# Use of Polyclonal Antisera and Monoclonal Antibodies to Examine Serological Relationships Among Three Filamentous Viruses of Sweetpotato

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## ABSTRACT

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Sweetpotato is susceptible to infection by several filamentous viruses of which only the aphid-transmitted sweetpotato feathery mottle virus (SPFMV) has been well-characterized. Sweetpotato mild mottle (SPMMV; transmitted by whiteflies) and sweetpotato latent (SPLV; for which no vector has been identified) viruses, although well-recognized, have not been definitively classified. The serological relationships among these viruses were re-examined with SPFMV-, SPLV- and SPMMV-specific polyclonal antisera and potyvirus cross-reactive monoclonal antibodies (MAbs). No significant relationships were detected between SPMMV and SPFMV, SPLV, or any other aphid-transmitted potyvirus. None of the MAbs reacted to SPMMV. Several MAbs reacted with epitopes common to SPFMV and SPLV, whereas others reacted with epitopes on only one of these viruses. With the exception of one MAb specific for SPFMV, all MAbs used recognized epitopes present on other distinct potyviruses. An apparent distant relationship between SPFMV and SPLV was also revealed by probing Western blots with respective polyclonal antiserum. Thus, SPFMV and SPLV share some common epitopes but are easily differentiated when potyvirus cross-reactive MAbs are used. SPMMV appears to be serologically distinct from any of the aphid-transmitted potyviruses; it possesses none of the epitopes recognized by the MAbs used. These MAbs included PTY 1, which cross-reacts with almost every aphid-transmitted potyvirus (and SPLV) so far tested. We concluded that SPFMV, SPLV, and SPMMV are three separate but atypical potyviruses, and SPFMV and SPLV are more closely related to typical aphid-transmitted members of the potyvirus group.

Sweetpotato (*Ipomoea batatas* (L.) Lam.) may be severely affected by at least five viruses with elongated, flexuous particles (31). Only sweetpotato feathery mottle virus (SPFMV), which is found virtually everywhere the crop is grown, has been even partially characterized (1,5,28). Because SPFMV is widely distributed, it is frequently identified as a component of mixed infections, which further interferes with correct identification of these viruses. Two other viruses with long flexuous virions, sweetpotato latent virus (SPLV) and sweetpotato mild mottle virus (SPMMV), have been separated from SPFMV. Virions of the three viruses have distinct normal lengths, all within the acceptable limits for potyviruses, and each virus induces cytoplasmic inclusions characteristic of potyviruses (6,7,17). Neither SPLV nor SPMMV has been adequately characterized relative to SPFMV, and thus the extent of their serological relatedness to each other or to other potyviruses has not been established.

SPFMV has many of the biological and cytopathic characteristics of potyviruses, including aphid transmissibility, occurrence of pinwheel inclusions, and a relatively narrow host range. Structurally, SPFMV is significantly larger than most potyviruses. The size of virions ranges from 810 to 865 nm (30,33); the capsid protein is 35 kDa (1), and the RNA is estimated to have an MW of $3.65 \times 10^6$ Da (28). SPLV is more similar morphologically to typical potyviruses than either SPFMV or SPMMV, but neither aphids nor whitefly species have been shown to vector the virus (7; and J. W. Moyer, *unpublished*). SPLV occurs in Taiwan and mainland China and has a wider host range than SPFMV (7). Although SPMMV is structurally similar to SPFMV, it differs in many other characteristics. SPMMV is reportedly transmitted by *Bemisia tabaci*, has been mechanically transmitted to 45 plant species representing 14 families, and is known to occur only in East Africa (17). SPMMV is considered by some (e.g., 17,31) to be synonymous with sweetpotato virus B (34). A proposal to classify SPMMV in the monotypic genus Ipomovirus of the Potyviridae has been forwarded to the Plant Virus Subcommittee of the International Committee on Taxonomy of Viruses (4).

Previous studies (e.g., 7,17) using virus-specific polyclonal antisera have not revealed any serological relationships among these viruses or with any other potyviruses. SPFMV, SPLV, and SPMMV are of interest not only because of their importance to sweetpotato production, but because they provide an additional opportunity to compare serological relationships between nonaphid-transmitted potyviruses and the more numerous and thoroughly investigated aphid-transmitted potyviruses.

