Detection of Potato Spindle Tuber Viroid in Field-Grown Potatoes by an Improved Electrophoretic Assay

M. A. Pfannenstiel, S. A. Slack, and L. C. Lane

Research assistant and associate professor, respectively, Department of Plant Pathology, University of Wisconsin-Madison, Madison 53706, and assistant professor, Department of Plant Pathology, University of Nebraska, Lincoln 68583.

This research was supported by the College of Agricultural and Life Sciences and the Graduate School, University of Wisconsin-Madison, and the Wisconsin Potato Industry Board.

We are grateful to Bonnie J. Zaleski for technical assistance and to Steven Vicen for photographic assistance.

Accepted for publication 23 April 1980.

ABSTRACT

PFANNENSTIEL, M. A., S. A. SLACK, and L. C. LANE. 1980. Detection of potato spindle tuber viroid in field-grown potatoes by an improved electrophoretic assay. Phytopathology 70:1015-1018.

An improved polyacrylamide gel electrophoresis (PAGE) procedure was developed for more efficient detection of potato spindle tuber viroid (PSTV). Modification in sample preparation permitted sample processing in 3 hr and completion of PSTV detection in one day. Inclusion of xylene cyanole, which comigrates with PSTV in gels, eliminated confusion due to the occasional appearance of extraneous RNA bands or uneven migration in individual gels. Photography of gels stained with ethidium bromide and illuminated with ultraviolet light enhanced PSTV detection. Reliability of the procedure was investigated by assaying 14 cultivars of field-grown potatoes (*Solanum tuberosum*) at monthly intervals. When potatoes were grown from infected tubers, PSTV was detected in all assays. Detection of current-season infections increased from 20% at 1 mo to 70% at 2 mo postinoculation. In current-season infections, PSTV detection was not affected by different PSTV strains (mild or severe). The concentration of PSTV in stem apices of the 14 cultivars varied from 0.64 to 2.20 μg per gram of tissue and was not correlated with symptom severity.

The potato spindle tuber (PST) disease has been recognized since 1923 (10), but the nature of the pathogenic agent, a viroid (PSTV), remained obscure for many years (1,2). Although insect vectors have been reported (3), potato spindle tuber viroid (PSTV) is thought to be transmitted primarily by foliar or mechanical contact (4) or through true seed (12). Therefore, PSTV can spread through valuable germplasm sources during seed stock increase programs and is of most concern to seed potato certification programs, germplasm centers, and breeding programs.

Since the disease symptoms are often subtle, the discovery of the nature of viroids has been central to the problem of disease

0031-949X/80/10101504/$03.00/0

detection. Polyacrylamide gel electrophoresis (PAGE) was introduced as a sensitive biochemical assay to supplement PSTV detection in basic seed lots (5,6) and has been shown to be quicker and more reliable than tomato (*Lycopersicon esculentum* L. 'Rutgers') bioassay for detecting PSTV in greenhouse-grown potatoes (11).

The purposes of this study were to simplify sample preparation and gel interpretation of PAGE assays and to evaluate PAGE reliability for PSTV-infected field-grown potatoes (*Solanum tuberosum* L.) at different times during the growing season.

MATERIALS AND METHODS

Electrophoresis. For testing field-grown potatoes, electrophoretic separation of nucleic acid preparations was performed on 5% polyacrylamide, N, N′-methylene-*bis*-acrylamide (bis) (40:1, w/w) gels in 40 mM Tris (hydroxymethyl) aminomethane, 20 mM sodium acetate, 1 mM EDTA pH 7.2 (6). Electrophoresis was performed in cylindrical gels (0.6 × 9 cm) at room temperature or slab gels (18 × 14 × 0.3 cm, 4 mm diameter sample well width, 20 wells per slab) equipped with a water cooling apparatus for 3 hr at 6–8 mA per gel or 100 mA per slab. Gels were stained with either Toluidine Blue O (6) or ethidium bromide (11).

