# ELISA Detection of Various Tomato Spotted Wilt Virus Isolates Using Specific Antisera to Structural Proteins of the Virus

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**ABSTRACT**

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Antibodies to electrophoretically isolated 26,000 MW nucleoprotein (26K NP) and 78,000 MW membrane protein (78K MP) of the BL isolate of tomato spotted wilt virus (TSWV) were produced. The antibodies were tested in different ELISA assays, as was a previously produced antibody from rabbit immunized with virus preparations of the BL isolate. Thirty TSWV isolates (most from the United States, a few from other countries) were analyzed and compared by ELISA with the different antibodies. All 30 isolates reacted positively in double antibody sandwich (DAS) direct ELISA with antibodies to the whole virion. Nineteen isolates were consistently detected in different ELISA tests using various antibodies, while the other 11 isolates either were not detected or were inconsistently detected in these tests. Of the 11 isolates that gave inconsistent results, eight were purified and compared by western blot. They reacted similarly to antibodies to the virion and to anti-26K NP and anti-78K MP antibodies. Most isolates of TSWV from the United States were considered to be closely related serologically. The DAS direct ELISA using antibodies to the whole virion is recommended over the use of antibodies to structural proteins for detecting a diverse selection of isolates of TSWV.

Tomato spotted wilt virus (TSWV) is the sole member of the tomato spotted wilt virus group (17). The virus is distributed throughout the world and is transmitted by several species of thrips in a persistent manner. TSWV has a wide host range, infecting 157 species of 29 dicotyledonous families and six species of five monocotyledonous families (3). Newly discovered diseases caused by this virus in vegetables and ornamental crops are still being reported (1,2,18,23). Particles of TSWV are membrane-bound, isometric, and about 80 nm in diameter. Virus particles are apparently composed of a nucleoprotein (26,000 MW) and three membrane proteins (52,000, 56,000, and 78,000 MW) (21).

For more than 40 yr, isolates of TSWV were characterized primarily by symptoms on differential hosts and, when available, by hosts with resistant genes (4,11,14,22). The few attempts to do serological studies were frustrated by the difficulty of purifying the virus and producing reliable antisera (5,24). Using sucrose-gradient centrifugation, Verkleij and Peters (28) were able to separate nucleoprotein and 78K membrane protein and produce pertinent antisera. Because of its wide host range and structural complexity, concerns have been raised about the heterogeneity of TSWV isolates and, consequently, about the ability of serological techniques to detect all TSWV isolates. Gonsalves and Trujillo (12) recently reported purification of the virus, production of antibodies to purified virions, and the successful use of enzyme-linked immunosorbent assay (ELISA) for TSWV detection. However, the comparative effectiveness of using antibodies to the virion or antibodies to the different structural proteins to detect TSWV isolates has not been determined.

In the present study, we selected a range of TSWV isolates and then used various ELISA tests to compare the effectiveness of using antibodies to purified virions and antibodies to isolated 78K and 26K structural proteins to detect the TSWV isolates. Although the tested isolates varied in their reactivities to different antibodies in various ELISA assays, our results showed that all could be detected by double antibody sandwich (DAS) ELISA using antibodies prepared from rabbits injected with whole virions.

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## MATERIALS AND METHODS

**Virus isolates.** The 30 TSWV isolates used in this investigation were originally collected from Brazil, Australia, different regions of the United States, and southern Canada (Table 1). Infected *Nicotiana benthamiana* Domin. was the source of inoculum used in host range comparisons. Plant species included in the comparisons were *Datura stramonium* L., lettuce (*Lactuca sativa* L. 'Minetto'), tomato (*Lycopersicon esculentum* Miller), *N. glutinosa* L., cowpea (*Vigna sinensis* Endl.), *Chenopodium quinoa* Willd., and cucumber (*Cucumis sativus* L.). Virus isolates were maintained in *Gomphrena globosa* L. or freeze-dried in infected *N. benthamiana* leaf tissue and stored at −80 C. An isolate obtained from infected lettuce cultivar Batavia (BL) in Hawaii was used as the standard isolate and antigen for antibody production. Isolate BL is identical to isolate TSWV-L used by Gonsalves and Trujillo (12).

