Lack of Potato Virus S Transmission via True Seed in Solanum tuberosum

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ABSTRACT

Microprecipitin and single-radial-immunodiffusion serological methods combined with indicator hosts (*Nicotiana debneyi* and *Chenopodium quinoa*) did not detect potato virus S (PVS) in 1,028 greenhouse-grown seedlings derived from crosses of five PVS-infected parents. To insure accuracy of the negative tests, we saved tubers from these seedlings and repeated the tests through two subsequent tuber generations; PVS was not detected in any of the plants. Therefore, we conclude that PVS is not transmitted via true seeds of crosses between PVS-infected cultivars Atzimba and Katahdin and breeding lines GN70-3, A6334-19, or B6695. This evidence indicates that PVS probably is not transmitted through true seed in tetraploid potatoes.

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Additional key words: virus dissemination, PVS-free potatoes, serological detection.

Potato virus S (PVS) has been recognized as a virus disease of potato *Solanum tuberosum* L. since its discovery in 1952 by Van Slogteren (14). All potato-growing areas of the world have plants infected with this virus, and 100% of many of the older cultivars are infected (13). The wide distribution and high infection percentage of PVS have been attributed to: (i) PVS-infected plants produce a higher percentage of small tubers, and the use of these as seed has enhanced the propagation and dissemination of the virus (5, 6, 7, 10, 11), and (ii) PVS is latent in many cultivars and, frequently, the only discernible effects are slight yield reductions. This latency makes roguing based solely on symptomatology almost impossible. Moreover, eliminating infected plants by roguing in cultivars that show symptoms is further hampered by erratic symptom development depending on virus strain, cultivar, age of plant, and environment. Even serological assay, the method by which PVS was discovered and still the principal diagnostic method for detecting the virus, is not entirely reliable as an index for roguing, because the virus titer and distribution in the potato plant may vary with plant cultivar and stage of development. Therefore, infected plants are often missed, and can act as insidious sources of initial inoculum in this clonally propagated crop.

Since establishing and maintaining virus-free stocks have special significance in a program for potato germplasm development, we serologically assayed the advanced seedlings for the presence of several viruses, including PVS. We found that 33% of 831 second-generation plants and 35% of 1,233 third-generation plants were infected with PVS in spite of the use of phytosanitary methods. These results suggested that PVS possibly was transmitted through true seed used in the breeding program. Therefore, we conducted the study reported here to determine if transmission via true seed of the parental lines was the source of initial infection of PVS.

MATERIALS AND METHODS.—The methods for producing and increasing plants and the various stages at which assays were made are outlined in Fig. 1.

Parental lines.—The parental plants of crosses 8174 (Atzimba × Katahdin), 8179 (Atzimba × B6695), 8211 (A6334-19 × Atzimba), 8213 (A6334-19 × B6695), and 8364 (GN70-3 × Katahdin) were assayed for the presence of PVS. All parents were grown in the greenhouse. Each plant was assayed when it was about 16 cm tall, and again when it was in the early flowering stage. Tissue samples were a composite of three terminal leaflets from selected compound leaves. Throughout this study, the potato cultivar Saco and PVS-free cultivar Katahdin obtained from N. S. Wright (Research Station, Vancouver, British Columbia) were used as PVS-free controls. PVS-infected breeding line 41956 from E. S. Schultz's collection and the PVS-infected *S. tuberosum* 'Shoshoni' were used as PVS-infected controls.

Virus assays.—Three methods were used to assay extracted sap. Droplets of two-fold serial dilutions of PVS antiserum and normal serum obtained from D. Van Slogteren (Bulb Research Department, Lisse, The Netherlands) were evenly distributed across plastic petri dishes, and droplets of sap from the various plants were added to them. The volume of the sap was about half that of the antiserum. The droplets were covered with paraffin oil and then the dishes were agitated on an orbital shaker at 120 rpm for 20-30 minutes at 22 C. The drops were observed for flocculation with a dissecting microscope by use of side illumination and a dark background.

