Identification of an Isolate of the Andean Strain of Potato Virus S in North America

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ABSTRACT

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An unusual isolate of potato virus S (PVS-An) was recovered from potato (*Solanum tuberosum* 'Red La Soda'). In contrast with most described PVS isolates, PVS-An is aphid-transmissible and invades *Chenopodium quinoa* systemically after inducing chlorotic local lesions. PVS-An was transmitted (10 aphids per plant) at about 10% efficiency in tests that included transmission of purified PVS-An by aphids fed through artificial membranes. The systemic invasion of *C. quinoa* is of interest because the PVS isolates reported from Europe and North America induce only a local lesion response. Recent evidence indicates that aphid-transmissibility and systemic invasion of *C. quinoa* are common features of isolates of the Andean strain of PVS. PVS-An conforms to the description of the Andean strain of PVS.

Additional key word: carlavirus

Potato virus S (PVS), a carlavirus that is generally symptomless in potato (*Solanum tuberosum* L.), is found in *Solanum* spp. throughout the world (18). Although efforts are under way in North America to eliminate PVS and other latent potato viruses from seed stocks, PVS is still common in most of the established potato varieties. Also, data indicate that PVS recontamination of seed stocks may be rapid in some potato varieties and generally more rapid than recontamination with potato virus X (PVX), another latent potato virus (4,12,13). Although some PVS isolates are aphid-transmitted, PVS spread in potato cultivars is thought to be primarily by contact (8,18,21).

Particle morphology and serology are the usual criteria for PVS identification because the response of indicator hosts has varied with the PVS isolates studied. In general, the PVS isolates studied in North America and Europe have induced a local lesion response in *Chenopodium quinoa* Willd. and guar (*Cyamopsis tetragonoloba* (L.) Taub.) and have not been able to infect tomato (*Lycopersicon esculentum* Mill.) (1,3,7,11).

The apparent inability of PVS to infect tomato has been used as a basis for separating PVS and potato virus M (PVM) in mixed infections (1,19). Others, however, have shown that tomato is susceptible to many PVS isolates (10,20). In addition, Hinostroza-Orihuela (5) reported recovery of PVS from germ plasm from Peru that invaded *C. quinoa* systemically. Recent work at the International Potato Center has demonstrated that some Andean isolates of PVS frequently invade *C. quinoa* systemically and are aphid-transmissible (15). These isolates are considered isolates of the Andean strain of PVS (C. E. Fribourg and R. A. C. Jones, *personal communication*).

In a 1975 survey of potato cultivars in Wisconsin, an isolate of PVS was detected that invaded *C. quinoa* systemically. Because the response of *C. quinoa* to this PVS isolate, PVS-

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An, was unique and appeared to indicate some significance as to the possible origin of PVS-An, a comparative study of PVS-An with type strains of PVS (PVS-T) and PVM was initiated. A preliminary report has been presented (16).

MATERIALS AND METHODS

The PVS-An isolate was obtained from the potato cultivar Red La Soda. Three sequential single-lesion transfers of the PVS isolate obtained from the original Red La Soda plants from Wisconsin were made prior to further characterization studies. Comparative studies were made with type strains of PVS and PVM obtained from the Schultz potato virus collection (17) and maintained in USDA potato seedling 41956 and in the potato cultivar Saco, respectively. Saco is immune to PVX and highly resistant to PVS and USDA 41956 is immune to PVX (1). All test plants were inoculated by dusting leaves with corundum (600-mesh), then rubbing leaves with a cotton swab that had been dipped into an extract of infected leaves triturated in 0.03 M phosphate buffer, pH 8.0 (1:10, w/v).

Viruses were purified from frozen leaf tissue. All purification steps were performed at 4 C. After blending in three volumes of 0.5 M borate (pH 8.2) containing 0.1% 2-mercaptoethanol and 1.0% sodium sulfite, the homogenate was expressed through cheesecloth and chloroform (30% final volume) was added while stirring. The extract was then centrifuged for 10 min at 10,000 *g* and polyethylene glycol (6,000 mol wt) and NaCl (6 and 1.8%, w/v, respectively) were added to the recovered supernatant. After 1 hr, the centrifugation step was repeated and the resultant pellets were resuspended overnight in one-third the volume of 0.05 M borate (pH 8.2). The final steps consisted of two cycles of differential centrifugation. In high-speed cycles, virus was being sedimented through 10 ml of a 20% sucrose cushion containing 0.5% Triton X-100 at 78,000 *g* for 2.5 hr.

Purified virus (1–2 mg/ml) was emulsified with Freund's complete (first injection) or incomplete (subsequent injections) adjuvant (1:1, v/v). New Zealand white rabbits were given two or three primary subcutaneous injections at weekly intervals followed by a booster injection 3 wk after the last primary injection. Rabbits were bled 1 wk after

Fig. 1. (Right) *Chenopodium quinoa* plant with systemic chlorotic mottle after inoculation with potato virus S-An and (left) a healthy control plant.