**Purification of virus, 26K viral proteins, and 78K viral proteins of TSWV.** The BL isolate was propagated in *D. stramonium* and purified as described by Gonsalves and Trujillo (12). Viral proteins were dissociated and separated by electrophoresis in 12% SDS-polyacrylamide gels as described by Laemmli (19) and then isolated from the gels according to the methods of Hager and Burgess (13), as described by Yeh and Gonsalves (29). Purified 26K and 78K proteins were suspended in 6 M guanidine-HCl; their concentrations were measured by spectrophotometry, assuming an extinction coefficient ($A_{280}$) of 1.

**Antibody production.** Antibodies to purified virions were produced in a previous study (12). To produce antibodies to separated viral proteins, 1 mg of purified nucleoprotein (NP) or 78K membrane protein (MP) was emulsified with complete Freund's adjuvant (1:1) and then injected intradermally into separate rabbits (New Zealand white). Subsequent injections (1 mg of purified 26K NP or 78K MP antigens mixed with incomplete Freund's adjuvant, 1:1) were given 2 wk and 3 wk later. Antisera titers were determined from bleedings taken at weekly intervals, beginning 1 wk after the final injection and continuing for a period of 4–5 mo.

**Serological assays by ELISA.** Direct and indirect ELISA procedures (10) were used to characterize the antibodies and compare the isolates. These procedures are summarized in Table 2. Antibodies and conjugates were incubated at 30 C for 4 hr; antigens were incubated at 4 C overnight. Antibodies to whole virions and their conjugates prepared for use in the simple direct or indirect ELISA were preabsorbed with healthy *D. stramonium* as described by Gonsalves and Trujillo (12) with one difference: the incubation time was 4 C overnight. Enzyme-substrate reactions were conducted at room temperature and recorded by a MICROELISA autoreader

at $A_{405}$. Reactions were considered positive when the $A_{405}$ readings were both above 0.10 and at least two times higher than the readings of the healthy control.

Crude extracts of TSWV-infected *N. benthamiana* were used as antigens in ELISA for serological studies. The ratio of tissue weight to extraction buffer used to prepare crude homogenates for ELISA testing is explained in Tables 3–5. Leaf tissues were ground in extraction buffer (phosphate-buffered saline [PBS] [10], 0.05% Tween 20, and 2% polyvinylpyrrolidone 40, pH 7.1) for DAS direct and indirect ELISA and in coating buffer (35 mM $NaHCO_3$, 15 mM $Na_2CO_3$, and 0.02% $NaN_3$, pH 9.6) for simple direct and indirect ELISA (Table 2). Serial fivefold dilutions up to 1:1,250 with respective buffers were made of each sample. A monoclonal antibody produced against the 78K membrane protein of an Oklahoma isolate by Hsu et al (15) was the second antibody in a DAS indirect ELISA using plates coated with anti-78K MP polyclonal antibody.

**Serological assays by western blotting.** A clear supernatant of purified virus preparation was frozen at −20 C, thawed, given a very brief low-speed centrifugation, and then used in western blot analyses. Gel electrophoretic analysis showed that this clear supernatant contained intact virus particles and was essentially free of host material. Western blotting was performed as described by Butnette (6). Separated proteins were electrophoretically transferred from polyacrylamide gel to nitrocellulose membranes using a BioRad "transblot" apparatus at 22 V overnight in a transferring solution containing 20% methanol, 25 mM Tris, 192 mM glycine, pH 8.3. A similar gel was stained with Brilliant Blue R 250 without blotting. After blotting, membranes were blocked in 4% nonfat milk powder in PBS buffer (MPBS) for 1 hr at 37 C. The membranes were incubated in antibodies to TSWV, NP, or MP antisera for 2 hr, then incubated for one more hour after adding goat antirabbit immunoglobulin alkaline phosphatase conjugate; the entire incubation took place in PBS containing 0.5% nonfat dry milk at 37 C. Substrates for western blotting were nitro blue tetrazolium chloride (NBT) and 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt (BCIP). Reactions were usually carried out for 5 min and stopped by soaking membranes in distilled water. Between each step, the membranes were washed in PBS, 0.05% Tween 20, and 2% polyvinylpyrrolidone 40, pH 7.1. The dilutions of anti-TSWV, anti-NP, and anti-MP sera were 1:1,000, 1:2,000, and 1:200, respectively, and the dilutions of goat globulin conjugate were 1:1,000, 1:2,000, and 1:500, respectively.

