Factors Affecting Bioassay of Potato Virus S in Chenopodium quinoa

C. Hiruki

Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada, T6G 2E3.

Financial supports provided by the Alberta Agricultural Research Trust Grant No. AR-55-28048, and the Alberta Potato Commission are gratefully acknowledged.

Accepted for publication 10 June 1975.

ABSTRACT

Various factors affecting production of potato virus S (PVS) lesions on leaves of *Chenopodium quinoa* were investigated to determine optimal conditions for the local lesion test. All bioassays were carried out using intact plants under controlled environment conditions. PVS extracts were obtained from infected leaves of potato (*Solanum tuberosum*) at a ratio of 1:10 in 1% K_2HPO_4 at pH 8.0. The maximum local lesion number was obtained at 27 C with a light period of 16 hours. Light intensities suitable for the assay were 5,380 to 10,760 lx. Dark treatment of *C. quinoa* for 24 hours before inoculation significantly increased the susceptibility of the assay plants. Highly susceptible *C. quinoa* plants, a Canadian selection, with four to six leaves of uniform size were obtainable for assay 40 to 60 days after seeding, and consistently produced numerous local lesions 8-10 days after inoculation at 25 C. In contrast, a European selection was less susceptible than was the Canadian selection and produced a very few lesions in 10-12 days under the same conditions.

Phytopathology 65:1288-1292

Additional key words: local lesion host, potato virus S infectivity test.

Potato virus S (PVS), a member of the carla virus group (7), is one of the most common viruses of potato (*Solanum tuberosum* L.). The virus is widespread in many old cultivars of potato (1), although it can be eliminated when thermotherapy is combined with nutrient culture of axillary buds (15). Reduction in yield due to PVS infection may reach 10-15%, but it varies according to cultivars, virus strains, and possibly weather conditions. Infected potato plants yield abnormally high proportion of small tubers in some cultivars (3), thus presenting serious problems in seed potato programs (13).

Several hosts including *Chenopodium quinoa* Willd. form local lesions following inoculations with PVS (4, 8, 10, 14, 16, 17). However, optimal assay conditions for these host plants have not been thoroughly investigated. Since *C. quinoa* is one of the frequently used indicator plants in the greenhouse for many viruses, and since an effective bioassay method is very important for establishing and maintaining virus-free seed potatoes, an investigation was carried out to evaluate factors affecting production of local lesions and to develop a reliable method for quantitative assay of PVS. Recently, a similar study was carried out on Red Kidney bean with potato virus M, another member of the carla virus group (9).

MATERIALS AND METHODS.—Three Canadian isolates of PVS, arbitrarily designated A, B, and N were obtained respectively from J. Letal, the Alberta Department of Agriculture, Edmonton, Alberta; N.S. Wright, the Canada Department of Agriculture, Vancouver, British Columbia; and R. H. Bagnall, the Canada Department of Agriculture, Fredericton, New Brunswick. Unless otherwise stated, the isolate A was mostly used in this investigation. The source potato plants for PVS were free from infections with other viruses including potato viruses X, Y, and M. Repeated assays by serology and indicator plants were made to ascertain the purity of PVS prior to and during the course of the investigation. The source plant of virus inoculum was 'Netted Gem' (Russet Burbank) potato that was vigorously growing 4 to 20 weeks in a greenhouse at about 22 C. The potato plants were twice cut back to induce axillary shoots during the course of this investigation. The seeds of *C. quinoa* were sown in a growth chamber at 22 C with daily light period of 16 hours at a light intensity of about 10,760 lx (1,000 ft-c). The seedlings were transplanted individually into 12-cm clay pots containing a soil mixture of loam, sand and peat in a ratio of 3:1:1 in the greenhouse 2 weeks after sowing. During summer the greenhouse was properly shaded on the roof and wall. In winter, a 16-hour light period per day was maintained with supplementary fluorescent and incandescent lights at an average intensity of 16,000 lx. Prior to inoculation, four-to-six well-developed leaves per plant were selected and were uniformly dusted with 22-μm (600-mesh) Carborundum. The remaining leaves were removed. At least 12 half-leaves were used in testing of a sample. Standard inoculum was obtained by grinding fresh infected potato leaves, with a mortar and a pestle, in 0.057 M phosphate buffer, pH 8.0 in a ratio of 1:10 (w/v). The sap was strained through two layers of cheesecloth and used for inoculation. Leaves were rinsed with distilled water immediately after inoculation. Inoculated plants were incubated in growth chambers. Standard conditions employed were: temperature, 25 C; light period, 16 hours, and light intensity, 10,760 lx. In certain experiments, the area of individual *C. quinoa* leaf was determined by an automatic area meter (Model AAM-5, Hayashi Denko