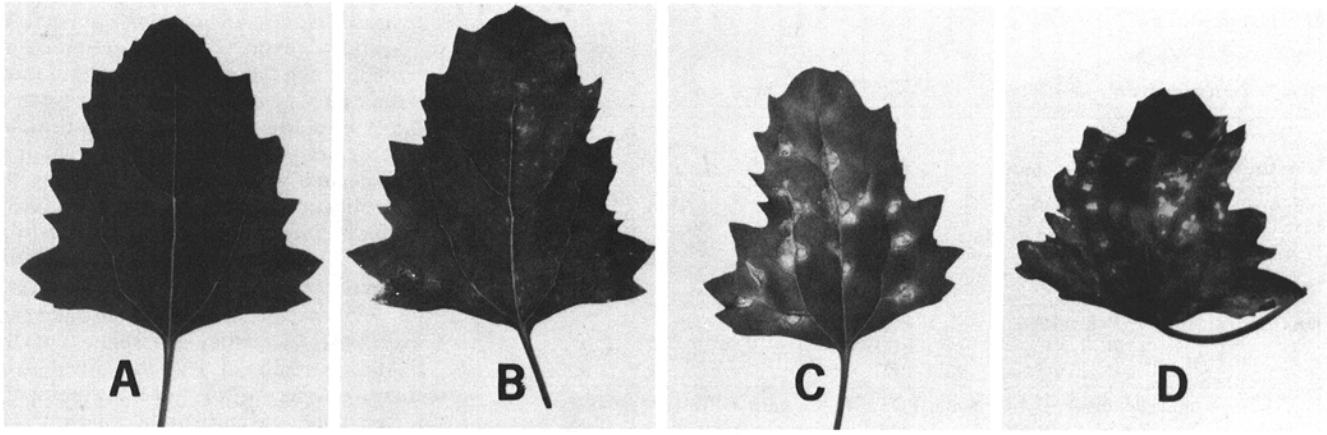

Fig. 2. Leaves from *Chenopodium quinoa* plants showing: **(A)** healthy control, **(B)** local lesions incited by the type strain of potato virus S (PVS), **(C)** local lesions incited by PVS-An, and **(D)** systemic symptoms incited by PVS-An.

the booster injection. Titers were determined by microprecipitin tests (2) and latex agglutination tests were performed as described previously (9).

For electron microscopy, diluted preparations of freshly cut virus-infected tissues were touched to a droplet of distilled water on carbon-coated Formvar films. After shadowing with carbon-platinum, samples were examined with a JEM 8 electron microscope calibrated with a carbon-grating replica.

A colony of nonviruliferous *Myzus persicae* (Sulzer) maintained on radish (*Raphanus sativus* L.) was used for aphid-transmission studies. Aphids were starved 1–3 hr before placement for 10 min on access hosts or on stretched Parafilm M membranes containing purified PVS (1 mg/ml) in 20% sucrose. Ten aphids were transferred to each test plant. After 24 hr, aphids were killed by spraying test plants with pirimicarb and by adding aldicarb to the soil.

Table 1. Serological relationship between potato virus S (PVS) strains and potato virus M (PVM)

Antigen[a]	Antiserum to PVM	PVS-An	PVS-T
PVM	1,024[b]	8	8
PVS-An	8	2,048	1,024
PVS-T	8	512	4,096
Healthy protein	0	0	4

[a] Antigens were purified from potato (viruses) or tobacco (healthy protein) before use.
[b] Number represents reciprocal titer of the diluted antiserum in microprecipitin tests.

RESULTS AND DISCUSSION

In a 1975 survey of seed potato fields in Wisconsin, PVS was recovered from 12 potato cultivars. All PVS isolates incited chlorotic local lesions on *C. quinoa* in 8–12 days. In addition, isolates from Red La Soda caused a systemic chlorotic mottle in 14–17 days. The latter plants became stunted in 3–4 wk and leaves showed necrotic lesions, epinasty, distortion, and premature abscission (Figs. 1 and 2). Similar PVS isolates were recovered from Red La Soda seed tubers obtained from Minnesota, Nebraska, and North Dakota. All Red La Soda plants observed have been symptomless under greenhouse and field conditions.

The identity of PVS-An as an isolate of PVS was confirmed on the basis of particle morphology and serological reactions. Measurement of 46 PVS-An virions and 32 PVS-T virions produced mean particle lengths of 672 ± 15 and 686 ± 15 nm, respectively. Microprecipitin serological tests determined that PVS-An was closely related to PVS-T and distantly related to PVM (Table 1). In latex agglutination tests, all PVS isolates tested reacted with sensitized latex preparations using antisera produced against either PVS-An or PVS-T but not with sensitized latex preparations using PVM antiserum. In contrast, PVM reacted with the sensitized latex preparations made with the PVM antiserum but not the PVS-An or PVS-T antisera.