**Table 1.** Origins of tomato spotted wilt virus isolates

| Isolate | Original host | Location |
|---|---|---|
| Agdia 0076 | *Capsicum* sp. | California |
| Arkansas | *Datura* sp. | Arkansas |
| Australia | *Lycopersicon esculentum* Miller | Australia |
| BC | *Chrysanthemum* sp. | British Columbia |
| Begonia | *Begonia* sp. | New York |
| BL | *Lactuca sativa* L. | Hawaii |
| Brazil | *L. esculentum* | Brazil |
| Cineraria | *Cineraria* sp. | New York |
| 74K Celery | *Apium graveolens* L. | Hawaii |
| Frio | *Arachis hypogaea* L. | Texas |
| Iowa Tomato 1 | *L. esculentum* | Iowa |
| Iowa Tomato 2 | *L. esculentum* | Iowa |
| LI Potato | *Solanum tuberosum* L. | New York |
| LI Tomato | *L. esculentum* | New York |
| 54W ML | *Lactuca sativa* L. | Hawaii |
| N3-TSWV | *L. esculentum* | New York |
| OK | *Datura* sp. | Oklahoma |
| ONA | *L. esculentum* | Ontario |
| ONF | *L. esculentum* | Ontario |
| P4-84 | *L. esculentum* | Hawaii |
| 10W Pakchoy | *Brassica chinensis* | Hawaii |
| Polaris 4A | *Chrysanthemum* sp. | California |
| Polaris House 1C | *Chrysanthemum* sp. | California |
| R27G | *Lactuca sativa* | Hawaii |
| Strain 19 | *Nicotiana glutinosa* L. | Hawaii |
| 75M Tomato | *L. esculentum* | Hawaii |
| Tomato 87-2-1 | *L. esculentum* | New York |
| TX Spinach | *Spinacia oleracea* L. | Texas |
| W162 | *N. glutinosa* | Hawaii |
| Westland | *Chrysanthemum* sp. | California |

**Table 2.** ELISA procedures used in the detection of isolates

| ELISA types | Antibody-antigen sequence[a] |
|---|---|
| DAS[b] direct | Antibody — antigen — antibody conjugate |
| DAS indirect | Antibody — antigen — mAB[c] — anti-antibody conjugate |
| Simple direct | Antigen — antibody conjugate |
| Simple indirect | Antigen — antibody — anti-antibody conjugate |

[a] Example: whole virion antibody — virus — anti-whole-virion antibody conjugate.
[b] DAS = double antibody sandwich.
[c] Monoclonal antibody to 78K MP.

## RESULTS

**Origin and biological properties of TSWV isolates.** Most isolates caused

**Table 3.** Results of double antibody sandwich (DAS) direct ELISA in evaluating antibodies to nucleoprotein and 78K membrane protein

| | Conjugate to virion[a] | | | | Conjugate to NP[b] | | | | Conjugate to MP[c] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Coating antibodies | H[d] | TSWV[e] | NP[f] | MP[g] | H[d] | TSWV[e] | NP[f] | MP[g] | H[d] | TSWV[e] | NP[f] | MP[g] |
| To virion (1 μg/ml) | −[h] | +++ | ++ | + | − | + | +++ | − | − | − | − | − |
| To NP (1 μg/ml) | − | +++ | + | − | − | + | +++ | − | − | − | − | − |
| To MP (1 μg/ml) | − | + | + | − | − | − | +++ | − | − | − | − | − |
| Buffer only | NT[i] | − | + | NT | NT | − | +++ | NT | NT | − | − | NT |

