

Concentration and Distribution of Mild and Severe Strains of Potato Spindle Tuber Viroid in Cross-Protected Tomato Plants

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Portion of a thesis submitted by the first author in partial fulfillment of the requirements for the M.S. degree at the University of New Brunswick.

This research was supported by a grant to R. P. Singh and J. Khoury by the Canadian International Development Agency and by the Canadian Potato Export Agency.

Accepted for publication 9 May 1988 (submitted for electronic processing).

ABSTRACT

Khoury, J., Singh, R. P., Boucher, A., and Coombs, D. H. 1988. Concentration and distribution of mild and severe strains of potato spindle tuber viroid in cross-protected tomato plants. *Phytopathology* 78:1331-1336.

Analysis by return polyacrylamide gel electrophoresis (R-PAGE) of a mild strain of potato spindle tuber viroid (MA-PSTV) and a severe strain (S-PSTV) showed that both strains replicated in the plant at a similar rate and could be distinguished from each other by different electrophoretic mobilities. In singly infected plants, both strains were detected 10 days after inoculation; in doubly infected plants they were detected 8 days after inoculation. MA-PSTV was detected in all the leaflets of tomato plants 14 days postinoculation, a duration often used before challenge inoculation. In MA-PSTV-protected plants, the challenge strain (S-PSTV) was first detected 21 days after inoculation, and its concentration increased with time. Symptoms of S-PSTV appeared 48 days after challenge inoculation. In unprotected plants, S-PSTV was detected 10 days and the symptoms

appeared 21–28 days after inoculation. In the top leaves of the MA-PSTV-protected plants, S-PSTV totally replaced the protecting strain in the later stages of infection. Both strains multiplied in the middle and bottom leaves of MA-PSTV-protected plants. In S-PSTV-protected plants, MA-PSTV as a challenge strain was detected only in the later stages of infection but was present in top, middle, and bottom leaves of doubly infected plants. Both strains were found to be present in sepals, petals, anthers, and pistils in MA-PSTV-protected and S-PSTV-challenged plants. Fruit pulp contained only S-PSTV in MA-PSTV-protected and S-PSTV-challenged plants but contained both strains in S-PSTV-protected and MA-PSTV-challenged plants. Only low percentages of seeds were infected with MA-PSTV, and none were infected with S-PSTV.

Additional keywords: breakdown of cross-protection, diagnosis, electrophoresis, replication.

The phenomenon of cross-protection, in which prior infection by a mild strain of a virus leads to the protection of the infected plant from disease caused by a severe strain, has been used in virus disease control (1,6,7). Studies with viruses have led to various hypotheses to explain this phenomenon based on capsid-protein involvement in cross-protection (2,4,15) and the breakdown of cross-protection by the RNA of the challenge strain (4,15). Results of recent experiments with transgenic plants expressing viral coat protein have supported these hypotheses (12,13).

Potato spindle tuber viroid (PSTV) is a low molecular-weight RNA (3,19) devoid of capsid protein. The phenomenon of cross-protection between mild and severe strains of PSTV has been demonstrated (5,9,20). This implies that factor(s) other than capsid proteins also can play a part in cross-protection in plants. Studies with viroids, therefore, can help to elucidate the mechanism of cross-protection.

Although it has been shown that a challenging severe strain of PSTV (S-PSTV) can be recovered from plants previously infected with a mild strain of PSTV (MA-PSTV) (5,9), the concentration of the individual strains in doubly infected, cross-protected plants is unknown. The physical similarity between mild and severe PSTV strains has made quantitative analysis of challenge strain accumulation difficult. However, a recently developed technique of return polyacrylamide gel electrophoresis (R-PAGE) (18) permits the separation of a mild and a severe strain of PSTV on the basis of their differential migration rates during electrophoresis. Using the modified R-PAGE technique, we report here on both the concentration and the distribution of these strains in cross-protected plants.

MATERIALS AND METHODS

Viroid culture and bioassays. A severe strain isolated locally and a mild strain obtained from S. A. Slack, University of Wisconsin, Madison (21), were propagated in potato (*Solanum tuberosum* L. 'Russet Burbank'). Indicator plants were tomato (*Lycopersicon esculentum* Mill. 'Sheyenne') or *Scopolia sinensis* Hemsl. (16). Seedlings were manually inoculated with leaf sap or partially purified nucleic acids (17). Sap inocula were prepared from viroid infected leaves ground with buffer (0.05 M glycine and 0.03 M K₂HPO₄, pH 9.2 [1:1, w/v]) in a centrifuge tube with a polytron PT-10-35 equipped with PT-10ST microgenerator (Brinkman Instruments, Rexdale, Canada). Partially purified nucleic acids were prepared from infected tissue and concentrated twofold on a tissue-weight basis by ethanol precipitation. Inoculated seedlings were maintained in a greenhouse under environmental conditions (24–28 C with a photoperiod of 16 hr) optimum for symptom development (16,20).