Both PVS isolates were purified from potato, and PVS-An was also purified from systemically infected leaves of *C. quinoa* and *Nicotiana* spp. The *Nicotiana* spp. were discontinued as propagation hosts because infection of inoculated plants was erratic. Virus yield for PVS ranged from 2 to 40 mg virus per 100 g infected tissue. Highest virus yields (23–40 mg virus per 100 g infected tissue) were obtained when PVS-An was purified from *C. quinoa* about 3 wk after inoculation. The A_{260}/A_{280} ratio for purified virus preparations ranged from 1.19 to 1.58, with a mean of 1.31. A single infectious virus zone was present in linear-log sucrose gradients.

A comparison of host reactions on differential indicator plants demonstrated that PVS-An differed from both PVS-T and PVM (Table 2). PVS-An differs from PVS-T and most reported PVS isolates in that it is systemic in *C. quinoa*, does not infect *C. tetragonoloba*, and infects tomato. In addition, Hiruki (6) reported *Phaseolus vulgaris* L. 'Red Kidney' to be a diagnostic local lesion host for PVM and immune to PVS. PVS-T, however, caused local infection in Red Kidney bean. Kowalska (10) also reported PVS isolates that incited local lesions on Red Kidney bean. It is apparent that unequivocal diagnosis of PVS or PVM based on indicator plant response is not possible. Consequently, I used serological assays for the routine detection of both viruses. Supplemental bioassays were on *C. quinoa* for PVS and *Datura metel* L. for PVM.

Table 2. Differential host reactions to potato viruses S (PVS) and M (PVM)

Host	Virus PVS-An	PVS-T	PVM
Chenopodium album L.	CL,Vn[a]	CL	(−)
C. amaranticolor Coste & Reyn.	CL,CM	CL	(−)
C. murale L.	CL,CM	CL,CM	(−)
C. quinoa	CL,CM	CL	(−)
Cyamopsis tetragonoloba	(−)	NL	(−)
Datura metel L.	Vc	Vc	CL,E,Ld,St
Gomphrena globosa L.	CL,NL	(−)	(−)
Lycopersicon esculentum 'Rutgers'	S	(−)	S
Phaseolus vulgaris 'Red Kidney'	(−)	NL	NL

[a] C = chlorotic, E = epinasty, L = local or primary lesion, Ld = leaf drop, M = mottle, N = necrotic, S = symptomless, St = stunting, Vc = veinclearing, Vn = vein necrosis. Reactions were systemic unless designated by L. (−) = test plant not infected.

Table 3. Transmission of two strains of potato virus S (PVS) by *Myzus persicae*

Virus strain	Access source[a]	Test host[b]	Assays (no.)	Transmission (No.)	Transmission (%)
PVS-An	POT-RLS	POT	69	7	10
	POT-NR	POT-NR	30	0	0
	C. quinoa	*C. quinoa*	23	3	13
	Purified	POT-NR	26	7	27
	Purified	*C. quinoa*	25	0	0
PVS-T	POT-41956	POT	103	0	0
	POT-NR	POT-NR	30	0	0
	Purified	POT-NR	29	0	0

[a] POT-RLS = potato cultivar Red La Soda, POT-NR = potato cultivar Norgold Russet, POT-41956 = USDA seedling 41956, and Purified = purified virus (1 mg/ml) in 20% sucrose and within stretched Parafilm M membrane.
[b] After 5–10 min on access host, 10 aphids per plant were transferred to test host for 24 hr.

The role of aphid transmission in the epidemiology of PVS is not clear but could be important in establishing PVS in PVS-free crops (8). The PVS-T strain in USDA seedling 41956 was the basis for early reports that PVS was not transmitted by aphids (18,21), and this study supports those reports (Table 3). In contrast, this study is consistent with others that show that isolates of the Andean strain of PVS are transmitted by aphids (15; C. E. Fribourg and R. A. C. Jones, *personal communication*). Transmission of purified PVS-An was of interest because many viruses require an accessory factor to be aphid-transmitted in a nonpersistent manner and therefore are not transmitted once purified (14). Current evidence does not exclude the possibility that an accessory factor may have remained associated with PVS virions during the purification process. Data do indicate that an interesting virus-vector relationship exists that merits additional investigation.

Evidence presented here indicates that PVS-An is similar to isolates of the Andean strain of PVS described from South America. It is not known how the Red La Soda cultivar became infected with PVS-An, but the clonal propagation of PVS-An-infected seed stocks of this

cultivar appear responsible for maintaining this virus isolate in North America.

PVS-An does not appear to pose a significant threat to the North American potato crop because infected potato plants are symptomless and PVS-An has not been recovered from PVS-free cultivars grown in the vicinity of PVS-An-infected plants (S. A. Slack, *unpublished*). As PVS-free potato cultivars become more prevalent in the future, however, aphid-transmissible strains may pose a more significant threat to the contamination of PVS-free stocks than will non-aphid-transmissible PVS strains. In any event, the Red La Soda stocks known to be infected with PVS-An will be systematically replaced. In itself, PVS-An is a valuable research tool because it can be purified in large quantities from *C. quinoa* and can be used to study the mechanisms underlying the nonpersistent aphid-transmissibility of plant viruses.

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