Polyclonal antiserum to the trypsin-resistant core of the capsid protein from Johnson grass mosaic virus revealed the possibility of epitopes common to the whitefly-transmitted SPMMV, the mite-transmitted wheat streak mosaic virus, and aphid-transmitted potyviruses (35). This contrasts with a study in which the monoclonal antibody (MAb) PTY 1, specific for aphid-transmitted potyviruses (22), reacts with SPFMV and SPLV but not with SPMMV.

Our goal in this research was to define the serological relationships among these three atypical potyviruses. A relatively large number of MAbs with differing cross-reactivities to aphid-

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transmitted potyviruses was used in enzyme-linked immunosorbent assay (ELISA) and Western blot analysis of the three viruses as well as in homologous and heterologous combinations of virus and polyclonal antiserum.

## MATERIALS AND METHODS

**Viruses.** Two serologically distinct strains of SPFMV (5,30) and a single isolate each of SPLV (7) and SPMMV (17) were used in this study. SPFMV-C and SPFMV-RC were obtained from naturally infected sweetpotato in North Carolina and were separated by single aphid transfers (23); both were propagated in *Ipomoea nil* 'Scarlet O'Hara'. These isolates of SPFMV do not infect *Nicotiana* spp. (29). SPLV originated in Taiwan, and SPMMV was from Kenya. The isolates of SPLV and SPMMV were provided by A. A. Brunt (AFRC Institute of Horticultural Research, Littlehampton, England). SPLV and SPMMV were propagated in *N. benthamiana* and *N. tabacum* 'NC 95', respectively, under USDA-APHIS PPQ permit 59048. SPFMV was purified by the method of Cali and Moyer (5). SPLV and SPMMV were purified by a method previously used for other potyviruses (10). Polyclonal antiserum to each virus was produced in New Zealand white rabbits immunized with preparations of native virions emulsified in Freund's complete adjuvant as described previously (5).

Previously described isolates (13,22) of SPFMV (Ibadan isolate, SPV-I), potato virus Y (PVY-3), and bean yellow mosaic virus (BYMV-GDD), with known reactions with the MAbs for aphid-transmitted potyviruses (22), were used as internal controls for ELISA assays with MAbs.

**Serological analysis.** Polyclonal antisera were cross-absorbed with partially purified extracts from healthy plants of the species used for virus propagation, and the IgG fraction was purified by protein-A affinity chromatography as previously described (10). The purified IgG fraction from each viral antiserum was titrated on Western blots to yield bands of similar intensity for each of the respective homologous reactions. At least two blots were probed with each of the standardized antisera.

The aphid-transmitted potyvirus-reactive MAbs PTY 1–43 have been described; MAbs PTY 1–5 and 8–10 have differential spectra of cross-reactivity with diverse potyviruses (22). MAb TBV 27C2H2 was raised against tulip breaking virus and also cross-reacts with a number of distinct potyviruses (14,18,19). MAb FMV 7H8 was prepared against an admixture of SPFMV-C and Nigerian isolate SPV-I of SPFMV and reacts strongly with SPV-I but weakly with SPFMV-C (J. Hammond, H. T. Hsu, and G. Thottappilly, *unpublished*). Tissue culture supernatant fluids of the PTY MAbs were diluted 1:10 for ELISA as previously described (22); TBV 27C2H2 and FMV 7H8 ascitic fluids were diluted 1:4,000 and 1:5,000, respectively. For Western blots, ascitic fluids containing PTY 1 (1:25,000) or culture supernatant fluids of PTY 1, 2, 4, or 8 were diluted 1:15. TBV 27C2H2 and FMV 7H8 ascitic fluids were diluted as for ELISA. Polyclonal antisera to each of the viruses were used as positive controls in each of the ELISA experiments.