Single-radial-immunodiffusion method. — Immunodiffusion plates were prepared by the addition of 3 ml of PVS D-protein antiserum to 5 ml of 2% agarose (12). The agarose was melted in 0.2 M [Tris(hydroxymethyl)-aminomethane]-HCl buffer pH 7.2 containing 0.85% NaCl buffer brought to 50 C, and 0.4% sodium azide was added. Two μliters of crude sap was extracted with 30 μliters of 3.5% pyrrolidine. An aliquot of each extract-pyrrolidine mixture was placed into the depot of the Ionagar PVS D-protein plate with pasteur pipettes.

Nicotiana debneyi Domin. and *Chenopodium quinoa* Willd. (5) were used as diagnostic hosts to detect PVS, for further assay in those plants that showed questionable reactions in the above methods. Leaves of these plants were dusted with Carborundum and rubbed with crude sap of the individual test plants (10). By electron microscope, we examined infected and control test-plant leaves using the dip method of Brandes (2, 3).

Seedlings.—To remove seeds, we cut each fruit in half, squeezed seed and pulp into water, and washed the seeds from the pulp. The first 1,028 seedlings assayed were

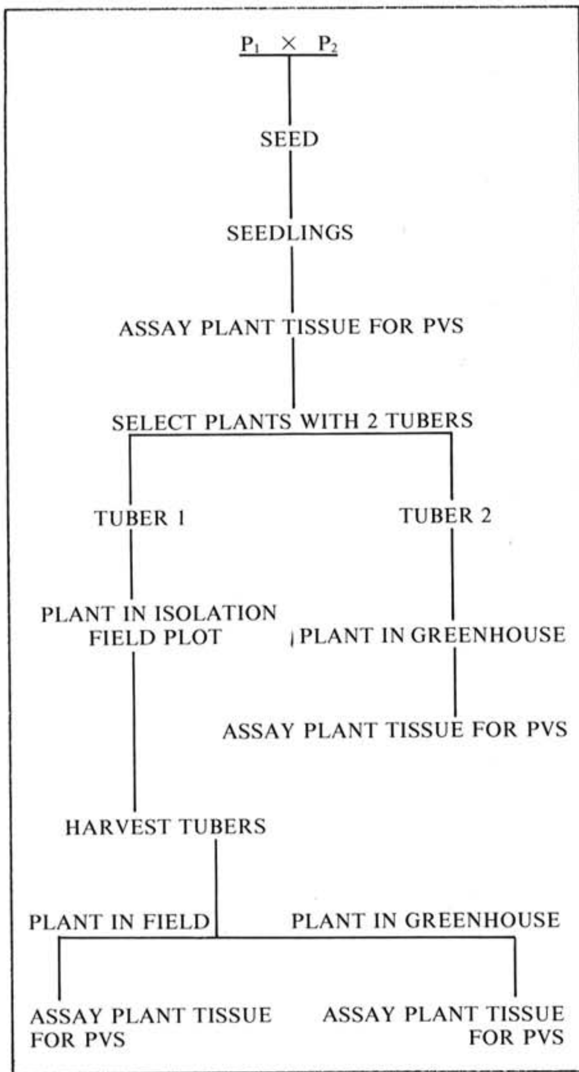

Fig. 1. Schematic outline of procedure used to study possible seed transmission of potato virus S.

started in small flats in the greenhouse and later transplanted to individual 10.2-cm (4-inch) diameter pots in steam-sterilized soil. We assayed each seedling twice with the single-radial-immunodiffusion method, first when it was about 16 cm tall and second, 2 weeks before it was harvested. Two tubers were harvested from each of the 638 plants that formed tubers.

Greenhouse–first tuber generation.—The tubers were stored at 4 C. Six weeks after harvest, one tuber was removed from storage, kept at 22 C for 1 week, treated with gibberellic acid to break dormancy (4), and planted in the greenhouse in 20.3-cm (8-inch) diameter pots with steam-sterilized soil. Each of the 581 plants that developed was assayed twice for PVS by the single-radial-immunodiffusion method. We assayed the plants when they were 16 cm tall and again when they flowered, using composite tissue samples of two terminal leaflets from selected compound leaves.