Extraction of nucleic acids. Total nucleic acids were prepared (11,17) by grinding 1 g of tissue (leaves, floral and fruit parts) in 3 ml of extracting buffer (0.53 M NH₄OH, 0.013 M disodium ethylenediaminetetraacetate [EDTA] adjusted to pH 7.0 with Tris, 4 M LiCl, and 1% purified bentonite [19]) and 4 ml 0.05 M Tris-saturated phenol (containing 0.1 g of 8-hydroxy quinoline/100 ml). Samples and extraction buffers were maintained at 4–5 C throughout the extraction procedure. The polytron-homogenized samples were centrifuged (15 min, 7,710 g) at 4 C, and nucleic acids from the upper aqueous layer were precipitated (–20 C, 30 min) by adding 2.5 vol of ethanol and 50–80 μ l/sample of 4 M sodium acetate solution. The precipitate was collected by centrifugation as above, dried with a current of air, and dissolved in high salt buffer (14) (100 μ l/g of tissue for gel electrophoresis, or 0.5 to 1.0 ml/g in glycine-phosphate buffer [17] for inoculation).

Gel electrophoresis and recovery of infectious viroids. Return polyacrylamide gel electrophoresis (14) as modified (18) was used. Nucleic acids were dissolved in 100 μ l of "high-salt" buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.3 [14]) containing 40% glycerol and 10 μ l of a solution consisting of 1% xylene cyanol FF and 1% bromophenol blue. First electrophoretic separation of the nucleic acids was at constant current 46 mA for 2.5 hr using an SE 600 series apparatus (Hoefler Scientific Instruments, San Francisco, CA) on 5% nondenaturing slab gels (5% polyacrylamide, 0.125% bisacrylamide, 14 \times 16 \times 0.15 cm) in high-salt buffer, using 6 μ l of sample in each well. The buffer in both the upper and lower reservoirs was replaced with a "low-salt" buffer (1:8 dilution of the high-salt buffer). About 2 L of the low-salt buffer heated to 87–90 C was poured into the lower electrophoresis chamber in which the gel was immersed. These conditions denature the viroid. The remainder of the lower and upper reservoirs was filled with 3 L of buffer heated to 70–71 C. The polarity was reversed and the second electrophoresis was performed at 70–71 C (46 mA constant current, 2.0 hr).

Gels were stained using silver nitrate as modified (14). Gels were shaken 2 \times 5 min in a solution of 10% ethanol and 0.5% acetic acid, then 15 min in 0.2% silver nitrate, followed by 4 \times 15 sec washes in distilled water. Gels were incubated in a fresh solution of 375 mM NaOH, 2.3 mM Na₂BH₄, 0.5% HCHO (37% w/v) for 7 to 15 min. A final 5-min treatment with 70 mM Na₂CO₃ was used to preserve the gel. Gels were placed on a light box and photographed with Polaroid B/W-type 55 film using a Polaroid 4 \times 5 Land Camera.

Infectivity of various viroid bands from the gel was tested by excising the bands representing MA-PSTV or S-PSTV, grinding them in a mortar and pestle with glycine-phosphate buffer (17), and inoculating the suspension onto tomato seedlings. Plants were observed for symptom development for 3 wk, pruned to promote axillary growth, and tested after an additional 2–3 wk with R-PAGE for the presence of viroid strains.

Standardization of viroid concentration. For the determination of viroid concentration, MA-PSTV was purified by PAGE (5% nondenaturing gel). A small vertical lateral strip was removed, stained with silver, then aligned with the remaining gel. A 1-cm horizontal strip from the remaining unstained gel corresponding to the viroid zone (18) was excised and viroids were electroeluted, using a 5% cylindrical gel (6-mm diameter) into a dialysis tubing, at 2.5 mA for 4 hr. Eluted nucleic acids were ethanol precipitated and recovered by centrifugation. Concentration was calculated on the basis of an extinction coefficient of 20 (mg/ml)⁻¹ cm⁻¹ at a wavelength of 258 nm for nucleic acids (10). Various twofold dilutions were electrophoresed, and a concentration standard was always included with each gel.

Experiments with MA-PSTV-protected plants. Three sets of tomato seedlings were used in each experiment. Seven plants in the first set were inoculated with MA-PSTV (protecting strain) initially, then challenge inoculated with buffer 14 days later; in the second set, seven plants were initially rubbed with buffer, then challenge inoculated 14 days later with S-PSTV; in the third set, seven plants were initially inoculated with MA-PSTV, then challenge inoculated 14 days later with S-PSTV. The first inoculation was carried out when tomato seedlings had two to three true leaves. Challenge inocula (nucleic acid extracts or buffer) were rubbed on all the leaves (usually 5 to 6 leaves), including those initially inoculated. Combined samples for viroid analysis were taken from seven plants every week. The samples were collected from the lower one-third of the plant (bottom leaves), the middle third of the plant (middle leaves), and the upper one-third (top leaves) of the plant. Appropriate uninfected and infected controls were always included. The experiments were repeated twice. The only difference between the two repetitions was the nature of the initial inoculum. It was leaf sap from MA-PSTV infected plants in the first experiment and a nucleic acid extract in the second experiment.

