Detection and Spread of Potato Virus S

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ABSTRACT

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Enzyme-linked immunosorbent assay (ELISA) detected potato virus S in purified virus preparations at concentrations as low as 100 ng/ml and in extracts from naturally infected potato plants. The best stage of plant development for virus detection was at or before flowering with samples from middle leaves of the plant. Potato virus S could be detected in composite samples of leaves from 20 to 30 plants. By ELISA, spread by leaf contact was shown in greenhouse and field conditions. In plots initially containing 10% infected plants, the number of infected plants increased approximately twofold in one season.

Additional key word: epidemiology

Potato virus S (PVS) is one of the most common latent viruses in potatoes in North America (17). Most commercially important potato seed stocks are universally infected (16). PVS may decrease yield of potato tubers by 3–20% (12,21).

Rapid, reliable, and sensitive indexing techniques for PVS are essential to any program for the production of virus-free potatoes and potato seed certification. Lack of a suitable indicator host (2), low concentration of PVS in infected potato plants (14), and variable concentration of PVS in infected plants throughout the growing season and among varieties (6,10) complicate detection of PVS. Several serologic methods have been used for detection of PVS (9,10,15,18), but none is as sensitive as the enzyme-linked immunosorbent assay (ELISA) for detection of viral antigens.

This paper reports the use of ELISA to detect PVS in purified preparations, tuber sprouts, and foliage samples collected at different stages of plant growth. The feasibility of detecting PVS in composite plant samples was also investigated.

ELISA was also used to study the spread of PVS. Some PVS isolates can be transmitted by the aphid *Myzus persicae* Sulz (3,11); however, aphid transmission has been discounted by some investigators (5,8,22). The discrepancies in these reports prompted us to study the spread of PVS with special attention to contact transmission.

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MATERIALS AND METHODS

Virus isolate. PVS was obtained from greenhouse-grown, naturally infected

potato (*Solanum tuberosum* L. 'Targhee'), provided by D. H. Hall, University of California, Davis. The virus produced chlorotic local lesions on *Chenopodium quinoa* Willd 21 days after mechanical inoculation and produced systemic vein clearing and mosaic symptoms in *Nicotiana debneyi* Domin 20 days after inoculation. Identity of the virus was confirmed by electron microscopy and in serologic tests using PVS antiserum provided by S. A. Slack, University of Wisconsin.

Virus purification. Infected foliage was homogenized with a Waring Blendor in cold (4 C) 0.5 M borate (boric acid-NaOH) buffer, pH 8.2, containing 1% sodium sulfite at 1 g of tissue per 4 ml of buffer solution. Crude extracts were squeezed through a double layer of cheesecloth. The filtrate was centrifuged for 10 min at 8,000 rpm using a GSA rotor in a Sorvall centrifuge. Pellets were discarded and the supernatant fluid was clarified by vigorous shaking for 5 min with an equal volume of chloroform. The resultant emulsion was broken by centrifugation at 8,000 rpm for 10 min in a GSA rotor. The aqueous (upper) phase was collected by aspiration, and the virus was precipitated from it by adding solid polyethylene glycol (PEG, mol wt 6,000) to 7% (w/v). The extract was stirred until the PEG was completely dissolved and then placed in a refrigerator (4 C) for 1 hr. The precipitate was collected by centrifugation for 10 min at 10,000 *g*. The supernatant was discarded and the precipitates redissolved overnight in one-half the original volume of 0.05 M borate buffer, pH 8.2, in a refrigerator. Insoluble components were removed by centrifugation at 10,000 *g* for 10 min.

Virus in the supernatant fluid was reprecipitated by the addition of 4 g of NaCl and 5 g of PEG per 100 ml as described previously and resuspended in one-quarter of the original volume of 0.05 M borate buffer, pH 8.2, in a refrigerator overnight. After clarification, the virus was sedimented by two cycles of differential ultracentrifugation (38,000 rpm for 2 hr in a Beckman 65 rotor) and resuspended in a small volume of 0.05 M borate buffer, pH 8.2. Virus concentration was determined with a Beckman 25 UV spectrophotometer and an extinction coefficient of 3.0 cm^2/mg at 260 nm (15).