The double antibody sandwich (DAS) ELISA was performed as previously described (9). We conducted assays, designed to quantitatively compare serological relatedness, by titrating SPFMV-C and SPFMV-RC antisera against their respective viruses to determine the appropriate antiserum dilutions that would result in similar responses for homologous reactions. A range of standardized antiserum dilutions for each virus, which would result in ELISA $A_{405nm}$ values extending to those observed in negative controls, was identified. The shapes of the dose response curves for both virus-antiserum combinations were similar. The range of $A_{405nm}$ values for each combination of virus and antiserum was used to calculate the dilution at which 50% of the signal was lost, which is essentially the midpoint of the linear portion of the dose response curve. Calculations were performed by probit analysis (SAS computer program, SAS Institute, Cary, NC).

Indirect ELISA analyses were conducted on plates coated with sap extracts from healthy and virus-infected plants as previously described (22). The Western analysis protocol of Gray et al (10) was used for polyclonal antisera and similarly for MAbs, except that alkaline phosphatase was used in the detection system, as previously described (11). Highly purified samples of SPFMV-C, SPFMV-RC, and SPLV were obtained. The SPMMV preparation contained other, presumably host-derived, bands and was used at maximum concentration, which was less than that attainable for the other viruses. Virus sample concentrations were standardized by adjusting aliquots of purified virus to give a similar intensity of silver-stained capsid protein bands on polyacrylamide gels. A single, standardized preparation of each virus was used to prepare multiple replicas of blots onto nitrocellulose membranes (Schleicher & Schuell, Keene, NH).

## RESULTS

**Serological relationships among SPFMV, SPLV, and SPMMV.** Serological analyses by DAS-ELISA and immunobinding assays (i.e., native virus and antisera specific for each virus) revealed no cross-reactivity between viruses (data not shown). Heterologous and homologous DAS-ELISA assays at nonlimiting dilutions of antisera did not distinguish between SPFMV-C and SPFMV-RC in any combination of coating and conjugated antibody. When the assays were conducted over a range of antiserum dilutions beginning with standardized homologous reactions, the distinct serological nature of the two strains was revealed. The calculated antiserum dilutions, at which the intensity of the DAS-ELISA

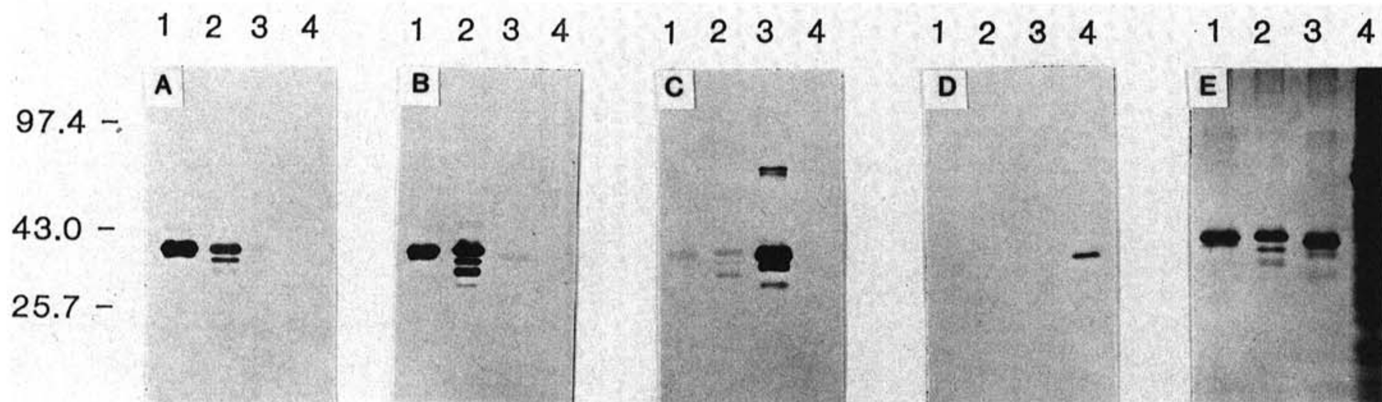


**Fig. 1.** Total protein stain and Western blots (10) of the virus preparations with virus-specific polyclonal antisera. Virus sample concentrations were adjusted to yield capsid protein bands of similar intensity in silver-stained gels (E). Purified IgG fractions were previously titrated to give bands of similar intensity for the homologous antigen. **A,** SPFMV-C antiserum; **B,** SPFMV-RC antiserum; **C,** SPLV antiserum; **D,** SPMMV antiserum; and **E,** silver-stained total protein. The lanes in each panel are 1, SPFMV-C; 2, SPFMV-RC; 3, SPLV; and 4, SPMMV.

reaction was at the 50th percentile, were 1:791 and 1:699 for the SPFMV-C and SPFMV-RC homologous reactions, respectively. These reactions were in contrast to the calculated dilutions for the heterologous mixtures, 1:163 for the SPFMV-C/SPFMV-RC antisera combinations and 1:88 for SPFMV-C/SPFMV-RC antisera combinations, required for the same response.