Experiments with S-PSTV-protected plants. These experiments were carried out in parallel with the MA-PSTV-protected plants, except that S-PSTV was the protecting strain and MA-PSTV was the challenging strain. Because of the severe symptoms caused by

S-PSTV on the plants, only electrophoretic viroid analysis was used to assess the nature of the infection. Experiments were carried out twice.

RESULTS

Symptom development of the strains used. MA-PSTV-infected tomato plants developed slight stunting, rugosity, and slight bunching of the top leaves, but no veinal or petiole necrosis. S-PSTV-infected plants developed severe stunting, bunching of top leaves, and extensive veinal and petiole necrosis. These symptoms were typical of the strains (18,21) and appeared within 12–21 days postinoculation when plants were inoculated at the two- to three-leaf stage. Symptom appearance was delayed 21–28 days if plants were inoculated at the five- to six-leaf stage (at the time of challenge inoculation).

Sensitivity of R-PAGE and distribution of viroid in the plant. The R-PAGE method employed in this study detected MA-PSTV amounts as low as 0.39–0.19 ng (Fig. 1). Stained bands of the lower amounts were visible by eye when illuminated from beneath. In the photograph, the only bands visible contain more than 0.78 ng of MA-PSTV (Fig. 1).

Four tomato plants inoculated at the three-leaf stage with MA-PSTV from both sap or nucleic acid extracts were tested for PSTV at 2 wk postinoculation, before challenge inoculation. All leaflets representing 23–26 leaflets per plant were tested. Viroid was detected in all leaflets of each plant and the concentrations were higher than 1 ng/6 μ l extracts in most of the leaflets. The leaflets from the nucleic acid-inoculated plants had about 1.5-fold to twofold higher concentration of viroid than those from the sap (data not shown). The amount of viroid detected from each leaflet ranged from 200 to 400 ng/leaflet in top leaves and from 25 to 200

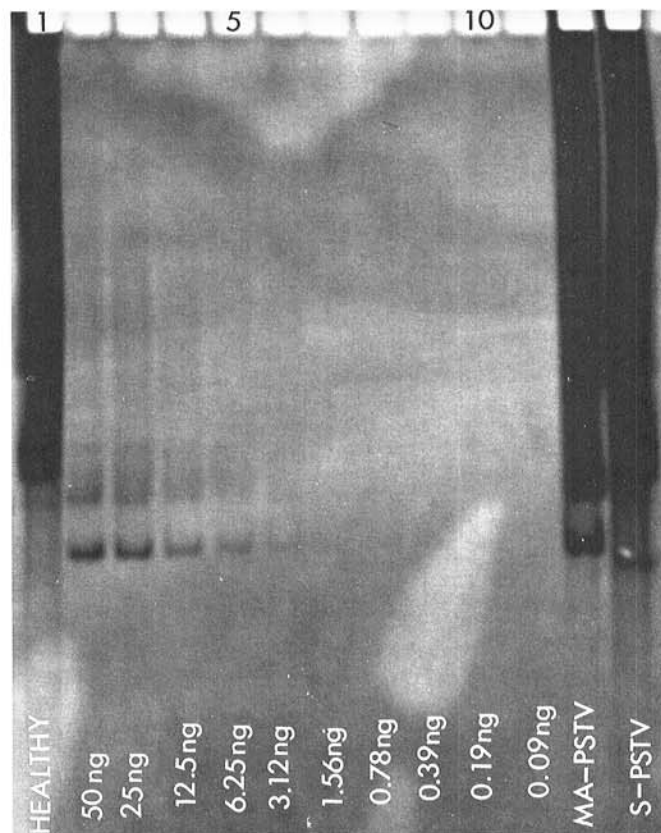


Fig. 1. Return polyacrylamide gel electrophoresis of purified potato spindle tuber viroid (PSTV) nucleic acids. Lane 1, extract from healthy tomato leaves; lanes 2–11, with various amounts of purified viroid (50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39, 0.19, and 0.09 ng); lane 12, mild strain of PSTV; lane 13, severe strain of PSTV. First electrophoresis was on 5% gels under nondenaturing conditions at 46 mA for 2.5 hr and the second was under denaturing conditions at 46 mA for 2 hr.

ng/leaflet in bottom leaves, in both types of inoculation.