Antiserum production. Initial immunization was achieved by injecting 1.5 mg of virus in the marginal ear vein and 2 mg of virus emulsified in an equal volume of Freund's complete adjuvant into a leg muscle. Four additional intramuscular injections each of 1.5–2.0 mg of virus were made at weekly intervals. Animals were bled 10 days after the last injection and at 10-day intervals thereafter. The serum was separated and cross-absorbed with healthy potato protein. The titer, after absorption, was 1/512 by using purified virus (0.1 mg/ml) in microprecipitin tests.

ELISA. ELISA was done as described for detecting plant viruses (4,19). Gamma-globulin was purified from the PVS-absorbed antiserum by precipitation with half-saturated ammonium sulfate followed by filtration through a DE22 cellulose column. Gamma-globulin was conjugated with alkaline phosphatase (Type VII, Sigma Chemical Co., St. Louis, MO 63178) using 0.06% glutaraldehyde (4) and was stored at 4 C with bovine serum albumin after thorough dialysis.

Test samples were prepared in a grinding buffer containing phosphate-buffered saline, pH 7.4; 0.5% Tween 20; 2% polyvinyl pyrrolidone (mol wt 40,000); and 0.2% ovalbumin.

Results were considered positive if the average absorbance at 405 nm in a Titerket Multiskan ELISA reader (Flow Laboratories Inc., Inglewood, CA 90302) was at least twice that of healthy control samples in the same plate (20).

Crude extracts for ELISA tests were obtained by grinding 2 g of plant material (leaves or tuber sprouts) in 2 ml of the grinding buffer with a pestle and mortar and squeezing the material through a double layer of cheesecloth.

Detection of PVS at different stages of plant growth. Sprouts were tested 4 wk after the tubers were placed at room temperature. PVS-infected and virus-free potato cultivars were planted in late July 1980 in the Delta area near Stockton, CA, and plants were sampled about 1, 2, and 3 mo after planting. Three plants were randomly selected from each cultivar on each sampling day. A leaflet was collected from near the top, middle, and bottom of each plant. Each leaflet was tested separately by ELISA.

Aphid transmission. Green peach aphids, *Myzus persicae* Sulz, were reared on turnip and starved in a glass dish for 1 hr before use. They were given an acquisition feeding period of 1 or 20 min on infected potato leaves and then transferred to test plants (virus-free potato or *N. debneyi* plants) at the rate of five aphids per plant. Aphids were left to feed on test plants overnight and were then killed with nicotine. Six weeks later, test plants were assayed for PVS by ELISA.

Spread of PVS by leaf contact. Thirty virus-free half tubers (provided by S. A. Slack) were planted in 20-cm pots arranged in groups of six in an insect-free greenhouse. The central pot contained a PVS-infected Targhee tuber. Maximum opportunity for contact between infected and virus-free foliage was assured by staking the groups of plants so that foliage of the infected plant in the center pot and the five virus-free surrounding plants mingled thoroughly. Each plant was tested by ELISA 2 mo after staking.

Spread of PVS in the field. In late July 1980, three plots were planted with virus-free potato tubers in the Delta area near Stockton, CA. Each plot contained four rows of 10 plants; four PVS-infected Targhee tubers were planted at random among 36 healthy tubers. Plants were spaced 25 cm apart in rows, with 100 cm between rows. The foliage of each plant was tested for PVS by ELISA 3 mo after planting.

RESULTS

Sensitivity and specificity of ELISA for PVS. The optimum concentration of gamma-globulin for the coating step was 1 μg/ml. The dilution of enzyme-antibody conjugate that produced the most intense reaction with the virus samples without appreciable reaction of the control was 1/800. With a purified preparation of PVS, positive readings were obtained with 100 ng/ml but not with 10 ng/ml (Fig. 1). PVS was detected in leaf extracts of Targhee and several other PVS-infected potato cultivars but not from healthy potatoes or from PVX-infected tobacco.

Detection of PVS at different stages of plant growth. ELISA readings of the tuber sprouts were in the range of the readings of leaflets (Table 1). At 1 mo (early season), there were some differences in ELISA readings among samples collected from the top portion of the plant and samples collected from the middle or bottom of the same plant. Samples collected after 2 mo (at or before flowering) showed higher ELISA readings than those taken after 1 or 3 mo. At this stage (2 mo after planting), samples collected from the middle part of the plant showed the highest ELISA readings.

Detection of PVS in composite samples. Samples were prepared by punching disks (1 cm diameter) from PVS-infected and virus-free potato plants 2 mo after planting (optimal time for virus detection). One leaf disk from a PVS-infected plant was homogenized with varying numbers of disks from healthy potato plants in grinding buffer at the ratio of 1 ml/g of tissue. PVS was detected in composite samples of one infected disk in 20 with five cultivars and one infected disk in 30 with four cultivars (Table 2).