Polyclonal antisera to the four virus isolates were also used to probe electrophoretically separated, denatured capsid proteins on nitrocellulose membranes (Fig. 1). Homologous and heterologous reactions between the strains of SPFMV (Fig. 1A,B) were consistent with differences observed in the DAS-ELISA. Slight, but consistent differences were observed in the intensity of the full-length capsid protein bands as well as in the faster migrating bands between the homologous and heterologous reactions of the two SPFMV strains (Fig. 1A,B; lanes 1,2). The SPFMV-C antiserum did not react with either SPLV or SPMMV (Fig. 1A). The SPFMV-RC antiserum produced a weak reaction with SPLV and no detectable reaction with SPMMV (Fig. 1B). In reciprocal tests (Fig. 1C), SPLV antiserum weakly detected capsid protein from both strains of SPFMV. SPLV antiserum did not detect SPMMV, whereas SPMMV antiserum only reacted with the SPMMV capsid protein.

**Reactions of SPFMV, SPLV, and SPMMV with MAbs for aphid-transmitted potyviruses.** In the indirect ELISA assay on sap samples, eight of 13 PTY MAbs previously shown to cross-react with multiple distinct potyviruses (21,22) reacted with either SPFMV-C and SPFMV-RC and/or SPLV. None of these MAbs clearly distinguished between SPFMV-C and SPFMV-RC (Table 1). Mab TBV 27C2H2 reacted strongly only with SPLV, and MAb FMV 7H8 reacted reliably only with SPFMV-RC. None of the MAbs had a significant reaction with SPMMV (Table 1). A positive reaction with SPMMV polyclonal antiserum (0.4 OD at 405 nm after 45 min of substrate incubation, compared to 0.04 OD for the healthy control) confirmed the presence of serologically reactive antigen in the sap extract tested.

The four PTY MAbs with the strongest reactions to one or more virus isolates in indirect ELISA (PTY 1, 2, 4, and 8; Table 1) and MAbs TBV 27C2H2 and FMV 7H8 were tested on Western blots. In general, the results matched those of the ELISA, except that the Western blots revealed some reactions not readily observable by indirect ELISA. None of the MAbs produced a significant reaction against SPMMV. A minor reaction (to a band migrating slower than the major coat protein band) was observed with MAb FMV 7H8 (Fig. 2B) and with MAb PTY 1 on two replicates probed with ascitic fluid (Fig. 2G). This reaction was not observed on two replicates with PTY 1 tissue culture supernatant fluid (Fig. 2F) and was not considered significant, because the coloration was faint and pinkish unlike the purple-blue of the other bands. Intense reactions with both isolates of SPFMV and SPLV were obtained with MAbs PTY 1, 4, and TBV 27C2H2. MAb

TABLE 1. Use of monoclonal antibodies reactive with various aphid-transmitted potyviruses in indirect antigen-coated plate enzyme-linked immunosorbent assay differentiation of sweetpotato feathery mottle virus (SPFMV), sweetpotato latent virus (SPLV), sweetpotato mild mottle virus (SPMMV), potato virus Y (PVY-3), and bean yellow mosaic virus (BYMV-GDD)

