 6 to the end of the experiment). Healthy P. maidis were also collected at each sampling date. Each sample was stored at −20 °C until all P. maidis could be individually tested for the MStpV 32K protein by ELISA. Individual P. maidis were considered positive for the 32K protein when their A_{405} values were greater than three times the S.D. plus the mean of healthy controls.

Individual inoculative P. maidis were compared for the ability to transmit MStpV and for the presence of MStpV proteins by allowing a group of 40 P. maidis a 5 day AAP to MStpV-infected Z. mays. These were then transferred as groups of 10 P. maidis per plant every 3 to 4 days to healthy Z. mays. After 17 days on the healthy Z. mays, 24 P. maidis survived. These were then separately caged, each on a single Z. mays seedling, for a 96 h inoculation access period. The 20 surviving P. maidis were removed, numbered and individually stored at −20 °C until they were tested for MStpV proteins. The 20 test Z. mays were numbered correspondingly and observed for symptom development indicative of infection by MStpV.

**Serological detection of MStpV proteins.** MStpV 32K protein was recovered by purification of MStpV nucleoprotein from infected Z. mays as described previously (Falk & Tsai, 1984). The 16.5K protein was purified from infected Z. mays by differential pH precipitation and electro-elution from SDS-polyacrylamide gels (Falk & Tsai, 1983). Antisera were produced by intramuscular injection of purified proteins into New Zealand white rabbits following the protocol used previously (Falk & Tsai, 1983). We emulsified 1 mg of purified protein with Freund's complete adjuvant (Difco) for the first injection, and with Freund's incomplete adjuvant for the second and third injections. Bleedings began 10 days after the third injection.

ELISA tests were done for the MStpV 32K protein using the double antibody sandwich method as described by Clark & Adams (1977). Microtitre plates (Immunol II; Dynatech Laboratories, Alexandria, Va., U.S.A.) were coated with purified immunoglobulins at 1 μg/ml and alkaline phosphatase-conjugated antibodies were used at a 1:1000 dilution. Samples were extracted in PBS (0.05 M-phosphate pH 7.4, 0.15 M-NaCl) containing 0.05% Tween 20 and 2% polyvinylpyrrolidone 40. Individual P. maidis (approx. 1-7 mg) were prepared for ELISA by titration in 1 ml of buffer.

Immunological analysis of Western blots was done by the method of Burnette (1981). Blots were washed for 15 min in PBST (0.02 M-phosphate, 0.15 M-NaCl, pH 7.4, 0.3% Tween 20) containing 3% bovine serum albumin, followed by two 20 min washes in PBST. Antiserum dilutions of 1/500 and 1/375 in PBST for the 32K and 16.5K antisera, respectively, were added to the washed blots which were incubated with shaking for 1.5 h. Blots were given two washes of 20 min in PBST and then incubated in Protein A conjugated with horseradish peroxidase (Boehringer) diluted 1/2000 in PBST for 1 h. Blots were again washed, 5 to 10 ml of substrate (0.1 mg/ml o-phenylenediamine in 0.006% hydrogen peroxide) was added and blots were kept in the dark for 30 to 60 min. Blots were removed from the substrate, briefly rinsed in PBST, allowed to dry and then photographed.

**SDS-PAGE and Western blotting.** Initially, proteins were extracted from uninoculated and MStpV-infected Z. mays and P. maidis by two different methods. Proteins were extracted after the method of White & Brakke (1983) as modified by Falk et al. (1987). This was done to determine whether the 16.5K protein was associated with the virion-containing fraction of plant sap, or if it was a soluble protein. The differential pH extraction method (Gingery et al., 1981; Falk & Tsai, 1983) was used when attempting to concentrate the MStpV 16.5K protein selectively. Two g of plant tissue or 10 to 20 P. maidis were used for each extraction.

To assay individual P. maidis for MStpV proteins, each insect was triturated directly in electrophoresis sample buffer (one insect per 200 μl). Samples were then immediately heated at 100 °C for 2 to 3 min, centrifuged at 12000
**MStpV proteins**

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*g* (max.) for 5 min and further processed for SDS–PAGE. Proteins from 1/10th to 1/20th of an individual insect were applied to a single gel line. As the mean weight of a single *P. maidis* was 1.7 mg, to obtain equivalent samples of *Z. mays* tissue, a single disc was taken from a leaf using a no. 2 cork borer and processed for SDS–PAGE as described for single *P. maidis*.