[a] Dilution of conjugate to TSWV virion is 1:2,000.
[b] Dilution of conjugate to NP is 1:1,000.
[c] Dilution of conjugate to MP is 1:1,000.
[d] H = 1:100 (w/v) dilution of health *Datura stramonium* crude extract.
[e] TSWV = 1:100 (w/v) dilution of *D. stramonium* extract infected with TSVW isolate BL.
[f] NP = Purified nucleoprotein (10 μg/ml).
[g] MP = 78K membrane protein (10 μg/ml).
[h] +++ = $A_{405}$ greater than 0.7; ++ = $A_{405}$ between 0.7 and 0.4; + = $A_{405}$ between 0.3 and 0.1, − = $A_{405}$ less than 0.1. Results taken 45 min after adding substrate.
[i] NT = not tested.

similar symptoms on test plants; however, there were a few exceptions. The Iowa Tomato 2 isolate induced both local and systemic infections in cowpea; the others produced only local lesions. In *D. stramonium,* the Australia and Begonia isolates produced sporadic systemic chlorotic spots followed by recovery, but other isolates caused local chlorotic lesions followed by systemic chlorosis. *G. globosa* responded to the Australia, Begonia, and Brazil isolates with localized infections only. All other isolates also caused systemic chlorotic mottle.

**Evaluation of antibodies to nucleoprotein and 78K membrane protein.** We could not consistently distinguish the 52K and 56K membrane proteins (21) by SDS-gel electrophoresis or western blotting (Fig. 1). As a result, these proteins are designated as 52-56K MP. In western blots, antibodies to NP reacted strongly with NP, weakly with 52-56K MP, and gave no reaction with 78K MP. These data indicate that the injected NP antigens were slightly contaminated with 52-56K MP. Antibodies to 78K MP reacted strongly with 78K MP and only weakly to 52-56K MP after a long incubation period in substrate. Anti-NP antibodies did not react with the 78K membrane protein or vice versa. As expected, antibodies to virions reacted with all structural proteins (Fig. 1).

Reactions of the antibodies were also evaluated by ELISA. In DAS direct ELISA, anti-NP conjugate reacted strongly with purified NP antigens under all coating conditions, including when only coating buffer was used (Table 3). Weak but positive reactions were obtained with anti-NP conjugate against crude extracts of infected plants when plates were coated with antibodies to virions or NP, but not when plates were coated with antibodies to 78K MP. Anti-78K MP conjugate did not react with the TSWV-infected tissue extracts or with purified NP or 78K MP proteins. However, antivirion conjugate reacted with 78K MP purified antigen in DAS direct ELISA.

Results for simple direct and indirect ELISA are summarized in Table 4. Antibodies to virions reacted positively with all antigen types in both ELISA tests. Antibodies to NP reacted to infected crude extracts and purified NP but not to 78K MP. The antibodies to 78K MP had weak positive reactions to purified 78K MP only in simple indirect ELISA.

**Serological reactions of isolates in ELISA assays and western blotting.** DAS direct ELISA has been routinely used for virus detection (7,8,9,12). In some instances, however, other types of ELISA tests (Table 2) may have advantages over DAS direct ELISA (15,20,27). Because of this, the reactions of antibodies to virions, NP, and 78K MP in various ELISA tests were compared with 30 isolates collected from different areas (Table 2). Only limited tests were done with antibodies to 78K MP protein because previous data (Tables 3 and 4) indicated that our conjugated polyclonal antibodies to 78K MP were not active in ELISA. Instead, a monoclonal antibody that reacted to the 78K MP was used in certain ELISA tests. ELISA reactions were considered positive when the readings were both twice of that of the healthy control and higher than 0.10 at $A_{405}$.