| Virus[a] | Tissue culture supernatants[b] | | | | | | | Ascitic fluids[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PTY 1 | PTY 2 | PTY 3 | PTY 4 | PTY 8 | PTY 10 | PTY 43 | PTY 1 | FMV 7H8 | TBV 27C2H2 |
| SPV-I PV | +++ | ++ | + | + | – | – | – | +++ | +++ | +++ |
| SPFMV-C sap | + | (+) | – | – | – | – | – | + | ND | (+) |
| SPFMV-C lyo | +++ | + | (+) | + | – | – | – | +++ | + | + |
| SPFMV-RC sap | ++ | + | – | + | – | – | – | ++ | ND | (+) |
| SPFMV-RC lyo | +++ | +++ | + | ++ | – | – | – | ++ | ++ | + |
| SPLV sap | +++ | – | – | +++ | +++ | + | – | +++ | ND | +++ |
| SPLV lyo | +++ | – | – | +++ | –[c] | + | – | +++ | – | +++ |
| SPMMV sap | – | – | – | – | – | – | – | – | – | – |
| PVY-3 sap | +++ | + | – | ++ | + | ++ | – | +++ | – | – |
| BYMV-GDD sap | +++ | + | + | ++ | + | – | +++ | +++ | – | – |

[a]Sap extracts (1:100 dilution) and extracts of lyophilized leaf (0.05 g ground in 1.2 ml of coating buffer plus 2% polyvinyl pyrrolidone; 1 ml of cheesecloth filtered extract diluted to 10 ml with coating buffer) were used to coat plates. SPFMV-SPV-I was purified virus diluted to 2 μg/ml in carbonate coating buffer; SPFMV-C and -RC were in *Ipomoea nil*, SPLV and BYMV-GDD in *Nicotiana benthamiana*, SPMMV in *N. tabacum* NC 95, and PVY-3 in potato. One hundred microliters per well of sample and reagents was used to perform antigen-coated plate ELISA as described (22).

[b]– = <2× Healthy control; (+) = <0.2 $OD_{405}$ and >2× healthy control; + = 0.2–0.6; ++ = 0.6–1.2; +++ = >1.2. Values are $OD_{405nm}$; ND = not done.

[c]The difference between sap and lyophilized tissue suggests that the epitope is affected by lyophilization.

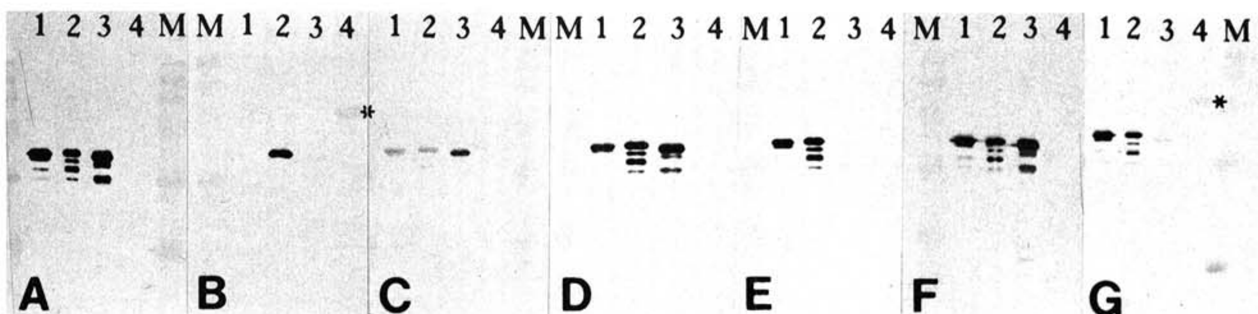


**Fig. 2.** Western blots developed with monoclonal antibodies (MAbs) as described (10), except that the substrate used was a mix of Nitro Blue Tetrazolium and bromochloroindolyl phosphate (11). Virus sample concentrations were adjusted to yield capsid protein bands of similar intensity on silver-stained gels (see Fig. 1E). The lanes in each panel are as follows. 1, SPFMV-C; 2, SPFMV-RC; 3, SPLV; 4, SPMMV; and M, prestained molecular weight standards. The MAbs used to develop each blot were **A**, TBV 27C2H2 (ascitic fluid, 1:4,000); **B**, FMV 7H8 (ascitic fluid, 1:5,000); **C**, PTY 8 (culture supernatant [cs], 1:15); **D**, PTY 4 (cs, 1:15); **E**, PTY 2 (cs, 1:15); **F**, PTY 1 (cs, 1:15); and **G**, PTY 1 (ascitic fluid, 1:25,000). The bands marked with an asterisk (*) in lanes B4 and G4 were pinkish rather than purple-blue and were interpreted as nonspecific artifacts due to the ascitic fluid. No such band was observed in F4 with PTY 1 culture supernatant, although the other bands were of equal or greater intensity in F compared to G.

