Virus Strain Differences in Eradication of Potato Viruses X and S

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ABSTRACT

Virus-free plants of 18 cultivars of potato (*Solanum tuberosum*) were developed by eradicating potato virus X (PVX) and potato virus S (PVS) from infected plants. The viruses were eradicated by heat treatment followed by nutrient culture of axillary buds 0.3-0.8 mm long. Potato virus X was usually eradicated more readily than PVS, but ease of eradication of both viruses varied with the source plant. Unusually heat-tolerant strains of PVX occurred in two cultivars; heat-tolerant strains of PVS in seven. For eradication of some of the most heat-tolerant strains of PVS, the size of the bud excised was of critical importance but, within the limited size range used, bud size usually had little influence on virus eradication. A treatment period of 6-8 weeks was optimal. Longer treatment periods actually increased the proportion of infected plantlets in some instances. Phytopathology 60:1587-1590.

Additional key words: heat therapy, meristem culture.

In an earlier paper on virus eradication by nutrient culture of buds excised from heat-treated plants, we reported the influence of length of heat treatment and size of bud excised on eradication of potato virus X (PVX) and potato virus S (PVS) from potato, *Solanum tuberosum* L. 'White Rose' (7). Success with the White Rose potato prompted attempts to develop virus-free plants of other cultivars, and to investigate further the factors that influence both bud survival and virus eradication. Factors affecting the development of excised buds into rooted plants have been reported (2). This paper presents the results of treating 21 selections, with comparisons of the relative ease of eradication of PVX and PVS from several sources.

MATERIALS AND METHODS.—The selections treated for virus eradication included 18 cultivars licensed for sale in Canada, all infected with PVX and PVS, and USDA seedling 41956, infected with PVS only. A single tuber of each cultivar usually was used to provide plants for treatment. Small plants were developed either from single-eye tuber pieces or from shoot tips 5-8 cm long, rooted in sand and transplanted to soil 2-4 weeks before treatment. In both cases the treated plants were small and ready to start active growth. The planting medium was John Innis Mix No. 2, in 4-inch clay pots.

Plants were exposed to air temp which alternated daily from 33 to 36 C, and soil temp which alternated from 30 to 32 C. Fluorescent lights provided a 16-hr photoperiod. At intervals during heat treatment, young shoots were removed from the treated plants and sterilized in a weak sodium hypochlorite solution.

The tip of each axillary bud was dissected out by the technique previously described (7) and transferred to a 10-ml tube containing 3 ml of liquid nutrient medium. The culture media were modifications of the one described by Murashige & Skoog (3). A simplified version of this medium, omitting the agar, growth-promoting substances, and rooting hormones, was adequate for most of the cultivars (2). The buds in culture were kept at 23 to 25 C under a 16-hr photoperiod. When the excised bud developed shoots and roots 2-3 cm long, the plantlet was transferred to soil and covered with a beaker for several days to conserve moisture.

Soon after the plantlets were established in soil they were indexed for PVX using the local lesion host, *Gomphrena globosa* L. A small leaflet from each potato plant was ground in a well of a porcelain spot plate, and rubbed on a young leaf of the indicator host previously dusted with Carborundum.

Potato virus S assays were made, first by serological tests. Antiserum was prepared by injecting rabbits with a purified preparation of PVS prepared from a clone of Netted Gem from which PVX was eradicated but in which PVS remained. Rabbits were given two intramuscular injections of 0.3 mg of virus each, 2 weeks apart. The rabbits were bled a week after the second injection, and at weekly intervals for 6 weeks. The antiserum, mixed 1:1 with glycerine, was pooled and stored at −20 C. The antiserum had a reaction of 1:800 against clarified sap from infected potato and did not react against clarified sap from healthy plants. Either the microprecipitin test which was used for PVS detection by Samson & Taylor (6), or the tube precipitin test, was used. From each indexed plant, 0.2 g of leaf tissue was ground in a glass tissue grinder in 2 ml physiological saline containing 0.01 M sodium diethyldithiocarbamate and 0.01 M Tris buffer [tris(hydroxymethyl) amino methane], pH 7.4. The homogenate was centrifuged 10 min at 10,000 rpm in a Spinco No. 50 rotor. The supernatant was diluted with physiological saline solution and used at dilutions of 1:10, 1:20, and 1:40. Antiserum, diluted to 1:200 with saline, was added to each tube and the tubes were held at 37 C in a serological bath for 3 hr before reading. Any plants that gave doubtful reactions were retested.