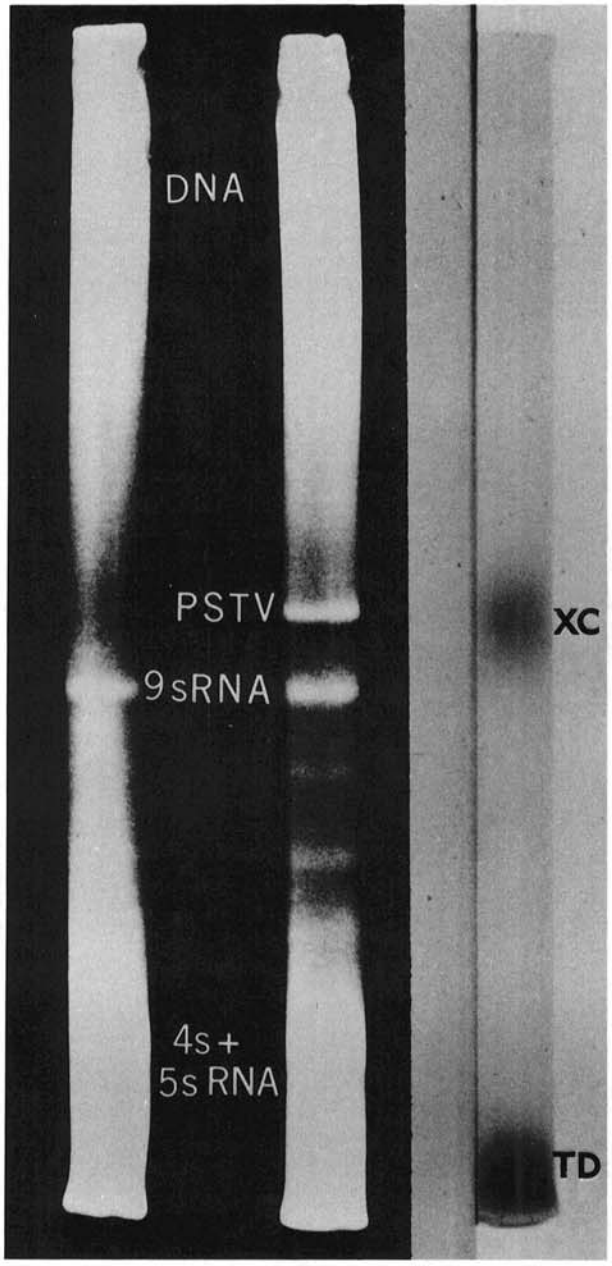

Fig. 1. Five percent polyacrylamide cylindrical gels (acrylamide: bisacrylamide [40:1 w/w]) containing nucleic acid extracts of 0.5-g stem apices from healthy (left) and PSTV-infected (middle) Katahdin potatoes. Gels were stained with ethidium bromide and photographed under ultraviolet light. The gel on the right, photographed in visible light, shows the position of the bromophenol blue tracking dye (TD) and xylene cyanole (XC), which comigrates with PSTV.

Photography. Gels stained with ethidium bromide were placed on a dark background and illuminated with short-wave ultraviolet lights placed to the side. Gels were photographed with Polaroid Type 57 film and by using Tiffen Series 6 Photar #3 and Red #1 filters. Exposures for 1–2 min gave best results.

Field plots. The following 14 potato cultivars were maintained on the University of Wisconsin Hancock Experimental Farm: Katahdin, Kennebec, LaChipper, Monona, Norchip, Norland, Norgold Russet, Oneida, Ontario, Red LaSoda, Russet Burbank, Sebago, Superior, and Wischip. Potatoes were inoculated with a severe strain, mild strain A (PSTV-S and PSTV-MA, respectively, obtained from E. D. Jones, Cornell University, Ithaca, NY) and mild strains B and C (PSTV-MB and PSTV-MC, respectively, obtained from W. J. Hooker, Michigan State University, East Lansing, MI). Unless otherwise indicated, PSTV-S was used in all experiments. Corundum-dusted leaves were inoculated by rubbing them with sap from homogenates (1:5, w/v in 0.1 M neutral phosphate buffer) of PSTV-infected tomato or by vigorously striking (switching) the potato foliage (4,9) five times with PSTV-infected Rutgers tomato plants. Inoculated plants were termed 1st-yr infections(s) (FYI). Plants from tubers harvested from FYI plants were called 2nd-yr infection(s) (SYI), and plants from tubers harvested from SYI plants were called 3rd-yr infection(s) (TYI).