PTY 2 had a strong reaction to the SPFMV isolates and a weaker reaction to SPLV. PTY 8 reacted more strongly to SPLV than to SPFMV. FMV 7H8 reacted strongly only with the major band of SPFMV-RC, weakly with the major band of SPFMV-C, and not with SPLV (Fig. 2).

## DISCUSSION

No relationship between isolates of SPFMV and SPLV was reported when polyclonal antisera was used in DAS-ELISA (7,31). Such results were confirmed in this study. However, Western blots with virus-specific polyclonal antisera revealed a reciprocal relationship between SPFMV and SPLV, and several MAbs reacted with both SPFMV and SPLV in indirect ELISA (Table 1) and on Western blots (Figs. 1,2). The more sensitive Western blot analyses revealed clear relationships, whereas only weak or insignificant reactions were obtained in indirect ELISA with some of the MAbs (Table 1; Fig. 2).

SPLV has an epitope (recognized by MAb PTY 1) common to almost all of the aphid-transmitted potyviruses tested, including SPFMV (21,22; R. L. Jordan and J. Hammond, *unpublished*). PTY 1 has no apparent reaction with nerine virus Y, nerine yellow stripe virus, or a potyvirus from *Gloriosa* (24), or with some isolates of peanut mottle virus (25) and papaya ringspot virus type W (3); PTY 1 reacts with other isolates of these latter two viruses (3; R. L. Jordan and J. Hammond, *unpublished*). Other epitopes are shared by SPLV and SPFMV with such diverse potyviruses as bean yellow mosaic virus (BYMV), iris mild mosaic virus, potato virus Y, and maize dwarf mosaic virus B (reactions with MAbs PTY 1, 2, 4, and 8), tobacco etch (TEV) and turnip mosaic viruses (MAbs PTY 1, 2, and 8), and zucchini yellow mosaic virus (MAbs PTY 1 and 4) as well as others (21,22).

In contrast, MAb FMV 7H8 (selected against a Nigerian isolate of SPFMV) reacted strongly only to SPFMV-RC, weakly to SPFMV-C, and did not detect SPLV or SPMMV. In prior tests (J. Hammond, H. T. Hsu, and G. Thottappilly, *unpublished*), FMV 7H8 reacted strongly to some isolates of SPFMV from different parts of the world and weakly or not at all to other SPFMV isolates that gave clear reactions with polyclonal SPFMV antiserum. The reactions obtained with MAb FMV 7H8 were primarily to the major coat protein band, with little, if any, reaction to the degradation products. Dougherty et al (8) and Shukla et al (36) have demonstrated that most of the virus-specific epitopes of potyviruses are surface-located in the amino-terminal portion of the coat protein, which is easily cleaved from the virion by trypsin (16) or during storage (15,27). Dougherty et al (8) showed that an MAb with strain specificity for TEV was reactive with an exterior, trypsin-cleavable portion of the coat protein, presumably the N-terminal domain. Previously, we showed that an MAb reactive with a single isolate of BYMV reacts with a surface-located epitope (22) in the N-terminal portion (12,20). It is, therefore, highly likely that the virus and strain specificity of MAb FMV 7H8 is due to the location of an epitope in the N-terminal domain of SPV-I, SPFMV-RC, and other isolates with which the MAb reacts, and that this epitope is altered in SPFMV-C and other weakly or nonreactive SPFMV isolates. The location of the epitope is consistent with the band pattern observed (Fig. 2B).