Field–first tuber generation.—The remaining tuber of each selected seedling was kept at 4 C until May, when it was planted by hand in an isolated field plot at Chapman Farm, Presque Isle, Maine. To reduce the possibility of mechanically spreading the virus, rows in these plots were planted on 2-m centers, and blank rows were left for tractor movement. Cultivating and hilling were completed before the vines were large enough to come into contact with the machinery. All insecticide sprays and vine-killing chemicals were applied aerially. To decrease the amount of contact, we did not assay these plants for PVS during the growing season. Instead, two tubers were harvested from each of 410 plants, individually wrapped and returned to Beltsville where they were assayed using radial immunodiffusion serology.

Greenhouse–second tuber generation.—These tubers also were stored at 4 C for 6 weeks. Each tuber was indexed and proper precautions to prevent virus spread were used. The cut section to be planted was treated with gibberellic acid and planted in the greenhouse, and the remaining part was returned to storage. Each resulting plant was assayed twice for the presence of PVS by the single-radial-immunodiffusion method. The plants were assayed first when they were about 16 cm tall and again when they were in the early flowering stage.

Field–second tuber generation.—The remaining part of the numerically indexed tuber returned to storage was planted in a field plot at Beltsville, Md. Again each plant was assayed for the presence of PVS by the single-radial-immunodiffusion method when it was about 16 cm tall and at early flowering.

RESULTS AND DISCUSSION.—PVS was not detected in the leaf tissue of the 1,028 seedlings first assayed (Table 1). The tuber progenies from the 638 plants that tuberized and produced at least two tubers were then assayed. We wanted to determine if PVS infections in these seedlings could have been missed in the first assay, either because of low virus titer or other factors. Such a repetition seemed necessary because results of previous studies have shown that current-season PVS infections are often not detected, but tuber infections by this latent virus may progress very quickly (8).

The possible effect of environment on detection of PVS was studied by growing these tubers in two different environments. The tubers originating from the 638 plants previously found to be PVS-free were planted in the greenhouse 2 weeks after harvesting. PVS was not detected in any of the resulting plants. Sister tubers of the 638 used in the greenhouse study were planted in an isolated plot at Presque Isle, Maine. To simulate normal cultural practices and yet minimize possible mechanical transmission by handling, we did not assay these plants for PVS during their growth. Only 410 of these first-tuber-generation plants produced two or more tubers. Two tubers from each of these plants were indexed, and one piece of each tuber was planted in the greenhouse. The virus was not detected in any of the resulting 786 plants in the greenhouse. However, treatment of these tubers with gibberellic acid and planting them in the greenhouse only 6 weeks after harvest might have accounted for the lack of detection. Therefore, the remaining parts of these tubers were planted in the field by usual practices and, in the resulting 734 plants, PVS was not detected.

TABLE 1. Response of potato *Solanum tuberosum* plants to assays for potato virus S

					Reaction[a]	
Source	Location	Planted (no.)	Plants evaluated (no.)	Tubers harvested (no.)	Plants positive (no.)	Plants negative (no.)
Parents	GH[b]	28	28	0	28	0
Seedlings	GH	1,150	1,028	1,276[c]	0	838
Tuber generation						
1st	GH	638	581	0	0	581
	Field[d]	638	0	820[e]	NT[f]	NT
2nd	GH	820	786	726	0	786
	Field[g]	820	734	0	0	734

[a]Reaction based on indicator plants and microprecipitin and radial-immunodiffusion tests.
[b]Greenhouse at Beltsville, MD.
[c]Two tubers from each of 638 plants.
[d]Field at Presque Isle, ME.
[e]Two tubers from each of 410 plants.
[f]NT = not tested.
[g]Field plot at Beltsville, MD.