Samples were collected for each cultivar in early June, July, and August of 1977, 1978, and 1979. Samples consisted of 0.5, 1.0, or 2.0 g of stem apices and were frozen at −20 C until tested.

Viroid quantification in potato cultivars. The relative PSTV concentration was determined for each cultivar by assaying 0.5-g samples from stem apices about 3 wk after emergence. Three SYI tubers per cultivar were grown in a greenhouse at 24 C with a 16-hr daylength. The PAGE method described in this paper was used except that xylene cyanole was omitted. Unstained cylindrical gels were scanned in quartz tubes at 280 nm with a Model 1310 ISCO gel scanner (ISCO, Lincoln, NE 68505). The PSTV concentration was determined by comparing the area under PSTV peaks to a standard curve prepared from known concentrations of yeast transfer RNA (tRNA) (Sigma Chemical Co., St. Louis, MO 63178) (5).

PROCEDURES AND RESULTS

A procedure was developed that enabled PSTV detection by PAGE to be completed within 1 day. The method is shorter than those previously described and requires only one-half as much tissue for PSTV detection.

The procedure is as follows:

Step 1. In a 15-ml Corex test tube, add 0.5 g of apical stem tissue to 0.5 ml of distilled H_2O, 0.2 ml of 4 M NH_4OH, 0.2 ml of 0.1 M ethyleneglycol-*bis*-N, N′-tetraacetic acid (adjusted to pH 7.0 with

TABLE 1. Detection of potato spindle tuber viroid (PSTV) in field-grown potatoes by polyacrylamide gel electrophoresis in 1977[a]

Date of sample collection	Healthy		Second-year infection	
	PSTV-positive (%)	Assays (no.)	PSTV-positive (%)	Assays (no.)
Prior to planting[b]	0	0[c]	50	34
31 May	0	21	50	42
27 June	0	7	24	41
1 August	0	9	14	42

[a] Assays were by the procedure of Morris and Smith (5).

[b] Date of planting was 28 April 1977. Seed lot was assayed in a greenhouse before planting.

[c] PSTV-free tubers were obtained from the Wisconsin Elite Foundation Seed Potato Farm.

Tris), 0.6 ml of 10 M LiCl, and 2 ml of water-saturated phenol containing 0.1% 8-hydroxyquinoline and homogenize the mixture with a Polytron equipped with a PT10-ST microgenerator (Brinkman Instruments, Des Plaines, IL 60016) for 30 sec. Samples must remain cold throughout the procedure.

Step 2. Centrifuge the homogenate in the same tubes at 7,710 *g* for 15 min.

Step 3. Withdraw the supernatant fluid and add two volumes of 95% ethanol. Precipitate nucleic acids at −20 C for 30 min.

Step 4. Centrifuge the suspension at 7,710 *g* for 5 min. Decant the supernatant fluid and wash the pellet gently with several milliliters of 95% ethanol. Decant the ethanol and vacuum dry the pellet.

Step 5. Resuspend the pellet in 150 μl of sterile distilled water. Add 50 μl of a solution containing 0.025% xylene cyanole (8), 0.025% bromophenol blue, and 40% sucrose. Samples may be stored at −20 C or electrophoresed immediately. Steps 1–5 require about 3 hr.

Step 6. PAGE is performed on 5% polyacrylamide gels as described earlier. Apply 200 μl of samples to cylindrical gels or 50 μl to slab gels.

Step 7. Stain gels in ethidium bromide (20 μg/ml) in 0.001 M EDTA for 15 min and destain for 15 min in 0.001 M EDTA (11).

Step 8. Add fresh EDTA and view gels against a black background while illuminated from the side with an ultraviolet light source.

Step 9. Photograph the gels as described in Materials and Methods.