Infection pattern of the strains. The infection pattern of MA-PSTV and S-PSTV strains was followed for 63 days using R-PAGE analysis. Composite leaf samples were collected on 4, 6, 8, 10, 12, 14, 21, 28, 35, 42, 49, 56, and 63 days from six plants inoculated at the five- to six-leaf stage. Viroid strains were studied in singly inoculated plants, as well as in plants inoculated with both strains (equal concentrations). Analysis of the nucleic acids from MA-PSTV- or S-PSTV-inoculated plants did not show any visible viroid bands up to 8 days postinoculation. However, the samples from the top leaves of co-inoculated plants contained viroid bands of both strains by 8 days (Fig. 2A, lane 15). By 10 days, in both singly (MA-PSTV or S-PSTV) and doubly (MA-PSTV + S-

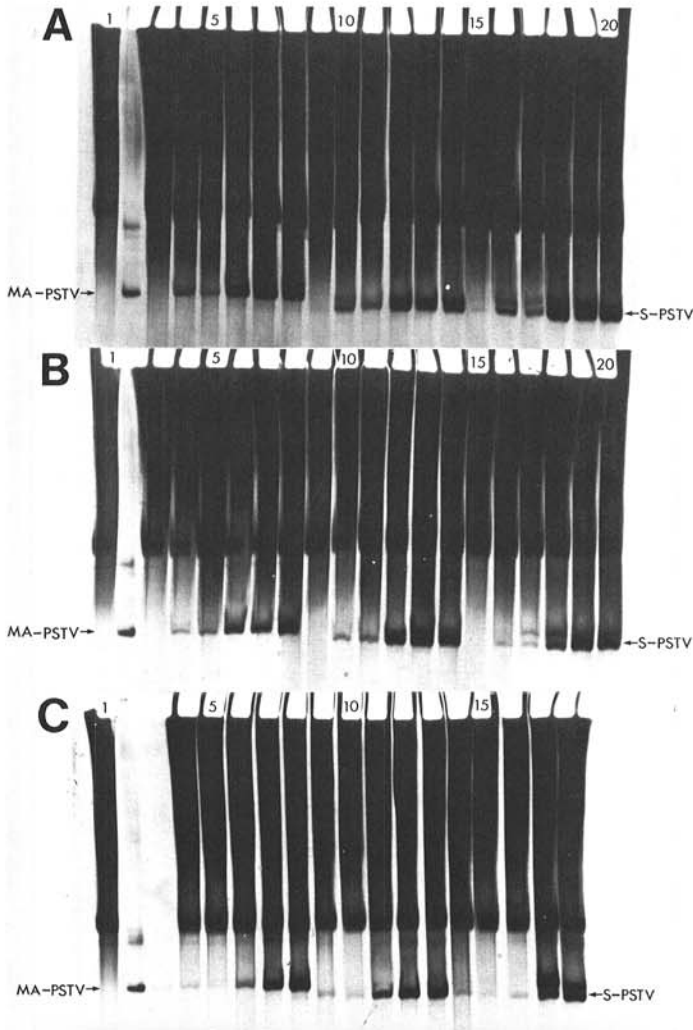


Fig. 2. Analysis by return polyacrylamide gel electrophoresis of potato spindle tuber viroid (PSTV) strains from plants inoculated with mild (MA-PSTV), severe (S-PSTV), or mild and severe (MA-PSTV + S-PSTV) at the five- to six-leaf stage. **A**, Top leaves: lane 1, nucleic acid extracts from healthy tomato; lane 2, purified MA-PSTV (50 ng); lanes 3–8, MA-PSTV 8, 10, 12, 21, 42, and 63 days after inoculation, respectively; lanes 9–14, S-PSTV 8, 10, 12, 21, 42, and 63 days after inoculation, respectively; and lanes 15–20, MA-PSTV + S-PSTV 8, 10, 12, 21, 42, and 63 days after inoculation, respectively. **B**, Middle leaves: same arrangements as for the top leaves. **C**, Bottom leaves: lane 1, nucleic acid extract from healthy tomato; lanes 2 and 3, purified MA-PSTV at 50 and 6.25 ng, respectively; lanes 4–8, MA-PSTV 10, 12, 21, 42, and 63 days after inoculation, respectively; lanes 9–13, S-PSTV 10, 12, 21, 42, and 63 days after inoculation, respectively; and lanes 14–18 MA-PSTV + S-PSTV 10, 12, 21, 42, and 63 days after inoculation, respectively. The electrophoresis conditions were similar to those described in the caption for Figure 1. (Lanes 6–8 of A and lane 8 of B and C show a faint S-PSTV band. This was not observed during the weekly testing but was observed in this particular summary presentation. Therefore, there is a possibility that S-PSTV contamination occurred during this run.)

PSTV) inoculated plants, viroid bands were detected in the samples from the top, middle, and bottom leaves (Fig. 2A, B, and C).

Concentration of MA-PSTV or S-PSTV in singly or doubly inoculated plants continued to increase, first in the top leaves, then in the middle, and later in the bottom leaves (Fig. 2A, B, and C). However, in co-inoculated plants, concentrations of the MA-PSTV started declining after 42 days in the top leaves. By day 63, the amount of MA-PSTV was less than that observed at day 42 in each leaf position (Fig. 2A and B, lanes 20, and Fig. 2C, lane 18).