Representative plants that indexed negative for PVX and PVS were checked by electron microscopy and by rub transmission to indicator plants. For electron microscopy, a small leaflet from the potato plant was cut and dipped a few times into a drop of water on a carbon-coated grid. The grid was then dried and shadowed with palladium for examination in a Philips EM 100 microscope. The indicator plants used to detect PVS were *Chenopodium amaranticolor* Coste

& Reyn., and *C. quinoa* Will., both of which have been reported as good indicators for PVS (8). Any plant that was negative for PVS by serology but positive by electron microscopy or mechanical transmission was retested serologically.

RESULTS.—*Virus detection.*—Potato virus X was detected readily by rub transmission to *G. globosa.* Virtually all PVX infections were detected in the initial indexing when the plants were less than 10 cm tall. A second test of 186 plants that indexed PVX-negative revealed only one infected plant that had escaped detection in the first test.

Potato virus S was more difficult to detect, particularly in the presence of PVX. When PVS occurred alone, approximately 90% of the infections were detected in the first indexing, whether by indicator plants, electron microscopy, or serology. The presence of PVX, however, precluded the use of indicator plants for PVS detection, since the lesions induced by PVX appear earlier and obscure those of PVS. Electron microscopy was also impractical, since the relatively small difference in particle length between PVS and PVX would necessitate measurements of many particles to differentiate the two viruses. Serology was therefore the only practical method of detecting PVS in plants which were also infected with PVX. Potato virus S was detected by serology as readily in plants infected with both viruses as in those infected with PVS alone.

Results of serological tests for PVS were sometimes uncertain, particularly when leaves of the plant being tested were homogenized in unbuffered physiological saline. Infected plants varied in the strength of the serological reaction. Some leaf homogenates caused a visible precipitate in the first tube only, whereas others caused a precipitate in all three tubes (i.e., dilutions of 1:10, 1:20, and 1:40). Furthermore, sap from healthy control plants sometimes caused a nonspecific reaction. This was particularly prevalent when lower leaves were used as test material.

The nonspecific reactions were lessened and the specific reactions intensified when leaves were homogenized in saline buffered with 0.01 M Tris buffer, pH 7.4. Inclusion of the antioxidant sodium diethyldithiocarbamate, 0.01 M, in the buffered saline sometimes improved the intensity of the reaction. But even with the buffered saline, PVS infections were not always detected, and nonspecific reactions occasionally gave misleading results. The microprecipitin test for detecting PVS gave results comparable to those of the standard tube precipitin test. PVS was detected by either method in most infected plants, and infected plants that gave negative results with the microprecipitin test also gave negative results with the tube precipitin test.

A single serological test usually gave a clear indication of infection with PVS, but in certain plants this virus was difficult to detect. In one Norland clone, for example, more than 50% of the PVS infections escaped detection in the first serological indexing, whereas with most cultivars, less than 10% of the infected plants were missed in the first test.

Virus eradication.—Two previous reports (1, 7), each based on experience with a single cultivar, showed the influence of length of heat treatment and size of excised bud on the eradication of PVX and PVS. Results of the present series of tests, based on experience with more than 900 plantlets of 21 potato selections, show that ease of eradication of both viruses varied with the source plant (Table 1). Moreover, the relative heat tolerance between the two viruses varied considerably. For lack of a better word, we used the word "strain" to designate virus isolates showing marked differences in heat stability, although the isolates from various source plants caused no appreciable symptom differences on indicator hosts, nor were differences detected in serological tests.

Differences in ease of virus eradication from various cultivars are only relative, since length of heat treatment and size of bud are not taken into account. Within each cultivar, however, the difference in number of infections with each virus is absolute, and provides a valid comparison of the relative heat tolerance of the particular virus strains present in each clone. Potato virus X was usually more heat-labile than PVS, but there were exceptions. In the cultivars Early Rose, Norgold Russet, Columbia Russet, and Cherokee both viruses were equally heat-labile, while in Netted Gem and in one source of Gold Coin, PVX was more heat-stable than the concomitant strain of PVS.