The bromophenol blue dye front will be near the bottom and the xylene cyanole marker dye will be near the center of the gels. If PSTV is present, it will appear as a fluorescent band in the middle of the xylene cyanole band (Fig. 1). In this gel system, xylene cyanole comigrates with PSTV at pH 6.0–8.0. Varying the acrylamide-to-*bis* ratio or adding sodium dodecyl sulfate will change the migration of xylene cyanole relative to PSTV. The marker dye is essential because nucleic acid migration may vary between gels or the 9S RNA band may be faint which can lead to confusion or false positives (7). The extraction procedure described was designed to be rapid. Therefore, attempts were not made to remove extraneous nucleic acids which did not interfere with PSTV visualization.

The volume of nucleic acid extract that produced distinct bands in cylindrical gels varied from 25–200 μl, which represents 0.06–0.5 g of tissue. Overloading of cylindrical gels was not a problem, but slab gels were easily overloaded. An aliquot of 50 μl of the total 200 μl resulted in optimum band visualization, but slab gels consistently were overloaded with 75–100-μl aliquots.

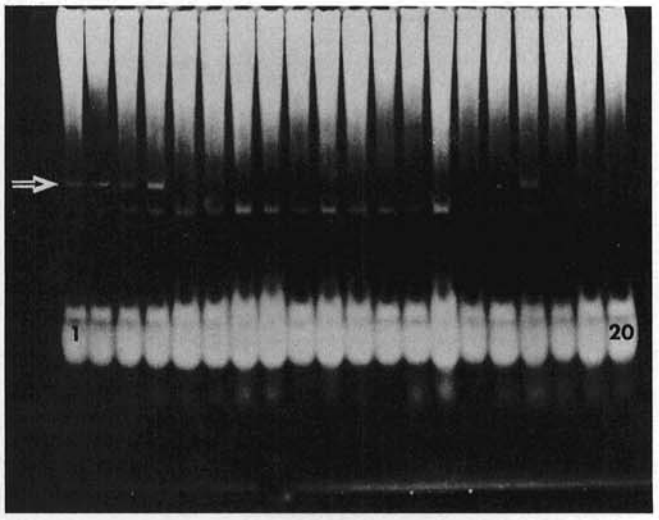

Fig. 2. Five percent polyacrylamide slab gel (acrylamide:bisacrylamide [40:1, w/w]) containing nucleic acid extracts from healthy and PSTV-infected field-grown potato plants sampled 1 August 1979. The gel was stained with ethidium bromide and photographed under ultraviolet light. The PSTV bands are apparent in lanes 1–4 and 17 (opposite arrow).

When samples from dormant tuber tissue were processed according to the described procedure, a low percentage of infected tubers was detected. However, PSTV was reliably detected in stem apices of greenhouse-grown plants and etiolated tuber sprouts from as little as 0.1 g of tissue.

PAGE detection of PSTV. Tissue samples (1.0 g) were collected from stem apices of SYI in June and July, 1977, and PAGE analysis was performed according to the procedure of Morris and Smith (5). June assays agreed with the known level of PSTV infection (50%) in the plot (Table 1). In July, only 24% of the samples tested positive for PSTV and PSTV bands were much fainter in the gels. Therefore, 2.0-g samples were collected in August, but the percentage of infected plants detected decreased further and PSTV bands in positive assays still were faint.

In 1978, the PAGE method (with cylindrical gels) described in this paper was adopted. Three TYI plants of the cultivars Katahdin, Kennebec, LaChipper, Russet Burbank, and Superior and one TYI plant of each of the remaining nine cultivars were sampled on 12 June, 7 July, and 1 August. All assays were positive in June and July, but two of 42 assays were negative in August. In addition, PSTV bands were fainter in August than earlier in the season. Healthy controls of these cultivars maintained in the same plot did not test positive for PSTV.