To ensure that the strains observed on gels were still associated with the appropriate symptoms in tomato plants, 20 silver-stained bands containing either MA-PSTV or S-PSTV were excised, homogenized with buffer, and inoculated onto tomato seedlings. After 21 days, symptoms of S-PSTV were observed on two plants. No symptoms were observed on any plants inoculated with MA-PSTV bands. New axillary growth analyzed 19 days after plants were decapitated showed MA-PSTV symptoms and MA-PSTV bands on gels in seven of the 10 plants inoculated with MA-PSTV, and S-PSTV bands in the same two inoculated plants that had shown symptoms earlier.

Experiments with MA-PSTV-protected plants. In plants inoculated first with MA-PSTV and 2 wk later with S-PSTV, characteristic symptoms of S-PSTV were not detected up to 42 days post-challenge inoculation (dpi), either in sap-inoculated or nucleic acid-inoculated plants. This compares with 21–28 days for symptom development of S-PSTV in singly inoculated plants. However, S-PSTV symptoms were eventually observed 49 dpi in the apical part of the protected and challenged plants regardless of the type of inoculum (Fig. 3).

At 28 dpi, the detection of PSTV strains by R-PAGE in plants subjected to different treatments showed that the concentration of the protecting strain (MA-PSTV) in protected and S-PSTV-challenged plants (Fig. 4, lanes 2–4) was similar to MA-PSTV-protected but unchallenged plants (lanes 5–7). The concentration of challenge strain (S-PSTV) was higher in unprotected and challenged plants (lanes 8–10) than in MA-PSTV-protected and challenged plants (lanes 2–4). The changes in accumulation pattern at various leaf positions for the entire duration (77 dpi) are summarized below (Fig. 5).

Challenge strain was first detected 21 dpi in the top (Fig. 5A, lane 3) and the middle leaves of protected and challenged plants (Fig. 5B, lane 3). For comparison, singly inoculated plants (in the five- to six-leaf stage) showed S-PSTV as early as 10 dpi, similar to Figure 2A (lane 10). By 35 dpi, S-PSTV was detected in all leaf positions of protected and challenged plants (Fig. 5A, lane 5, and Fig. 5B, lanes 5 and 15), and the concentration of both strains appeared equal in the top leaves. The concentration of S-PSTV increased whereas that of MA-PSTV decreased at later times in the top leaves (Fig. 5A, lanes 6–9). There was no significant change in

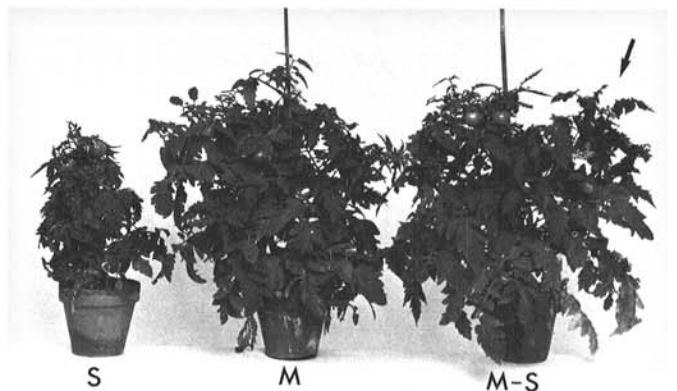


Fig. 3. Potato spindle tuber viroid (PSTV) symptoms on tomato cultivar Sheyenne plants that were unprotected and challenged with a severe strain of PSTV (S); protected with a mild strain of PSTV (M); and protected (M) and challenged (S) (M-S). Arrowhead denotes the location of severe PSTV symptoms developed at 56 days post challenge inoculation.

concentration in the plants infected with MA-PSTV alone (Fig. 5A, lanes 12–19). In the middle leaves of the protected and challenged plants, equal concentrations of each strain were observed up to 63–77 dpci, and no detectable decrease of MA-PSTV was observed. In the bottom leaves of such plants, S-PSTV was detected by 35 dpci and concentration continued to increase until the termination of the experiment, that is, by 77 dpci (Fig. 5B, lane 19).

Experiments with S-PSTV-protected plants. In plants protected with S-PSTV and challenged with MA-PSTV, 14 days post protection inoculation (dppi), the former was detected on gels at all sampling times and was essentially unaffected by the challenge strain, which was detected in the top, middle, and bottom leaves between days 63 to 77 after challenge inoculation, indicating that infection and replication had taken place throughout the plant (Fig. 6, lanes 5–7, 11–13, and 17–19) in spite of the previous infection with S-PSTV.