All 30 of the TSWV isolates were consistently detected by DAS direct ELISA using anti-TSWV serum (data not shown for all isolates; see Table 5). Nineteen of the 30 isolates gave consistently positive reactions in all ELISA tests using antibodies prepared to the whole virion or to specific structural proteins (data not shown). These reactions were typified by that of the BL isolate; the other 11 isolates gave variable reactions. Data on these reactions are summarized in Table 5. Because of the variation in the reactions, data are presented by showing the maximum reaction of the

**Table 4.** Results of simple direct and simple indirect ELISA for evaluating antinucleoprotein or anti-78K membrane protein antibodies

| Coating antigens | Simple direct ELISA with antibody conjugates[a] | | | Simple indirect ELISA with antibodies[b] | | |
|---|---|---|---|---|---|---|
| | To virion | To NP[c] | To MP[d] | To virion | To NP | To MP |
| Healthy (1:100) | −[e] | − | − | − | − | − |
| TSWV-BL (1:100) | +++ | + | − | +++ | ++ | − |
| NP (10 μg/ml) | +++ | +++ | − | +++ | +++ | − |
| 78K MP (10 μg/ml) | ++ | − | − | +++ | − | + |

[a] Dilution of conjugate to virion was 1:2,000; dilution of conjugate to NP and MP was 1:1,000. Conjugates were preabsorbed with extracts from healthy *Datura stramonium* at 4 C overnight.
[b] Dilution of antibodies to virion and NP was 1 μg/ml; dilution of antibodies to MP was 5 μg/ml. Antibodies were preabsorbed with extracts from healthy *D. stramonium* at 4 C overnight.
[c] NP = purified nucleoprotein.
[d] MP = 78K membrane protein.
[e] +++ = $A_{405}$ greater than 0.7, ++ = $A_{405}$ between 0.7 and 0.4, + = $A_{405}$ between 0.3 and 0.1, − = $A_{405}$ less than 0.1. Results taken 45 min after adding substrate.

**Table 5.** Summarized results of reactions of TSWV isolates in different ELISA tests

| | DAS direct ELISA | | | | | | Simple direct ELISA | | | | | | DAS indirect ELISA | | | Simple indirect ELISA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Virion As | | | Nucleoprotein As | | | Virion As | | | Nucleoprotein As | | | Membrane Protein As | | | Nucleoprotein As | | |
| | $A_{405}$ | | | $A_{405}$ | | | $A_{405}$ | | | $A_{405}$ | | | $A_{405}$ | | | $A_{405}$ | | |
| Isolates[a] | Max | Avg. | Reactions[b] | Max | Avg. | Reactions | Max | Avg. | Reactions | Max | Avg. | Reactions | Max | Avg. | Reactions | Max | Avg. | Reactions |
| Australia | 0.41[c] | 0.37 | 4/4[d] | <0.10 | 0.02 | 0/3 | <0.10 | 0.01 | 0/3 | <0.10 | 0.03 | 0/3 | <0.10 | 0.04 | 0/3 | <0.10 | 0.03 | 0/3 |
| Begonia | 0.36 | 0.27 | 4/4 | <0.10 | 0.01 | 0/3 | <0.10 | 0.02 | 0/3 | <0.10 | 0.02 | 0/3 | <0.10 | 0.05 | 0/3 | <0.10 | 0.05 | 0/3 |
| BL[e] | 1.50 | 1.35 | 7/7 | 0.55 | 0.50 | 7/7 | 0.51 | 0.43 | 7/7 | 0.78 | 0.34 | 5/5 | 1.19 | 1.00 | 4/4 | 1.32 | 0.69 | 4/4 |
| Brazil | 0.83 | 0.72 | 4/4 | <0.10 | 0.03 | 0/3 | <0.10 | 0.03 | 0/3 | <0.10 | 0.04 | 0/3 | 0.78 | 0.63 | 3/3 | <0.10 | 0.05 | 0/3 |
| 74K Celery | 1.27 | 0.95 | 6/6 | 0.15 | 0.08 | 2/5 | 0.20 | 0.09 | 2/6 | <0.10 | 0.02 | 0/5 | <0.10 | 0.03 | 0/4 | 0.20 | 0.11 | 1/4 |
| Iowa Tomato 2 | 1.35 | 1.15 | 2/2 | <0.10 | 0.05 | 0/2 | 0.50 | 0.45 | 2/2 | 0.18 | 0.15 | 1/2 | 0.78 | 0.69 | 2/2 | 0.28 | 0.21 | 2/2 |
| LI tomato | 1.45 | 1.05 | 2/2 | 0.25 | 0.11 | 1/2 | 0.62 | 0.58 | 2/2 | 0.20 | 0.10 | 2/2 | 1.20 | 0.97 | 2/2 | 0.35 | 0.27 | 2/2 |
| 10W Pakchoy | 1.02 | 0.89 | 6/6 | 0.20 | 0.06 | 1/5 | 0.31 | 0.24 | 4/5 | 0.20 | 0.11 | 1/5 | 0.37 | 0.36 | 3/3 | 0.20 | 0.13 | 2/3 |
| R27G | 1.21 | 0.85 | 6/6 | 0.48 | 0.20 | 4/5 | 0.35 | 0.15 | 2/6 | <0.10 | 0.06 | 0/4 | 0.67 | 0.32 | 2/3 | 0.41 | 0.27 | 3/3 |
| Strain 19 | 1.00 | 0.81 | 6/6 | 0.15 | 0.02 | 1/6 | 0.81 | 0.25 | 5/6 | 0.41 | 0.18 | 3/5 | 0.48 | 0.20 | 2/3 | 0.29 | 0.25 | 3/4 |
| 75M Tomato | 1.28 | 1.10 | 6/6 | 0.30 | 0.25 | 3/5 | 0.29 | 0.12 | 2/6 | 0.11 | 0.01 | 1/4 | 0.48 | 0.15 | 1/4 | 1.20 | 0.55 | 2/3 |
| TX Spinach | 1.22 | 1.07 | 3/3 | <0.10 | 0.01 | 0/3 | 0.29 | 0.13 | 2/3 | 0.15 | 0.10 | 1/2 | 0.20 | 0.10 | 1/3 | 0.25 | 0.21 | 3/3 |
| Healthy CK | <0.10 | 0.04 | 0/8 | <0.10 | 0.03 | 0/8 | <0.10 | 0.02 | 0/8 | <0.10 | 0.06 | 0/8 | <0.10 | 0.01 | 0/4 | <0.10 | 0.04 | 0/4 |