We observed no significant heterologous activity of either polyclonal antisera or MAbs against SPMMV, which contrasts to the report by Shukla et al (35). Minor color development with a higher MW band in the SPMMV lane with MAbs FMV 7H8 and PTY 1 ascitic fluids was not considered significant, and no color development was observed in the region of the major SPMMV-specific band. The minor reaction with the higher MW band in these cases might have been due to nonspecific reactions of the ascitic fluid, because it occurred only with ascitic fluid and not with tissue culture supernatant of PTY 1, or to a direct activity of the band itself on the substrate. As noted above, the reaction was pinkish rather than purple-blue. Figure 1b of Shukla et al (35) shows an apparent dimer with an obvious reaction, but essentially an absence of reaction at the predicted position of the major coat protein band, except *outside* the lane. In contrast, there is an obvious reaction in the wheat streak mosaic virus lane of the same figure, which may represent a true cross-reaction. The absence of a clear reaction with the SPMMV capsid protein monomer in their experiments precludes definitive conclusions but suggests that the reaction they observed may also have been nonspecific. The absence of a cross-reaction in our experiments with polyclonal antisera also points to a lack of significant serological reaction between SPFMV or SPLV and SPMMV. As in the work of Shukla et al (35), we used polyclonal antisera to probe virus preparations on Western blots. The SPFMV and SPLV antisera contained antibodies reactive with dissociated subunits and the trypsin-resistant core of the coat protein as shown by the reactions with degradation products that migrated faster than full-length coat protein (Fig. 1A,B,C; lanes 2,3). Such antibodies potentially react to conserved linear epitopes in the trypsin-resistant core; Shukla et al (35) propose these epitopes as the basis for the broad cross-reactivity of their polyclonal antiserum. The presence of serologically reactive SPMMV in the sap extracts used for indirect ELISA with the MAbs was confirmed by the reaction with polyclonal SPMMV antiserum in the same assay system. The presence of SPMMV antigen on the Western blots was also confirmed with the polyclonal antiserum (Fig. 1D).

MAbs, cross-reactive with nine separate epitopes present on distinct subsets of aphid-transmitted potyviruses (14,18,19,21,22), have been used to demonstrate the presence of epitopes common to SPFMV and SPLV as well as other potyviruses and to distinguish SPFMV isolates from SPLV. None of the PTY MAbs or MAb TBV 27C2H2 discriminated between SPFMV-C and SPFMV-RC, whereas MAb FMV 7H8 reacted strongly with SPFMV-RC, very poorly with SPFMV-C, and differentially with other isolates of SPFMV (data not shown). No correlation has been recognized between FMV 7H8 reactivity and biological properties or geographical origin of SPFMV isolates. Other research (J. W. Moyer, *unpublished*), in which isolates of SPFMV from throughout the world have been examined, indicates that RC is the predominant serotype. None of the 10 distinct epitopes recognized by these MAbs was found to be present on SPMMV, and no evidence was obtained from reactions with polyclonal antisera to suggest any relationship between SPMMV and either SPFMV or SPLV.

One of the biological differences between SPFMV and SPLV isolates is that SPLV has no known aerial vector, whereas SPFMV is readily transmitted by several species of aphids (e.g., 7). Isolates of several other normally aphid-transmissible potyviruses that have lost aphid transmissibility, including isolates of BYMV (38), TEV (37), plum pox virus (26), and tobacco vein mottling virus (2) have been reported. It is conceivable that SPLV could be a nonaphid-transmissible, serologically distinct isolate of SPFMV. Because sweetpotato is vegetatively propagated and the lack of obvious symptoms (7) acts against rogueing of SPLV-infected plants, SPLV could have been perpetuated without vectored transmission. However, SPLV is a distinct potyvirus as is indicated by the clear differentiation of SPLV and SPFMV isolates with polyclonal antisera and the differential reactions of some MAbs. Sequence analysis of the SPLV capsid protein gene is currently underway and should clarify this relationship.

In this report, we examined the relationships of SPFMV, SPLV, and SPMMV to each other as well as to other potyviruses and conclude that they are three distinct and atypical potyviruses. SPFMV and SPLV appear to be more closely related to typical aphid-transmitted members of the potyvirus group. Our results can be used to begin resolving the synonymy that has plagued the identification of filamentous viruses that infect sweetpotatoes. This approach can also be applied to the other filamentous viruses of sweetpotato (e.g., sweetpotato vein mosaic virus [32] and sweet potato yellow dwarf virus [7]) to determine if they are distinct viruses or related to the three better characterized viruses examined here. In addition to the inherent taxonomic value, clarifi-

cation of relationships and establishment of definitive methods of identification will improve quarantine guidelines and facilitate the international movement of germ plasm of this important crop.

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