Multiplication of the challenge strain in the inoculated leaves. Detection of the challenge strain (S-PSTV) in the top leaves of the cross-protected plants before detection at other locations raises the possibility that viroids may not multiply to the same extent in older inoculated leaves as they do in faster growing new ones. It is possible that the challenging strain may move from inoculation sites to the growing points, replicate there, and translocate to the middle and the bottom leaves only in the later stages of infection. To examine the multiplication of the challenge strain in inoculated leaves, tomato seedlings at the five- to six-leaf stage were challenge inoculated as usual, and challenge-inoculated seedlings were divided into two groups. One group (seven plants) was prevented from developing new growth because the apical and axillary growth was periodically removed, and the other group (six plants)

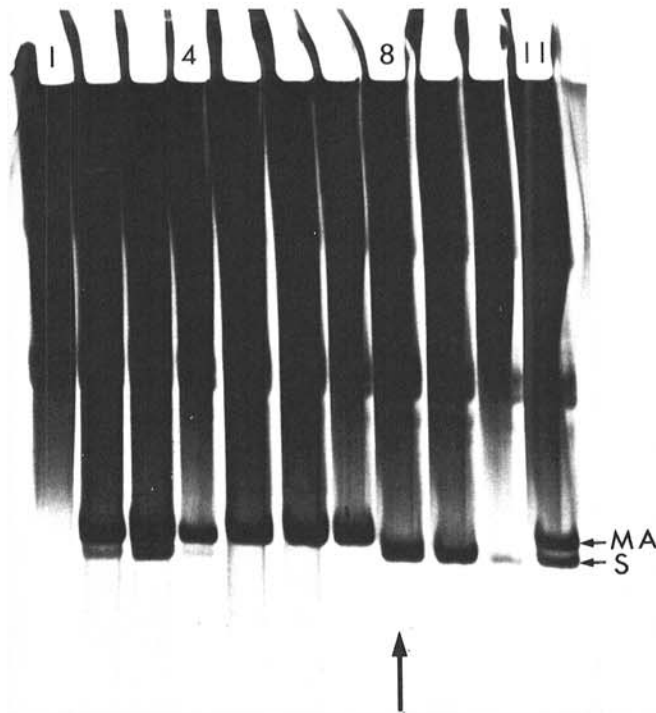


Fig. 4. Analysis of nucleic acid extracts from cross-protected and challenged or unprotected and challenged tomato plants by return polyacrylamide gel electrophoresis at 28 days post challenge inoculation (dpci). Plants were inoculated with a mild strain of potato spindle tuber viroid (MA-PSTV) at the three-leaf stage, followed by challenged at the five- to six-leaf stage with or without a severe strain of PSTV (S-PSTV) (14 days later). Lane 1, nucleic acid extracts from healthy plant; lanes 2–4, MA-PSTV-protected and S-PSTV-challenged top, middle, and bottom leaves; lanes 5–7, MA-PSTV-protected and unchallenged top, middle, and bottom leaves; lanes 8–10, top, middle, and bottom leaves unprotected and challenged with S-PSTV; and lane 11, MA-PSTV + S-PSTV mixture. Arrow denotes the appearance of S-PSTV symptom, 28 dpci, in the top leaves.