[a] Antigen dilution (g tissue: ml extraction buffer) used with nucleoprotein antiserum in DAS direct and simple direct ELISA; dilution with mAB to membrane protein in DAS indirect ELISA was 1:10. Dilution used with virion antiserum in simple direct ELISA and with nucleoprotein antiserum in simple indirect ELISA was 1:250. Antigen dilution used with virion antiserum in DAS direct ELISA was 1:1,250 except with Australia, Begonia, and Brazil isolates; the antigen dilution was 1:10 to these three isolates.
[b] First value is number of positive reactions; second value is total number of tests.
[c] Readings were taken 45 min after adding substrate.
[d] Reactions were considered positive when the ELISA readings were twice that of the healthy control and higher than 0.10 at $A_{405\ nm}$.
[e] The BL isolate was used as a standard in all tests shown in the table.

tests and the average of the different tests. For example, nine of 11 isolates gave erratic but generally negative reactions when anti-NP antibody was used in DAS ELISA (Table 5). Results from simple direct ELISA using antibody conjugates to whole virion or to NP also showed that there were differences among the isolates; i.e., some could be detected, others could not. Of 11 isolates, six or seven gave generally positive reactions in DAS indirect ELISA using monoclonal antibodies to 78K MP or in simple indirect ELISA using antibodies to NP. The results were more consistent from test to test in these two types of assays than in simple direct ELISA.

In summary, all 30 isolates gave consistently positive results in DAS direct ELISA using antibodies prepared to whole virions. Nineteen of the 30 isolates gave consistent, positive reactions in all ELISA tests. Of the 11 isolates that gave variable results, Australia, Begonia, Brazil, and 74K Celery isolates did not react to any of the antibodies in simple direct, indirect, and DAS indirect ELISA. The one exception was the Brazil isolate, which gave positive reactions in DAS indirect ELISA using antibodies to 78K MP.

Eight of 11 isolates were purified and reactions to anti-whole-virion, anti-NP, and anti-MP antibodies were tested in western blot. All eight isolates reacted positively with the different antibodies (result not shown). We were not able to purify the isolates from Australia, Brazil, or Begonia.