The same procedure was used with slab gels for field testing 14 potato cultivars in 1979 (Fig. 2). Also, slab gels were photographed which enhanced band visibility. All SYI infections assayed positive for PSTV in June, July, and August (Table 2). Only 20% of FYI assayed positive in July, but 70% assayed positive in August (Table 2) and PSTV bands were more intense. The remaining 30%, which assayed negative, were again tested for PSTV by sprouting harvested tubers and testing sprouts by PAGE. Infected plants that assayed negative in August were confirmed to be infected by these subsequent tests.

The influence of mild and severe PSTV strains upon detection efficiency was investigated by indexing FYI plants of Katahdin, Kennebec, LaChipper, Russet Burbank, and Norgold Russet in August. Approximately 80–90% of the FYI tested positive for PSTV regardless of PSTV strain (Table 3).

Quantification of PSTV. All potato cultivars tested contained PSTV concentrations well above the lower limit detectable by gel scans (0.2 μg/gel as determined with tRNA standards) and PSTV concentrations in stem apices varied among cultivars (Table 4). Norgold Russet, Oneida, Wischip, and Sebago yielded the most

TABLE 2. Detection of potato spindle tuber viroid (PSTV) in field-grown potatoes by polyacrylamide gel electrophoresis in 1979[a]

Date of Sample collection	Healthy		First-year infection[b]		Second-year infection	
	PSTV-positive (%)	Assays (no.)	PSTV-positive (%)	Assays (no.)	PSTV-positive (%)	Assays (no.)
7 June	0	14	...	...	100	32
2 July	0	15	22	95	100	25
1 August	0	15	70	90	100	34

[a] Assays were performed according to the procedure described in the text.
[b] Foliage was inoculated on 7 June 1979.

TABLE 3. Detection of mild and severe strains of potato spindle tuber viroid (PSTV) in potato by polyacrylamide gel electrophoresis[a]

Inoculum	PSTV strain	PSTV-positive (%)	Assays (no.)
Healthy	...	0	15
PSTV	Mild A	88	25
PSTV	Mild B	79	24
PSTV	Mild C	84	49
PSTV	Severe	90	50

[a] Assays performed according to procedure described in text. Foliage was inoculated 7 June 1979, and samples were collected 1 August 1979.

PSTV, while Kennebec, Norland, and Superior yielded the least PSTV. The relationship of PSTV concentration to symptom severity in the 14 cultivars was analyzed by using linear regression. A regression of decrease in tuber weight for SYI and TYI (9) vs PSTV concentration, resulted in correlation coefficients of 0.29 and −0.63, respectively. Thus, differences in symptom severity among potato cultivars were not correlated with increasing concentrations of PSTV.

DISCUSSION

The described procedure is rapid, reliable, and requires one-half the tissue and reagents required by previously reported procedures. The addition of lithium chloride in the initial homogenization reduces the number of preparative steps involved, and dialysis (which is included in other procedures) is omitted so that testing for PSTV may be completed in a single day. PSTV bands can be identified specifically because the marker dye xylene cyanole comigrates with PSTV, thus eliminating confusion due to gels that do not run evenly or to the occasional appearance of extraneous RNA bands. The efficiency of PSTV extraction according to this procedure was comparable to that reported by Morris and Smith (5) from Kennebec with a severe strain of PSTV under comparable greenhouse conditions.

Previously described PAGE detection methods are reported to be reliable in greenhouse testing (5,6,10), and were reliable in our greenhouse tests. Different results, however, were obtained with field-grown potatoes in Wisconsin. When we used Morris and Smith's method (5), detection of PSTV in SYI field-grown tissue decreased as the season progressed. Yellow pigments, which impaired visualization of nucleic acid bands, were observed in gels for samples collected late in the growing season. Further, all nucleic acid bands appeared fainter as the season progressed which appears to support Morris and Smith's report that both PSTV and total extractable nucleic acid concentrations are reduced in older tissue (5).