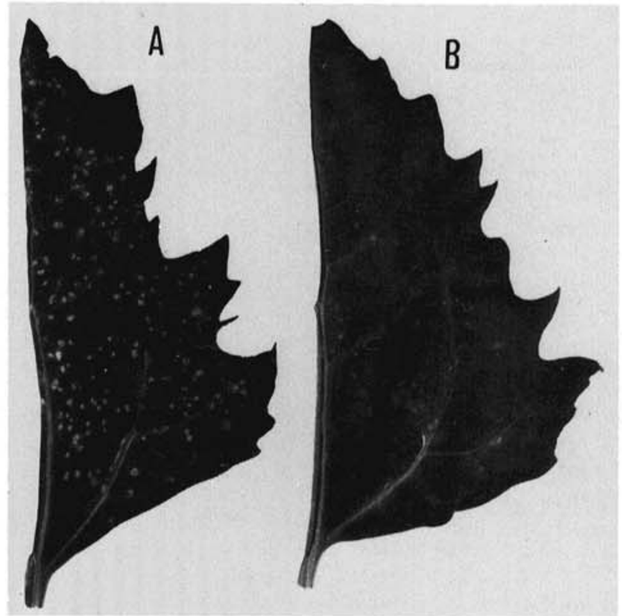

Fig. 1-(A, B). Half-leaves of *Chenopodium quinoa* showing local lesions induced by potato virus S (isolate A) 14 days after inoculation. **A**) the selection C, and **B**) the selection P of *C. quinoa*, respectively. (Photograph by T. Tribe).

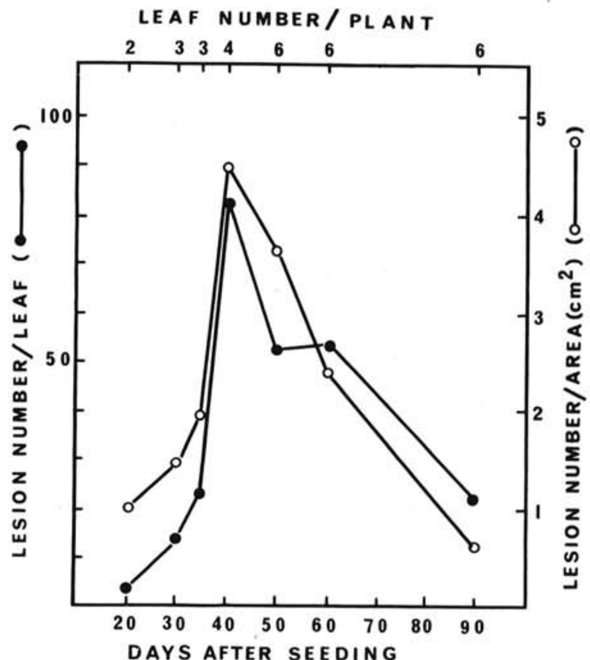

Fig. 2. Numbers of local lesions obtained in *Chenopodium quinoa* of different ages inoculated with potato virus S (isolate A). The number of local lesions were compared per leaf and per unit leaf area (cm^2) for plant having 2, 3, 4, and 6 leaves at different growth stages, respectively. Twelve leaves were used for each group.