Proteins were fluorescently labelled before electrophoresis analysis with 2-methoxy-2,4-diphenyl-3(2H) furanone or they were analysed as unlabelled proteins and subsequently detected by silver staining (Morrisey, 1981) or by immunological analysis of Western blots. Gels consisted of 5% and 12% (stacking and resolving gels, respectively) polyacrylamide, or were linear gradient gels of 10 to 20% acrylamide with a 3% stacking gel using the buffer system of Laemmli (1970). Proteins were visualized by exposing the gel to u.v. illumination (302 nm) or by silver staining (Morrisey, 1981). Fluorescently labelled proteins were photographed using Polaroid P/N 665 film and a Wratten 9 filter.

Western blotting of proteins separated by SDS–PAGE was done according to Burnette (1981). Proteins were transferred from SDS gels to 0.2 μm pore size nitrocellulose (BA83, Schleicher & Schüll) using an electroblotting chamber. Transfer was at 10 V/cm for 2 h, or 5 V/cm overnight, followed by 10 V/cm for 15 min. All transfers were done at 4 °C. Blots were then immunologically analysed for MStpV proteins.

**Analysis of MStpV RNA in vitro translation products.** RNAs were extracted from a mixture of the four sucrose density gradient-purified MStpV nucleoprotein components as described previously (Falk & Tsai, 1984). Virion RNA extracted from southern bean mosaic virus (SBMV) was used as a control. Rabbit reticulocyte lysate was obtained from Green Hectares (Oregon, Wisconsin, U.S.A.). The lysate was cleared of endogenous mRNA by treatment with micrococcal nuclease and calcium chloride (Pelham & Jackson, 1976).

A 100 μl lysate mixture was prepared according to Dougherty & Hiebert (1980a) with 6 to 8 μg of MStpV or SBMV RNA and 4 μCi of [35S]methionine (500 to 800 Ci/mmol). The reaction mixture was incubated at 30 °C for 1 h. Samples of the reaction mixture were treated with ribonuclease A and then adjusted to contain 10% glycerol, 2% SDS and 5% 2-mercaptoethanol. Samples were placed in a boiling water bath for 5 min and then analysed by SDS–PAGE in an 8 to 15% acrylamide gradient gel. Products were detected by fluorography.

Translation products were immunologically analysed by selective immunoprecipitation using antisera to the MStpV 32K and 16-5K proteins by the method of Dougherty & Hiebert (1980b). Antigen–antibody complexes were reacted with *Staphylococcus aureus* cell wall proteins (Sigma) and collected by centrifugation. Precipitates were analysed by SDS–PAGE and fluorography.

**RESULTS**

**Detection of MStpV proteins in Z. mays and P. maidis**

ELISA analysis of *P. maidis* after a 5 day AAP on MStpV-infected *Z. mays* showed that the percentage of ELISA-positive *P. maidis* per sampling date increased with time (Table 1). In three separate experiments, each using 12 to 15 *P. maidis* per sampling date, no insects were positive for the 32K protein until at least 8 days after the beginning of the AAP, which was 3 days after removal of the *P. maidis* from the MStpV-infected acquisition source plants. The number of 32K-positive *P. maidis* per sample averaged around 35% from day 12 to the end of the sampling date. The *A405* values for the individual ELISA-positive *P. maidis* at any given sampling date were variable, e.g. those from day 10 in experiment 2 ranged from 0.075 to 0.41. Uninfected *P. maidis* from the same test plate averaged 0.011.

Previous attempts to detect the MStpV 16-5K protein in *P. maidis* by indirect ELISA gave unclear results and double antibody sandwich ELISA is not an efficient way to detect the 16-5K protein, even in plant samples (Falk & Tsai, 1983). When *P. maidis* were tested by indirect ELISA, both healthy and MStpV-inoculative *P. maidis* gave strong non-specific reactions and could not be differentiated. Therefore, we used SDS–PAGE and immunological analysis of the SDS–PAGE-separated proteins to assay unequivocally both the MStpV 32K and 16-5K proteins in the same samples of *Z. mays* and *P. maidis*.