PVS epidemiology. No transmission occurred when aphids were given a 1-min acquisition feeding on infected potato leaves before being transferred to 65 potato and 15 *N. debneyi* plants, or of 20 min before being transferred to 25 potato and 15 *N. debneyi* plants. Also, virus was not detected by ELISA with homogenate of 50 aphids that had been given a 1-min probe on a PVS-infected potato leaf.

Leaves of weeds and native plants growing in and around potato fields were collected and tested for PVS by ELISA. No positive reactions were obtained from at least three plants each of *Amaranthus albus* L., *A. hybridus* L., *Chenopodium*

album L., *C. murale* L., *C. pumilio* R., *Erodium cicutarium* L., *Euphorbia esula* L., *Medicago sativa* L., *Polygonum aviculare* L., *P. argyrocoleon* Steud., *Portulaca oleracea* L., *Tribulus terrestris* L., and *Verbascum thapsus* L.

Spread of PVS. After 2 mo in contact with PVS-infected plants in the insect-free greenhouse with conditions that excluded insect participation, five of the 30 previously healthy plants were infected with the virus. In the field, 11 of the 108 previously virus-free plants were infected with PVS. All newly infected potato plants were adjacent to a virus source (Fig. 2).

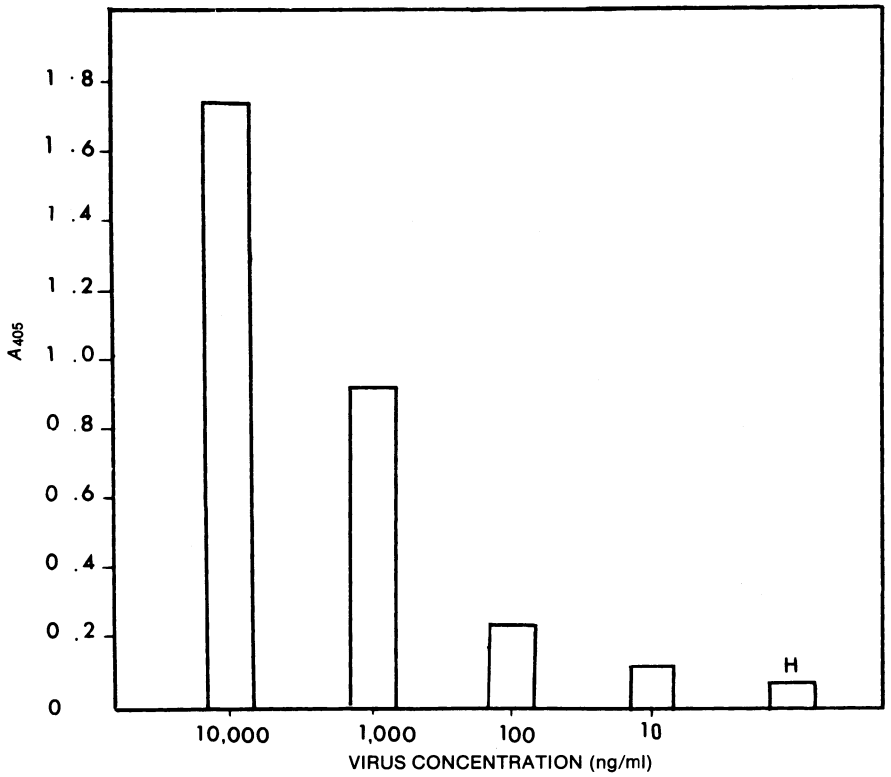

Fig. 1. Enzyme-linked immunosorbent assay absorbance values at 405 nm for a purified preparation of potato virus S. H = healthy control sap absorbance.