TABLE 1. Average numbers of local lesions per half-leaf formed at different postinoculation temperatures on leaves of *Chenopodium quinoa* plants of different ages inoculated with potato virus S

Days after sowing	Postinoculation temperature (C)			
	17	22	27	32
40[a]	7.3 ± 1.7	81.2 ± 4.2	81.3 ± 5.0	0
60[b]	2.8 ± 1.6	71.2 ± 7.4	61.9 ± 6.6	0
120[b]	2.7 ± 0.5	38.3 ± 3.8	63.9 ± 3.9	0

[a]Based on counts from 24 half-leaves 12 days after inoculation.
[b]Based on counts from 36 half-leaves 12 days after inoculation.

Co., Ltd., Bunkyo-ku, Tokyo, Japan). The area meter measured, photoelectronically, the total area of a test leaf by detecting how much the leaf shaded the scanning light beam.

RESULTS.—*Virus isolates.*—The lesions produced by the three PVS isolates on *C. quinoa* had first a water-soaked or chlorotic appearance 4-5 days after inoculation, and were usually well defined 8-10 days after inoculation at 25 C. Routine infectivity tests on 30- to 50-day-old plants showed consistent lesion reproducibility with all three PVS isolates.

Chenopodium quinoa selection.—A selection of *C. quinoa* maintained in this laboratory (C) and a Polish selection (P), obtained from A. Kowalska, Potato Research Institute, Mlochow, Poland, were compared. The reproducibility of results with the selection C was very good and a large number of lesions was consistently obtained (Fig. 1-A). This selection had a markedly serrated leaf edge with a smooth leaf surface, and a relatively dark green color. In contrast, the selection P with relatively less-serrated leaves was less sensitive to PVS than was the selection C: PVS lesions appeared 2 days later than on leaves of the selection C and slowly developed to give a number less than that on the selection C and the majority of lesions produced remained mostly chlorotic (Fig. 1-B, 4). For the selection P it took about 3 weeks to reach the maximum number of lesions after inoculation.

Plant age.—To determine the effect of age of test plants on sensitivity of the bioassay, groups of *C. quinoa* plants of different ages were inoculated with the A isolate of PVS. Twelve leaves were used for inoculation of each group of *C. quinoa* plants; 20-, 30-, 40-, 50-, 60-, and 90-days old. Fig. 2 shows a good correlation between the average local lesion number produced per half-leaf and that obtained per cm^2 area of the inoculated leaves. The highest local lesion number was obtained with 40-day-old plants having four inoculable, expanded leaves.

Dark treatment before inoculation.—The effect of a dark treatment on susceptibility was determined using 24 leaves for each treatment. The average lesion numbers for two experiments, I and II, were as follows; dark treatment for 24 hours, I, 227, II, 169; for 48 hours, I, 142, II, 132; untreated, I, 118, II, 55. The dark treatment of *C. quinoa* plants immediately before inoculation significantly increased susceptibility of the plants to PVS. The plants which were treated for 48 hours showed epinasty of petioles at the end of treatment and were not satisfactory for practical assay of PVS. The 24-hour dark treatment consistently produced the highest local lesion number and was most practical without any harm to the assay plants.

Postinoculation temperature.—Groups of three 40-day-old plants with a total of 12 inoculated leaves for each group were placed in controlled growth chambers which were individually set at different temperatures, 17, 22, 27, 32 C, with a 16-hour photoperiod (approximately 10,760 lx). At 32 C, no visible lesions were formed, and no PVS was recovered from the inoculated leaves of these plants. The plants kept at 27 C produced consistently the highest number of local lesions per half-leaf (Fig. 3). At 22 C lesions were rather chlorotic in the beginning of their development and then gradually became necrotic. At 17 C, only a few chlorotic lesions developed very slowly. In another experiment, groups of six 40-, 60-, and 120-day-old plants with a total of 12-18 inoculated leaves for each temperature treatment were placed at the temperatures given above. The results listed in Table 1 indicate that an optimal temperature range for the PVS assay is 22 to 27 C for 40- to 60-day-old plants and 27 C for 120-day-old plants. Therefore, in subsequent experiments, plants were incubated at this temperature range after inoculation.