Both the 32K and 16-5K proteins were detected when protein preparations from MStpV-infected *Z. mays* were analysed by SDS–PAGE (Fig. 1). No similar proteins were seen in preparations from uninoculated *Z. mays*. Proteins extracted from uninoculated and MStpV-viruliferous *P. maidis* contained too many proteins to determine visually whether the 32K or 16-5K proteins were present in the preparations. Serological analysis of the protein extracts after their transfer to nitrocellulose membranes showed that the 32K protein was detected primarily in the 145000 *g* pellet fractions of both MStpV-infected *Z. mays* and MStpV-inoculative *P. maidis* (Fig. 2). Somewhat less 32K protein was also detected in the 145000 *g* supernatant...
Fig. 1. SDS-polyacrylamide gel showing proteins extracted from healthy and MStpV-infected *Z. mays* and *P. maidis* samples. Proteins were extracted and fractionated by centrifugation at 145000 g for 90 min. The supernatant and pellet fractions were saved and analysed separately. Proteins were fluorescently labelled before electrophoresis and visualized after electrophoresis was complete by exposing the gel to u.v. illumination. HS and MS refer to healthy and MStpV-infected supernatant fraction proteins, respectively, and HP and MP refer to healthy and MStpV-infected 145000 g pellet fraction proteins, respectively. The first lane shows protein markers. Lanes designated C and NC show purified MStpV 32K capsid and 16.5K non-capsid proteins respectively.

Table 1. *ELISA detection of the MStpV 32K protein over time in P. maidis after acquisition access to MStpV-infected Z. mays*.

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* Groups of *P. maidis* were given a 5 day AAP on MStpV-infected *Z. mays*.
† Shows day of collection relative to the start of the AAP.
‡ The results of three separate experiments are shown. Fraction shows the number of positive *P. maidis* over the number tested.

fraction from MStpV-inoculative *P. maidis* and a strong reaction for the 32K protein was seen for the 145000 g supernatant fraction from MStpV-infected *Z. mays*. In contrast, the 16.5K protein was detected entirely in the 145000 g supernatant fraction from MStpV-infected plants.
Fig. 2. Immunologically analysed Western blot of SDS–PAGE-separated total proteins (see Fig. 1). Upper blot was probed with antiserum to the MStpV 32K capsid protein. Lower blot was probed with antiserum to the MStpV 16.5K non-capsid protein. C shows location of capsid protein reaction. NC and D show locations of non-capsid protein monomer and dimer reactions, respectively.

and not in the virion fraction. It can also be seen that the 16.5K protein represents a significant proportion of the proteins from the MStpV-infected plants. Lane MS of the Z. mays samples in Fig. 1 and 2 is adjusted so that the proteins represent only 50 μg of fresh weight starting material. All other Z. mays lanes (MP, HS and HP) represent the proteins from 500 μg starting material. Lanes MP, MS, HP and HS of the P. maidis samples all represent proteins from equivalent amounts (350 μg) of sample with respect to the starting material.

The 16.5K protein was not detected in the 145000 g protein extracts from pooled MStpV-inoculative P. maidis (Fig. 2), nor was it detected when attempts were made to concentrate this protein by differential precipitation (data not shown).
The serological reaction labelled D for the lanes MS and NC for the blot in Fig. 2 that was probed with antisera to the MStpV 16-5K protein represents the 16-5K dimer. This was a consistent reaction and was slightly above the reaction for the 32K protein. We interpret this as being a 16-5K dimer because it is about twice the mol. wt. of the 16-5K protein, and the original purified 16-5K protein used for antiserum production and for the 16-5K control (lane NC) on this gel was purified by electro-elution from SDS gels, and only samples with a mol. wt. of 16000 to 18000 were electro-eluted.

Because the 16-5K protein was not detected by us in pooled P. maidis samples, we analysed individual P. maidis for both the 16-5K and 32K proteins. Individual P. maidis were first tested for their ability to transmit MStpV as an indicator of MStpV multiplication in P. maidis. These same individuals were then tested for the presence of the MStpV 32K and 16-5K proteins by immunological analysis of SDS-PAGE-separated total proteins. Eleven of the 20 P. maidis tested transmitted MStpV to Z. mays. When total protein extracts from all 20 of these P. maidis were immunologically probed for the 32K and 16-5K proteins, the 32K protein was readily detected only in extracts from the inoculative P. maidis. Results for nine of the transmitters (V) and three non-transmitters (Nv) along with four healthy P. maidis not given an AAP on MStpV-infected Z. mays are shown in Fig. 3. The nine transmitters show positive reactions for the MStpV 32K protein. None of the P. maidis gave a reaction for the 16-5K protein.