Although the nucleic acid bands detected by the procedure described in the text also were fainter in August of 1978 and 1979, PSTV was detected in all SYI plants when the gels were photographed. Bands not visible to the eye were obvious in photographs. In FYI, however, PSTV was not detected in August in 30% of the infected plants assayed. Although all plants were inoculated on the same date and plants of the same cultivar developed similar tuber symptoms, viroid accumulation in stem apices apparently varies from plant to plant in FYI and this variation can affect assay results. Thus, the time interval before PSTV can be detected in field-grown potatoes in Wisconsin appears to be longer than the 20 days reported for greenhouse-grown tomatoes (5) and the 4–6 wk reported for greenhouse-grown potatoes (11). In FYI, the length of the latent period and detection efficiency did not seem to be influenced by PSTV strain.

The described procedure offers distinct advantages over systems described previously. It increases speed, reliability, and interpretation of sample processing and facilitates field indexing for PSTV.

TABLE 4. Concentration of potato spindle tuber viroid (PSTV) in potato cultivars[a]

Cultivar	PSTV concentration (μg/0.5-g samples)[b]
Katahdin	0.78 ± 0.10
Kennebec	0.32 ± 0.02
LaChipper	0.62 ± 0.08
Monona	0.46 ± 0.04
Norchip	0.66 ± 0.08
Norgold Russet	0.98 ± 0.14
Norland	0.41 ± 0.04
Oneida	1.10 ± 0.20
Ontario	0.66 ± 0.12
Red LaSoda	0.66 ± 0.14
Russet Burbank	0.56 ± 0.10
Sebago	0.98 ± 0.10
Superior	0.36 ± 0.06
Wischip	0.98 ± 0.02

[a]Samples were collected from stem apices of plants derived from PSTV-infected tubers and were assayed by the method described in the text. Plants were maintained in a greenhouse at 24 C with a 16-hr day and sampled 3 wk after emergence.

[b]PSTV concentration was determined by planimetry of curves generated by scanning the gels at 280 nm. Values represent an average of three determinations.

LITERATURE CITED

1. DIENER, T. O. 1971. Potato spindle tuber "virus." IV. A replicating, low molecular weight RNA. Virology 45:411-428.
2. DIENER, T. O., and W. B. RAYMER. 1967. Potato spindle tuber virus: A plant virus with properties of a free nucleic acid. Science 158:378-381.
3. GOSS, R. W. 1930. Insect transmission of potato virus diseases. Abstract Phytopathology 20:136.
4. MERRIAM, D., and R. BONDE. 1954. Dissemination of spindle tuber by contaminated tractor wheels and by foliage contact with diseased potato plants. Abstract Phytopathology 44:111.
5. MORRIS, T. J., and E. M. SMITH. 1977. Potato spindle tuber disease: Procedures for the detection of viroid RNA and certification of disease-free potato tubers. Phytopathology 67:145-150.
6. MORRIS, T. J., and N. S. WRIGHT. 1975. Detection on polyacrylamide gels of a diagnostic nucleic acid from tissue infected with potato spindle tuber viroid. Am. Potato J. 52:57-63.
7. MOSCH, W. H. M., H. HUTTINGA, F. A. HAKKAART, and J. A. DeBOKX. 1978. Detection of chrysanthemum stunt and potato spindle tuber viroids by polyacrylamide gel electrophoresis. Neth. J. Plant Pathol. 84:85-93.
8. NIBLETT, C. L., E. DICKSON, R. K. HORST, and C. P. ROMAINE. 1980. Additional hosts of four viroids and an efficient purification procedure. Phytopathology 70:610-615.
9. PFANNENSTIEL, M. A., and S. A. SLACK. 1980. Response of potato cultivars to infection by potato spindle tuber viroid. Phytopathology 70: (In press).
10. SCHULTZ, E. S., and D. FOLSOM. 1923. Transmission, variation, and control of certain degeneration diseases of Irish potatoes. J. Agric. Res. 25:43-117.
11. SCHUMANN, G. L., H. D. THURSTON, R. K. HORST, S. O. KAWAMOTO, and G. I. NEMOTO. 1978. Comparison of tomato bioassay and slab gel electrophoresis for detection of potato spindle tuber viroid in potato. Phytopathology 68:1256-1259.
12. SINGH, R. 1966. Studies on potato spindle tuber virus. Ph.D. Thesis, North Dakota State University, Fargo. 89 pp.