Light intensity.—Experiments were carried out using growth chambers equipped with fluorescent lamps providing light intensities of 5,380, 10,760, 16,140, 21,520 lx (500, 1,000, 1,500, and 2,000 ft-c), respectively. The results indicate that the light intensities between 5,380 to 10,760 lx were adequate for PVS assay (Table 2).

Concentration of phosphate buffer.—PVS was extracted in various molar concentrations of K_2HPO_4 buffer at pH 8.0. PVS extracted in distilled water was included as a control. The highest lesion number was obtained with inoculum prepared in 0.057 M phosphate buffer (Fig. 5).

TABLE 2. Effect of light intensity after inoculation of *Chenopodium quinoa* with potato virus S upon the number of local lesions

Experiment	Local lesions per half-leaf exposed to a light intensity (lx) of:			
	5,380	10,760	16,140	21,520
1	64.3 ± 9.4[a]	39.5 ± 4.5	0.0	0.0
2	102.5 ± 14.0	42.3 ± 5.2	0.0	0.0
3	70.6 ± 7.8	94.5 ± 7.7	0.5	. . .
4	81.1 ± 9.5	92.0 ± 5.6	. . .	. . .
5	88.0 ± 10.6	67.6 ± 5.7	0.0	0.0
6	33.7 ± 3.1	46.0 ± 7.1	0.0	0.0

[a]Average number of local lesions for 24 half-leaves. Temperature of the growth chamber was adjusted to 25 C. At higher light intensities (16, 140, and 21,520 lx), however, temperature at leaf levels often exceeded the set temperature by a few degrees.