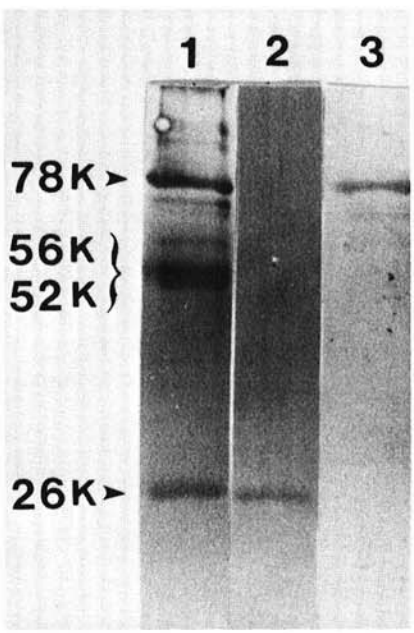


**Fig. 1.** Western blot analysis of TSWV proteins. Purified TSWV virions were dissociated with SDS. Proteins were separated by polyacrylamide gel electrophoresis and transferred electrophoretically to nitrocellulose membranes. TSWV proteins were probed with antivirus antibodies (1:1,000) and goat antirabbit alkaline phosphatase (GAR-E; 1:1,000) in lane 1, anti-NP antibodies (1:2,000) and GAR-E (1:2,000) in lane 2, and anti-MP antibodies (1:200) and GAR-E (1:500) in lane 3.

## DISCUSSION

This is the first report on comparative ELISA detection of TSWV isolates with antibodies to the whole virion and to 26K NP and 78K MP. It is also the first study with a wide geographic selection of TSWV isolates and the first serological analysis of TSWV by western blotting. Many of the isolates reacted similarly to standard isolate BL from Hawaii; a few isolates varied in their reactions in different ELISA assays. One each of the New York and Hawaii isolates and two foreign isolates showed distant relationships with other isolates tested in ELISA. However, all isolates could be detected by antibodies prepared to whole virions using DAS ELISA. Therefore, our results show that the majority of domestic isolates of TSWV are serologically closely related. Some of the foreign isolates may be categorized into distinct serogroups.

It is now possible to produce high-quality polyclonal antibodies to TSWV virions (12,28) (J. W. Moyer, *personal communication*), and the DAS ELISA test is widely used for plant virus detection. The antiserum prepared to whole TSWV virions has been distributed to many laboratories in the United States and around the world. It has been successfully used to detect TSWV in different plant species and even in vector thrips (1,7–9). Based on our data, we recommend that a combination of antibodies to whole virions and the DAS direct ELISA be used for routine detection of TSWV. However, a recent observation by J. W. Moyer (*personal communication*) suggests that an isolate from a floral crop may not react to our virus antiserum.

Since the 78K MP is in the outer envelope of the virion (25), it was somewhat surprising that the polyclonal 78K MP conjugate failed to show activity in ELISA tests while the monoclonal 78K MP did react. However, the polyclonal sera did react to 78K MP in western blot. It should be noted that these antibodies were induced with antigens that had been denatured with SDS prior to immunizing the rabbits. It is possible that antibodies were elicited primarily to internal epitopes (cryptotope) (26). In addition, antibodies may have lost their activities during the process of coupling and thus been rendered useless in DAS direct ELISA.

Antibodies to NP showed a broader reaction in ELISA tests than antibodies to 78K MP, but this reaction was less extensive than that of antibodies to whole virions. It was interesting to notice that gel-isolated NP antigen was "sticky"; it was bound to ELISA plates in extraction buffer and detected by anti-NP antibody conjugate regardless of coating conditions. This phenomenon was not observed with crude virus extract. Lommel et al (20) showed that, in certain instances, antigen can bind to plates without regular ELISA coating buffer with high pH.

Most virus samples (fresh or freeze-dried) received from overseas were biologically inactive by the time they arrived. This is probably due to the instability of TSWV (17). Our conclusions about the serological relationships of TSWV, therefore, are mainly based on isolates from the United States and southern Canada. It will be interesting to study the relationship of the Hawaii isolates to the Netherlands isolate with defective membrane protein (28) and the Japanese isolate that systemically infects cucurbits (18), in which TSWV normally produces only local infections (17).


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