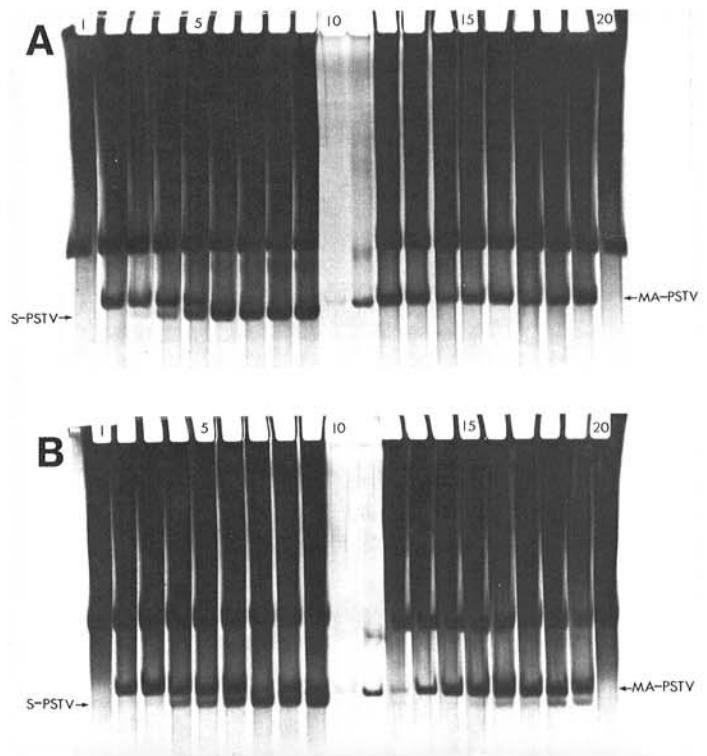


Fig. 5. Analysis of nucleic acid extracts from protected and challenged or protected and unchallenged tomato plants by return polyacrylamide gel electrophoresis. Plants were inoculated with a mild strain of potato spindle tuber viroid (MA-PSTV) initially at the three-leaf stage, followed by challenge inoculation with or without a severe strain of PSTV (S-PSTV) 14 days later. Electrophoretic analysis was done every week after challenge inoculation. **A**, Top leaves: lane 1, nucleic acid extracts from healthy tomato; lanes 2–9 from protected and challenged plants at 14, 21, 28, 35, 49, 56, 63, and 77 days post challenge inoculation (dpci), respectively; lanes 10 and 11, purified MA-PSTV at 6.25 and 50 ng, respectively; lanes 12–19, MA-PSTV-protected but unchallenged tomato plants at 14, 21, 28, 35, 49, 56, 63, and 77 dpci, respectively; and lane 20, nucleic acid extracts from healthy tomato. **B**, Middle and bottom leaves: lanes 1–11, middle leaves, similar to lanes 1–11 of **A**; lanes 12–19, bottom leaves at 14, 21, 28, 35, 49, 56, 63, and 77 dpci, respectively; and lane 20, nucleic acid extracts from healthy tomato. The electrophoresis conditions were similar those described in the caption for Figure 1.

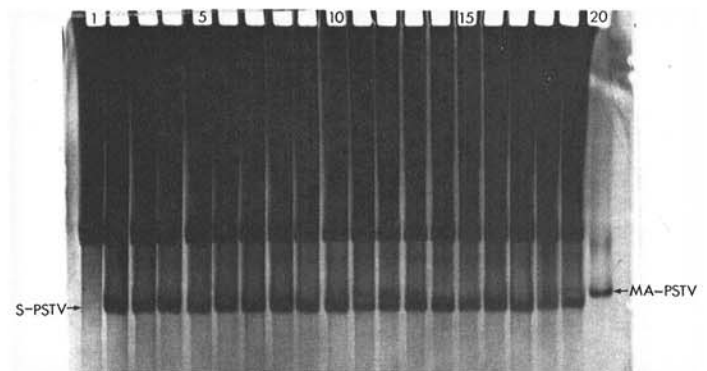


Fig. 6. Analysis of the nucleic acid extracts by return polyacrylamide gel electrophoresis from plants inoculated at the three-leaf stage with a severe strain of potato spindle tuber viroid (S-PSTV) followed by challenge inoculation with a mild strain (MA-PSTV) at the five- to six-leaf stage. Lane 1, nucleic acid extracts from healthy plants; lanes 2–7, nucleic acid extracts from protected and challenged plants (top leaves) at 21, 35, 49, 63, 70, and 77 days post challenge inoculation (dpci), respectively; lanes 8–13, similar to lanes 2–7, but from middle leaves; lanes 14–19, similar to lanes 2–7, but from bottom leaves; and lane 20, purified MA-PSTV (50 ng). The electrophoresis conditions were similar to those described in the caption for Figure 1.

was left untreated. S-PSTV was detected in the inoculated leaves of both groups of plants 21 days after challenge inoculation. The concentration of S-PSTV was higher in the inoculated leaves of pruned plants than in those allowed to develop new growth.

Presence of strains in floral and fruit parts. Because both strains were detected in the leaves of protected and challenged plants, it was of interest to determine if floral and fruit parts also contained both strains. Floral parts (sepals, petals, anthers, and pistils) were removed from protected and challenged plants at 21–28 dpci, and fruit pulp and seeds were removed 90 dpci. The presence of both strains was demonstrated in the floral parts (Fig. 7, lanes 2–5). Fruit pulp contained only S-PSTV in MA-PSTV-protected and S-PSTV-challenged plants but contained both strains in the S-PSTV-protected and MA-PSTV-challenged plants (Fig. 7, lanes 8 and 10). Nucleic acid extracts from 130 germinated seeds from MA-PSTV-protected and S-PSTV-challenged plants or 100 seeds from plants infected only with S-PSTV were tested by gel electrophoresis. None contained viroid band. In a subsequent test, 60 seeds from MA-PSTV-infected plants were tested after 8–10 wk of growth. Three seedlings were found to be infected with MA-PSTV (Fig. 7, lanes 12–14).

DISCUSSION

The present study demonstrates the usefulness of the R-PAGE in studying cross-protection using mild and severe strains of PSTV. The method is sensitive enough to detect nanogram amounts of viroid, which allows the detection of both strains in co-inoculated plants as early as 8–10 days postinoculation (Figs. 1 and 2).

We have presented evidence for the kind of cross-protection that results in delayed symptom development of a severe strain of PSTV as a consequence of prior infection of tomato with a mild strain. Tomato plants inoculated with a mild strain at the three-leaf stage before inoculation with a severe strain at the five- to six-leaf stage are slow to develop symptoms of the severe strain (not seen before 48 days after inoculation with the severe strain compared with 21–28 days for unprotected plants) and the physical presence of viroid in extracts is detected late (not before 21 days after inoculation with the severe strain, compared with 10 days for unprotected plants).

The detection of S-PSTV challenge strain in plants protected with MA-PSTV and its continued increase throughout the plant in

later stages of infection (Fig. 5) indicated that cross-protection was not complete. As noted earlier (5,9), symptoms of S-PSTV also were observed in protected plants in late stages of infection. Symptoms were well correlated with the increased viroid concentration of S-PSTV in the top leaves (Fig. 5).