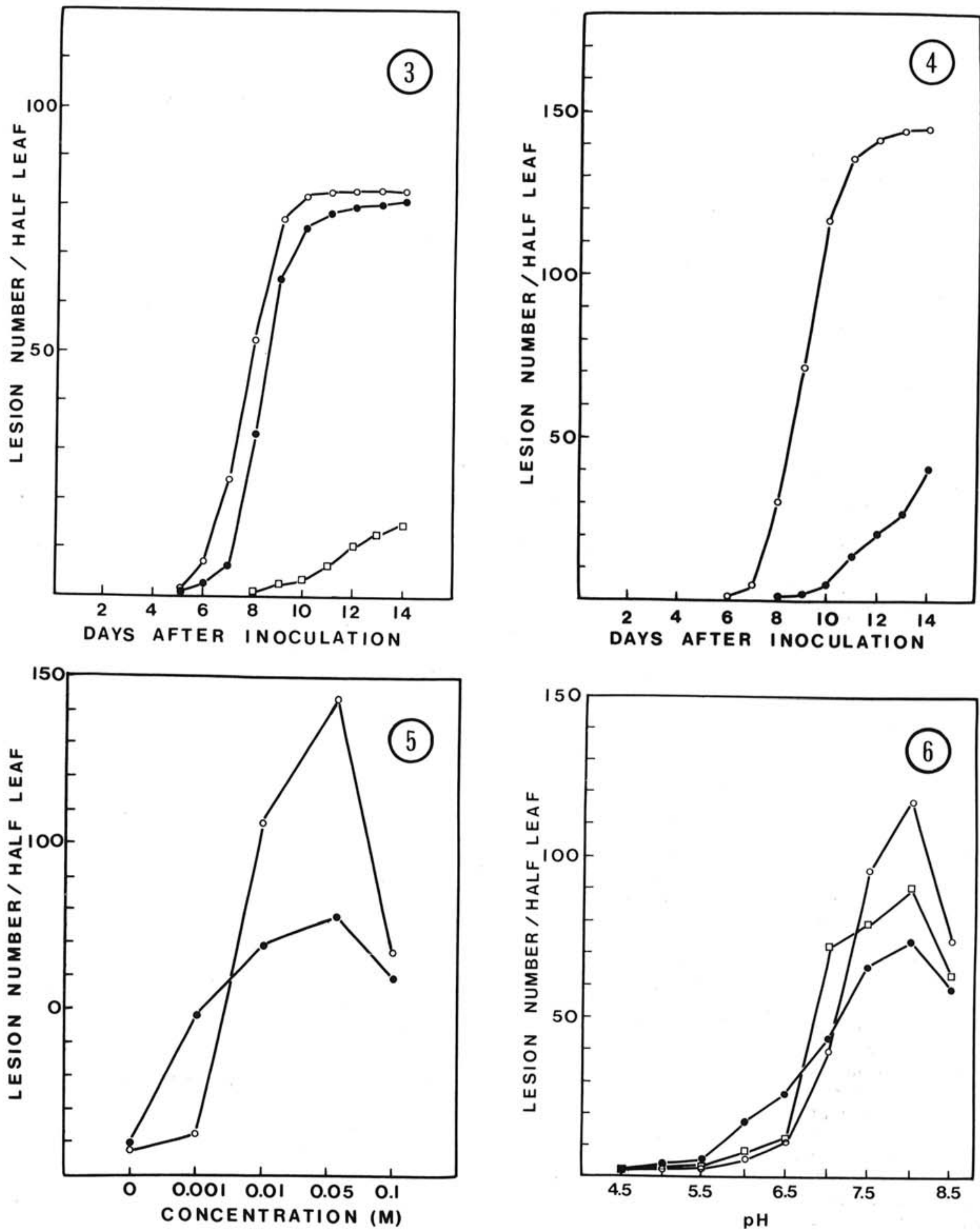

Fig. 3-6. 3) Differences in numbers of local lesions obtained from *Chenopodium quinoa* plants incubated at 17, 22, and 27 C, respectively after inoculation with potato virus S (isolate A). *C. quinoa* plants incubated at 32 C did not produce local lesions at all. □——□, 17 C; •——•, 22 C; o——o, 27 C. **4)** A difference in local lesion number obtained with two selections of *C. quinoa*. o——o, the selection C; •——•, the selection P. **5)** Effect of molar concentration of phosphate buffer, pH 8.0, used to extract potato virus S from infected leaf tissue upon the number of local lesions. The results of two experiments are shown. **6)** Effect of pH of 0.057 M phosphate buffer used to extract potato virus S from infected leaf tissue upon the number of local lesions. The results of three experiments are shown.

pH of phosphate buffer.—The pH of phosphate buffer used for extracting PVS from leaves also greatly influenced local lesion number. The results from three experiments (Fig. 6) suggest that pH 8.0 was optimal for PVS assay. In acidic buffers, the number of local lesions produced was considerably lower.

Dilution curve.—Infected potato leaves were separated into two half-leaves along the mid-rib. Two series of 10-fold dilutions of PVS extract were made with 0.057 M K_2HPO_4, pH 8.0, or with distilled water, adjusted pH 8.0. After adding 0.057 M K_2HPO_4 or distilled water in a ratio of 1 ml/g fresh weight of leaf tissue. The samples were ground and strained through two layers of cheese cloth. The infectivity of each sap sample was determined by inoculating 12 half-leaves of *C. quinoa* by the half-leaf method. Average numbers of local lesions per half-leaf produced by the original saps and the saps diluted 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} respectively were as follows: buffer extracts, 55, 104, 5, 1, 0, 0; distilled water extracts, 27, 52, 2, 1, 0, 0, respectively in order of dilution. The low infectivity of the initial inocula is perhaps due to inhibitory action of potato sap. Sap extracted in and diluted with 0.057 M K_2HPO_4 at 10^{-1} produced the highest number of local lesions.

DISCUSSION.—Heterogeneity in leaf morphology occurs in *C. quinoa* as well as in other species of *Chenopodium* (5. 6). Differences in susceptibility of *C. quinoa* strains to PVS have been reported (4, 8) and a correlation between a particular leaf shape and response to potato virus Y was noted (5). The selection C used in this investigation is similar to their strain in that high susceptibility to PVS was correlated with leaf morphology.