It was concluded that PVS was not transmitted via the true seed of these five crosses with PVS-infected Atzimba, Katahdin, GN70-3, A6334-19 or B6695 as parents. These results suggest that PVS is not transmitted readily, or at all, through true seed in potatoes. However, the 33% infection rate in second-tuber-generation plants in the field obtained in our original assays and the relatively low percentages of insect transmission reported by Bode and Weidemann (1), and more recently by MacKinnon (9), suggest that unknown sources of the virus provide the initial inoculum from which this readily mechanically transmitted virus spreads throughout a field of PVS-free seedlings. Lack of further PVS infection in the 410 plants from the Presque Isle field plot could perhaps have been caused by the extreme isolation of the field plot, which reduced the potential for insect transmission; by the wide row spacing (2 m); by not cultivating after the foliage was large enough to come into contact with equipment; or by the aerial application of the various chemicals routinely used in potato culture. More studies of insect transmissibility, virus strain relationships, and cultural practices are needed to determine the cause of the infiltration and spread of PVS in germplasm development programs. This needed information will be useful also in establishing PVS-free seed programs.

LITERATURE CITED

1. BODE, O., and H. L. WEIDEMANN. 1971. Untersuchungen zur Blattlausubertragbarkeit von Kartoffel-M-und-S Virus. Potato Res. 14:119-129.
2. BRANDES, J. 1957. Eine electronenmicroskopische Schnellmethode zum Nachweis faden-und stäbchenförmiger Viren, insbesondere in Kartoffeldunkelkeimen. Nachrichtenbl. Dtsch. Pflanzenschutzdienst. (Braunschweig) 9:151-152.
3. BRANDES, J. 1961. Einige Bermerkungen uber den Nachweis von Kartoffelviren mit Hilfe des electronenmikroskops. Pages 170-175 *in* Proc. 4th Conf. Potato Virus Dis. (Braunschweig) 1960.
4. BRUINSMA, J., A. SINNEMA, D. BAKKER, and J. SWART. 1967. The use of gibberellic acid (GA) and N-dimethylaminosuccinamic acid (B9) in the testing of seed potatoes for virus infection. Eur. Potato J. 10:136-152.
5. DE BOKX, J. A. 1967. Detection of virus S "Eersteling" isolate in glasshouse-grown plants with secondary virus infection. Overdruk Uit: Meded. Rijksfac. Landbouwwetensch. Gent. 32:915-921.
6. DE BOKX, J. A. 1972. Spread of potato virus S. Potato Res. 15:67-70.
7. EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION. 1963. Methods of diagnosis of potato virus diseases in the Soviet Union. Pages 81-91 *in* Potato Virus Diagnosis, Series A, Appendix VII.
8. HUNNIUS, W. 1969. Resistenzprufung und Resistenzverhalten von Kartoffelsorten gegenuber dem S-virus. Theor. Appl. Genet. 39:21-31.
9. MACKINNON, J. P. 1974. Detection, spread, and aphid transmission of potato virus S. Can. J. Bot. 52:461-465.
10. MACKINNON, J. P., and R. H. BAGNALL. 1972. Use of Nicotiana debneyi to detect viruses S, X, and Y in potato seed stocks and relative susceptibility of six common varieties to potato virus S. Potato Res. 15:81-85.
11. ROZENDAAL, A., and J. H. BRUST. 1955. The significance of potato virus S in seed potato culture. Pages 120-133 *in* Proc. 2nd Conf. on Potato Virus Diseases Lisse-Wageningen, The Netherlands, 1954.
12. SHEPARD, J. F. 1970. A radial-immunodiffusion test for the simultaneous diagnosis of potato viruses S and X. Phytopathology 60:1669-1671.
13. STACE-SMITH, R., and F. C. MELLOR. 1968. Eradication of Potato Viruses X and S by thermotherapy and axillary bud culture. Phytopathology 58:199-203.
14. VAN SLOGTEREN, D. H. M. 1955. Preparation of antisera against potato virus S with special reference to the preparation of non-toxic virus-suspensions for the immunization of rabbits from extracts of infected potato plants. Pages 35-39 *in* Proc. 2nd Conf. on Potato Virus Diseases. Lisse-Wageningen, The Netherlands, 1954.