Functional cross-protection (absence of the disease caused by severe strain) operated up to 35–42 days after challenge inoculation because symptoms of S-PSTV were not observed until after this time. Because of this, cross-protection has been used for strain identification for field testing (5,20). However, use of the cross-protection as a measure for viroid disease control in vegetatively propagated crops would be highly questionable in view of the incomplete cross-protection observed in this study, where the challenging strain replaced the protecting strain in the new growth (Fig. 5) which developed symptoms of S-PSTV.

Studies with plant viruses have shown that cross-protection is complete when virions are used as challenge inocula, but cross-protection breaks down (4,15) or is partial (22) when RNA (4,15) or a defective mutant (22) is used as the challenge inoculum. The results of our study also point to the inherent ability of RNA to superinfect. In the present study, the degree of infection of all cells by the protecting strain could not be determined, but all leaflets were infected at the time of challenge inoculation. Therefore, incomplete protection could not be the result of a slow spread of MA-PSTV but could be due to either the infection of uninfected cells or to the multiplication of both strains in the same cell.

Although co-inoculation of plants with both strains or simultaneous infection of separate plants by individual strains failed to disclose any difference in the rate of spread in various leaves, the amount of S-PSTV appears higher than the amount of MA-PSTV at any given date of infection (Fig. 2), which would indicate a more rapid synthesis of S-PSTV. This rapid synthesis of S-PSTV might explain the eventual disappearance of MA-PSTV in the co-inoculated or challenge-inoculated plants (Figs. 2 and 5). This increase in the concentration of S-PSTV at the expense of MA-PSTV is a phenomenon not previously reported for viroids.

The observation that MA-PSTV could establish itself in S-PSTV-protected plants is interesting, particularly in view of the more rapid rate of S-PSTV synthesis. Because MA-PSTV was detected considerably later (63–77 dpci) and the concentration was very low (Fig. 6), the possibility of both strains multiplying in the same cells is less likely. Thus, the establishment of MA-PSTV in cells unoccupied by S-PSTV may be a good possibility.

This study also demonstrated the presence of both strains in floral and fruit parts (Fig. 7). Absence of MA-PSTV in fruit pulp from cross-protected plants (Fig. 7, lane 8) and its presence in fruit pulp from S-PSTV-protected plants (Fig. 7, lane 10) can be explained by assuming that MA-PSTV was eliminated in MA-PSTV-protected and S-PSTV-challenged plants by the S-PSTV, in accordance with the trend observed in the top leaves of such plants (Fig. 5). Because MA-PSTV can multiply in S-PSTV-protected plants up to 77 days, its presence in fruit pulp of such plants could be the continuation of the MA-PSTV multiplication (Fig. 7). However, the absence of S-PSTV in the seeds either from a cross-protected or singly infected plant is intriguing. It may require a larger number of seeds than was tested in this study to find S-PSTV in seeds because S-PSTV is known to reduce viability of pollen (8).

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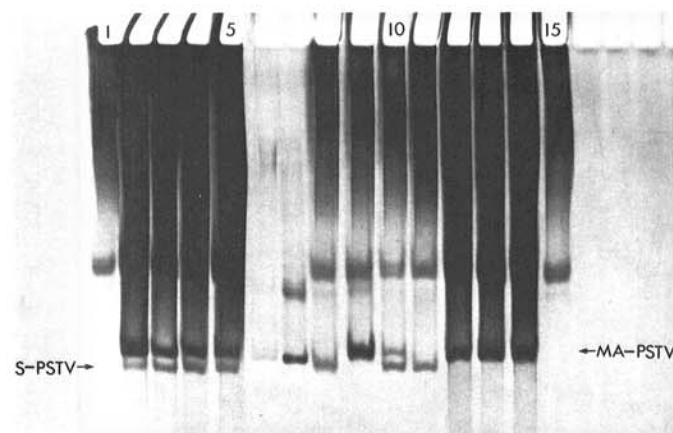


Fig. 7. Electrophoretic analysis of nucleic acid extracts from floral and fruit parts of cross-protected plants. Lane 1, nucleic acid extracts from healthy plants; lanes 2–5, a mild strain of potato spindle tuber viroid (MA-PSTV) + a severe strain (S-PSTV) from sepals, petals, anthers, and pistils, respectively, from MA-PSTV-protected and S-PSTV-challenged plants; lanes 6 and 7, purified MA-PSTV at 6.25 and 50 ng, respectively; lane 8, fruit pulp from protected and challenged plant; lane 9, fruit pulp from MA-PSTV-protected plant; lane 10, fruit pulp from S-PSTV-protected and MA-PSTV-challenged plant; lane 11, fruit pulp from S-PSTV-protected plant; lanes 12–14, tomato seedlings from MA-PSTV-infected seeds; and lane 15, nucleic acids from healthy fruit pulp. The electrophoresis conditions were similar to those described in the caption for Figure 1.

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