The incubation period of 4 to 6 days for the PVS isolates is considerably shorter than those reported by other workers (4, 8, 16). This discrepancy may be partly due to the following reasons. Firstly, in our tests, the assay plants were maintained under optimal environmental conditions by use of a controlled chamber immediately after inoculation. Secondly, the selection C is apparently more sensitive to the PVS isolates used than other strains of *C. quinoa*. Thirdly, the discrepancy may be due to the different isolates of PVS used in this investigation. It is known that differences in symptomatology occur in certain strains of *C. quinoa* plants in response to inoculations with various isolates of PVS (4, 8). Whether other isolates of PVS form local lesions on the selection C of *C. quinoa* can be assayed on this host remains to be seen, although all three Canadian isolates of PVS produced consistent results.

Unlike other test plants (1, 2, 11, 12), *C. quinoa* plants were infected only locally by the Canadian isolates in this investigation. Therefore, 6 to 12 PVS samples may be applied to a single plant. Furthermore, *C. quinoa* plants can be grown in less time and in less space than other test plants, and remain highly susceptible to PVS for a relatively longer time. The results presented in this paper suggest that *C. quinoa* plants would serve as a useful and reliable bioassay plant for the quantitative investigation of PVS if the various factors described here are controlled effectively.

LITERATURE CITED

1. BAGNALL, R. H., R. H. LARSON, and J. C. WALKER. 1956. Potato viruses M, S, and X in relation to interveinal mosaic of the Irish Cobbler variety. Wisc. Agric. Exp. Stn. Res. Bull. 198. 45 p.
2. BAGNALL, R. H., C. WETTER, and R. H. LARSON. 1959. Differential host and serological relationships of potato virus M, potato virus S, and carnation latent virus. Phytopathology 49:435-442.
3. BEEMSTER, A. B. R., and A. ROZENDAAL. 1972. Potato viruses: properties and symptoms. Pages 115-143 *in* J. A. de Bokx, ed. Viruses of potatoes and seed-potato production. PUDOC (Centre Agric. Pub. and Doc.), Wageningen, The Netherlands.
4. DE BOKX, J. A. 1970. Reactions of various plant species to inoculation with potato virus S. Neth. J. Plant Pathol. 76:70-78.
5. DELGADO-SANCHEZ, S., and R. G. GROGAN. 1966. Chenopodium quinoa, a local lesion assay host for potato virus Y. Phytopathology 56:1394-1396.
6. DEMSKI, J. W. 1968. Local lesion reactions of Chenopodium species to watermelon mosaic virus 2. Phytopathology 58:1196-1197.
7. HARRISON, B. D., J. T. FINCH, A. J. GIBBS, M. HOLLINGS, R. J. SHEPHERD, V. VALENTA, and C. WETTER. 1971. Sixteen groups of plant viruses. Virology 45:356-363.
8. HINOSTROZA-ORIHUELA, A. M. 1973. Some properties of potato virus S isolated from Peruvian potato varieties. Potato Res. 16:244-250.
9. HIRUKI, C., E. POUNTNEY, and K. N. SAKSENA. 1974. Factors affecting bioassay of potato virus M in Red Kidney bean. Phytopathology 64:807-811.
10. HIRUKI, C., and P. SHUKLA. 1973. Intracellular location of potato virus S in leaf tissue of Chenopodium quinoa. Can. J. Bot. 51:1699-1702.
11. 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.
12. ROSS, H. 1968. Lycopersicum chilense Dun., eine Testpflanze für die beiden Kartoffelviren M und S. Eur. Potato J. 11:281-286.
13. ROZENDAAL, A., and J. H. BRUST. 1955. The significance of potato virus S in seed potato culture. Pages 20-36 *in* Proc. 2nd Conf. Potato Virus Dis. (Lisse-Wageningen, 1954).
14. SAMPSON, P. J., and R. H. TAYLER. 1968. A comparison of the electron microscope, microprecipitin tests, and indicator plants for the detection of potato viruses S, X, and Y. Phytopathology 58:489-493.
15. 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.
16. VULIC, M., and W. HUNNIUS. 1967. Die Reaktionen verschiedener Pflanzenarten auf Blattinfektionen mit S- und M-virus der Kartoffel. Phytopathol. Z. 59:225-248.
17. YARWOOD, C. E., and A. H. GOLD. 1955. Guar as a local lesion host of potato virus S. Plant Dis. Rep. 39:622.