We quantitatively compared the levels of detection of the 32K and the 16-5K proteins in total proteins extracted from Z. mays and P. maidis samples of similar weight. When Z. mays and P. maidis samples representing 100 μg were analysed, the 32K protein was readily detected in both, and no reactions were observed for healthy samples. Based on the intensity of reactions for known amounts of the 32K protein, MStpV-infected Z. mays contained about 250 ng of 32K protein per 100 μg, and P. maidis had about 50 ng per 100 μg (Fig. 4). In contrast, the 16-5K protein was estimated to be about 10% of the total proteins in MStpV-infected plant samples or about 10 μg of the 100 μg sample. The 16-5K protein was easily detected when plant samples equivalent to only 2 μg of starting material were loaded onto the gel. The 16-5K protein was not detectable in P. maidis samples which were positive for the 32K protein. In some samples, a reaction was obtained just below the 16-5K region of the gels, but this occurred in a few healthy and in some MStpV 32K-positive P. maidis samples. This reaction also occurred when blots were probed with antisera to tobacco mosaic virus, beet yellows virus and SBMV and therefore is not a 16-5K reaction (data not shown).

Analysis of the MStpV RNA in vitro translation products

When the mixture of MStpV virion RNAs was incubated with the rabbit reticulocyte lysate in vitro, four MStpV RNA-specific prominent proteins of Mr larger than about 15000 were seen in the products (Fig. 5). (The prominent protein at 46000 is the endogenous globin mRNA product and can also be seen in the control lane.) Two of these co-electrophoresed with purified MStpV 32K (C) and 16-5K (NC) proteins. The protein co-electrophoresing with purified 32K protein was specifically immunoprecipitated with antiserum to MStpV 32K protein. Similarly, the translation product that co-electrophoresed with purified MStpV 16-5K protein was specifically immunoprecipitated by antiserum to MStpV 16-5K protein. No MStpV translation products were immunoprecipitated by antiserum to SBMV. Similarly, antiserum to the MStpV 32K protein did not immunoprecipitate any of the SBMV translation products.

Discussion

The data shown here provide evidence that MStpV multiplies in its vector P. maidis, that both the 32K and the 16-5K proteins are encoded by the MStpV virion RNAs, and that the 32K and 16-5K proteins are readily detectable in MStpV-infected Z. mays, yet only the 32K protein is detectable in MStpV-inoculative P. maidis. The ELISA detection of the 32K protein over time in P. maidis after AAP on MStpV-infected Z. mays supports the biological data suggesting the multiplication of MStpV in P. maidis. No 32K protein was detectable immediately after AAP, and in the blotting experiment (Fig. 3), the 32K protein was detectable only in transmitters.

Despite the sensitive, specific detection of the MStpV 16-5K protein from MStpV-infected Z.
Fig. 3. Total protein extracts from individual *P. maidis* after analysis by SDS–PAGE and Western blotting. Individual *P. maidis* were exposed to MStpV and individually tested for their ability to transmit MStpV as described in the text. V indicates that the individual transmitted MStpV and NV that it did not. H are healthy individual *P. maidis* that were not exposed to MStpV. (a) SDS–PAGE-separated proteins. (b) The above proteins after electrophoretic transfer to nitrocellulose and probing with antisera to the MStpV 32K capsid protein antiserum. Arrow shows location of 32K protein serological reaction. (c) Proteins after electrophoretic transfer and probing with antisera to the MStpV 16.5K non-capsid protein. Arrow shows location of 16.5K protein serological reaction. MS shows purified 32K and 16.5K MStpV proteins. Locations of protein standards are shown at the right.

*mays*, this protein was not detected in inoculative *P. maidis*, even by immunological analysis of total proteins on Western blots. In ‘spiked’ experiments we added small quantities of the partially purified 16.5K protein (2 μg) to individual insects during extraction of total proteins. Proteins representing 1/10 of the individual insects were analysed in a given gel lane and the 16.5K protein was consistently recovered and detected as well as when the 16.5K protein alone was tested (data not shown). This represents strong detection of only 200 ng protein. We could consistently detect as little as 100 ng of the 16.5K protein in spiked *P. maidis* extracts. In contrast, we estimated the amount of the 16.5K protein in symptomatic *Z. mays* leaves to be about 10 μg/100 μg of leaf tissue.
Fig. 4. Immunologically analysed Western blots for SDS–PAGE-separated total proteins from 100 µg samples of *Z. mays* and *P. maidis*. Both (a) and (b) show results for healthy (H) and MStpV-infected (M) *Z. mays*. Aliquots from the same samples were used for blots (a) and (b). Blots (c) and (d) show results for healthy (H) and MStpV-infected (M) *P. maidis*. Lanes 1, 2, 3 and 4 are healthy *Z. mays* (a and b) or healthy *P. maidis* (c and d) samples which contain 200, 100, 50 and 25 µg of purified 32K plus 16.5K proteins, respectively. Antisera were to 32K (a, c) or 16.5K (b, d) proteins.