Table 1. Detection of potato virus S by ELISA in samples of potato cultivars at different stages of growth

		Time from planting in field[b]								
		1 mo			2 mo			3 mo		
Potato cultivar	TS[a]	T[c]	M	B	T	M	B	T	M	B
Russet Burbank-58	1.384	0.952	1.251	1.390	1.293	1.453	1.351	1.531	1.482	1.501
Norgold Russet-54	1.125	0.738	1.052	1.169	1.183	1.499	1.382	1.409	1.098	1.198
Atlantic-53	1.325	0.531	1.239	1.303	1.509	1.643	1.520	1.130	1.333	1.371
Kennebec-51	1.254	0.935	1.357	1.381	1.307	1.534	1.352	1.313	1.252	1.234
Chieftain-52	1.351	0.359	1.391	1.432	1.513	1.508	1.407	1.398	1.209	1.403
Healthy potato	0.073	0.085	0.083	0.094	0.084	0.075	0.082	0.084	0.086	0.090

[a] TS = tuber sprouts tested 4 wk after tubers were submitted to room temperature.
[b] Data are average ELISA absorbance values at 405 nm of three samplings.
[c] Leaf samples were taken from T = top, M = middle, B = bottom of the plant.

Table 2. Sensitivity of ELISA for detecting potato virus S in a homogenate of disks from infected and healthy potato leaflets

	Ratio of infected/healthy disks[a]				
Potato cultivar	10/0	1/10	1/20	1/30	1/40
Russet Burbank-58	1.356	1.158	0.783	0.489	0.092
Norgold Russet-54	1.484	0.936	0.536	0.351	0.073
Atlantic-53	1.543	1.254	0.391	0.094	0.083
Kennebec-51	1.473	0.783	0.452	0.354	0.109
Chieftain-52	1.583	0.964	0.654	0.413	0.120
Controls					
Healthy potato	0.08				
Buffer	0.03				

[a] Data are average ELISA absorbance values at 405 nm of six samples.

DISCUSSION

Low concentrations of PVS were detected by ELISA in purified virus preparations, potato tuber sprouts, leaf extracts, and composite plant samples. In purified preparations, PVS was detected as low as 100 ng/ml, compared with 500 ng/ml by latex agglutination, which is considered to be more sensitive than agar diffusion (10).

Potato cultivar, position of the leaf on the plant, and plant growth stage influenced the detection of PVS under field conditions. Samples from the middle leaves of the plant at or before flowering gave the highest ELISA readings. These results confirm an earlier report that potato plants should be indexed just before flowering (16). Our results also indicated that PVS was easily detectable in potato sprouts by ELISA. Because ELISA readings for tuber sprouts were in the range of readings for leaves, it is possible to use tuber sprouts as a source of tissue for detection of PVS. The sensitivity of ELISA in detecting a single infected plant in batches of 20–30 samples indicates that ELISA is better than other methods now in use for indexing potato plants for PVS. Other reports have shown similar results for other viruses by ELISA (7,13).

Because of the widespread occurrence of PVS in most potato varieties, the virus is assumed to spread freely in nature. In our experiments, PVS was transmitted by foliage contact, but not by aphids as has

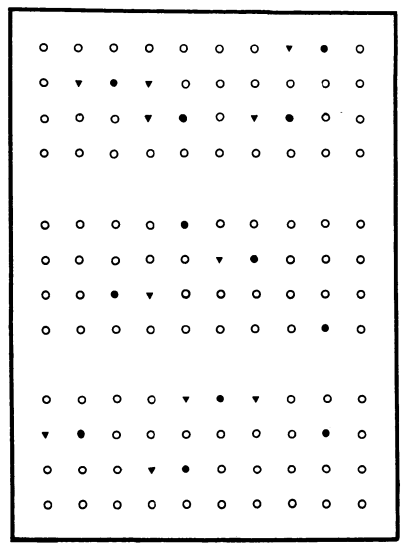

Fig. 2. Diagrammatic representation of three plots planted with potato virus S (PVS)-infected and healthy tubers showing plants infected 3 mo after planting in the field. Note that all newly infected plants are adjacent to source plants. • = source of virus, infected tuber planted; ▼ = plant infected at maturity, virus-free tuber planted; o = healthy plant at maturity, virus-free tuber planted.

been reported for some isolates (3,13). Cucumber mosaic virus has been detected by ELISA in a single aphid after probing on an infected plant (1). The failure to detect PVS in 50 aphids was probably not due to the insensitivity of ELISA. The apparent absence of PVS in the weed samples, the failure to transmit PVS from infected potato leaves to test plants when aphids were given 1- or 20-min acquisition feedings, and PVS transmission by foliar contact all indicate that it is unlikely that this isolate is aphid-transmissible.

Under field conditions, the number of infected plants increased approximately twofold in one growing season. Newly infected plants were always adjacent to a virus source. These results agree with reports of field spread of PVS in Alpha and Eersteling potatoes (5) and indicate that under local field conditions our PVS isolate was probably transmitted by foliar contact.

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