The 32K protein was estimated to be present in MStpV-infected *Z. mays* at 250 ng per 100 µg, while in *P. maidis* the 32K protein was present at about 50 ng per 100 µg. Therefore, the ratio of the 16.5K:32K proteins in MStpV-infected *Z. mays* is about 40:1. As the 32K protein is fivefold less by weight in *P. maidis* as opposed to *Z. mays*, if the 16.5K protein were present in *P. maidis* in similar amounts relative to the 32K protein, we could expect 2 µg of the 16.5K protein in a 100 µg *P. maidis* sample. But since the 16.5K protein is not detectable in *P. maidis* and the limit of detection is 100 ng in our system, this shows that if the 16.5K protein is present at all in *P. maidis* the ratio of 32K:16.5K protein is at least 20-fold less than it is in *Z. mays*. By weight the amount of 16.5K protein in *P. maidis* as opposed to *Z. mays* would be at least 100-fold less.
Fig. 5. Analysis of MStpV RNA and SBMV RNA in vitro translation products. [\(^{35}\)S]Methionine-labelled products were separated in an 8 to 15% SDS–polyacrylamide gel and detected by fluorography. Lane 1, MStpV translation products immunoprecipitated by MStpV 32K capsid protein antiserum; lane 2, MStpV products untreated; lane 3, MStpV products immunoprecipitated by MStpV 16.5K non-capsid protein antiserum; lane 4, SBMV translation products immunoprecipitated by SBMV capsid protein antiserum; lane 5, MStpV translation products immunoprecipitated by SBMV capsid protein antiserum; lane 6, no exogenous RNA; lane 7, SBMV translation products immunoprecipitated by MStpV 32K capsid protein antiserum; lane 8, total products of SBMV translation. C and NC show locations of MStpV 32K capsid and 16.5K non-capsid proteins, respectively.

These data would not be surprising if, as has been suggested (Jones et al., 1984), the 16.5K protein is a host-coded protein induced to high levels in Z. mays by MStpV infection. However, our data provide strong evidence that both the 32K and 16.5K proteins are coded for by the MStpV genome. A mixture of all the MStpV RNAs was used for the in vitro translations and therefore no assignment of the 32K or 16-5K proteins to a specific RNA can be made. The translation efficiency of the MStpV RNAs was poor relative to the control SBMV RNA. Similar results were obtained for the related RSV (Toriyama, 1986). Also, it is very unlikely that the proteins detected by our in vitro translation represent the total proteins which are encoded by the MStpV genome. However, the specific immunoprecipitation of proteins which co-electrophoresed with the 16.5K and 32K proteins from the translation products strongly suggests that the 16.5K and 32K proteins are virus-coded and not virus-induced host-coded proteins.

These data then raise the question: if the 16-5K and 32K proteins are encoded by the MStpV genome and MStpV infects both plants and insects (P. maidis), why then is the 16-5K protein so prominent in infected plant hosts and not detectable in MStpV-inoculative (infected) P. maidis? One possibility is that the 16-5K protein is present in MStpV-infected P. maidis, but in a much lower concentration, relative to the 32K protein, than it is in MStpV-infected plant hosts, and it was undetected by us. Another is that the 16-5K protein is not produced during MStpV infection...
of *P. maidis*, but is produced during MStpV infection of plant hosts. At this point, it is not clear which of these possibilities, if either, is correct.

The differences seen here between detection of the MStpV-specific proteins in MStpV-infected insect (*P. maidis*) and plant hosts are interesting but perhaps not unique. There has been little research on virus-specific proteins, other than capsid proteins, for many of the viruses that infect plant hosts as well as their insect vectors. Wound tumour virus, a member of the reoviridae which infects both plant and insect hosts, has some isolates which can only infect plants (Liu et al., 1975; Reddy & Black, 1977; Nuss & Summers, 1984). These transmission-defective isolates have deletions in specific genomic RNA segments that are required for infection of insect hosts but not plant hosts. Similarly Sindbis virus, an alphatogavirus that infects vertebrate hosts and its invertebrate vector, matures differently and establishes different types of infections in the two cell types (Gliedman et al., 1975). Also, actinomycin D inhibits the maturation of the virus in invertebrate cells but not in vertebrate cells (Scheeffers-Borchel et al., 1981). If MStpV replicates differently in invertebrate and plant hosts, we might then expect to see differences between the MStpV-specific proteins made in the different hosts.

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