Although PVX was often eradicated with ease, its heat tolerance varied with the source plant. A particularly heat-labile strain occurred in our sources of Avon

TABLE 1. Comparative persistence of potato virus X and potato virus S in buds excised from 20 infected potato selections after heat treatment

	Plantlets developed				
				Virus-infected[a]	
Cultivar	Total	Virus-free		PVX	PVS
	no.	*no.*	*%*	*no.*	*no.*
Early Rose	17	17	100	0	0
Norgold Russet	19	18	95	0	1
Sebago	35	33	94	0	2
Avon	79	74	94	0	5
Red Pontiac	37	33	89	1	4
Green Mountain	157	140	89	7	12
Fundy	102	87	85	4	14
Columbia Russet	38	32	84	4	4
Netted Gem	26	21	81	4	1
Warba	57	45	79	1	12
Lenape	38	30	76	0	8
Gold Coin (1)[b]	31	21	68	1	10
Gold Coin (2)	27	18	67	7	2
Keswick	52	35	67	5	12
Irish Cobbler	26	17	65	2	8
Cherokee	25	14	56	7	8
Norland (1)[b]	18	10	55	1	8
Katahdin	78	30	38	1	48
Norland (2)	33	11	33	1	22
Red La Soda	13	2	15	7	11
Totals	908	688		53	192

[a] Some plantlets were infected with both viruses; double infections appear under both PVX and PVS columns.

[b] Two source plants each of the cultivars Gold Coin and Norland were treated.

and Katahdin. In these two cultivars, only one plantlet was infected out of 157 developed from buds excised after heat treatment of 4-10 weeks. Usually PVX persisted in about 3% of the buds taken after 4-6 weeks and rarely in buds after longer treatment. Unusually heat-stable strains of PVX occurred in Netted Gem and in one source of Gold Coin. After 10 weeks' treatment, nearly 30% of the buds excised from these two source plants were still infected with PVX.

Potato virus S varied even more widely than PVX in its tolerance to heat. The most heat-labile strains occurred in the cultivars Early Rose, Norgold Russet, Sebago, and Avon. Potato virus S persisted in only 3% of buds excised from these source plants after 4-8 weeks' heat, and in none excised after treatment of 10-26 weeks. The most common pattern of PVS eradication is illustrated by the results from Green Mountain potato. The proportion of PVS-infected plantlets declined from 30% after 2 weeks' treatment to less than 10% after 4-16 weeks. After 26 weeks' heat, however, the incidence of PVS was unexpectedly high, with four infected plantlets among the 13 survivors (Table 2). Eradication of PVS from the cultivars Fundy, Irish Cobbler, Red Pontiac, and Warba followed a pattern similar to that for Green Mountain. The combined results for these five cultivars showed that the incidence of PVS-infected plantlets after 4, 6, and 8 weeks' treatment was 18, 8, and 4%, respectively, but after 18-26 weeks was 20%. The diminishing effect of prolonged heat treatment was even more pronounced in one source of Norland potato, where PVS was eradicated from nearly all the buds excised after treatment up to 7 weeks; from less than half excised after 10 weeks; and from none excised after 16 weeks. Strains of PVS from Katahdin and Red La Soda were also extremely heat-tolerant, persisting in 65% of the surviving plantlets.

The heat tolerance of virus strains that survived prolonged heat treatment was not necessarily a stable characteristic. Two Norland explants, still infected with PVS after 16 weeks' heat, were subjected to a second heat treatment. Many of the buds excised after treatment periods of 7-10 weeks developed into virus-free plantlets.

TABLE 2. Influence of treatment period on eradication of potato virus X and potato virus S from buds excised from Green Mountain potato at intervals during heat treatment

Treatment period	Plantlets developed			
			Virus-infected	
	Total	Virus-free	PVX	PVS
weeks	*no.*	*no.*	*no.*	*no.*
2	10	6	3	3
4	27	25	1	1
6	19	17	2	0
8	21	20	1	0
10	13	11	0	2
12	27	26	0	1
14	17	16	0	1
16	10	10	0	0
26	13	9	0	4
Totals	157	140	7	12

TABLE 3. Influence of bud size and treatment period on eradication of a heat-tolerant strain of potato virus S from buds excised from Katahdin potato at intervals during heat treatment

Length of bud (mm)	Treatment period (weeks)				
	4	6	8	10	Totals
0.3	1/1[a]	1/3		1/1	3/5
0.4		3/4	2/2	3/4	8/10
0.5	0/2	1/3	0/2	3/3	4/10
0.6	1/2	4/7	2/3	0/3	7/15
0.7	1/1	0/3			1/4
0.8			1/1	0/6	1/7
Totals	3/6	9/20	5/8	7/17	24/51

[a] Number of virus-free plantlets per number of plantlets developed.

Within the relatively narrow size range of buds excised, length of bud usually had little or no influence on virus eradication. In a few cultivars, however, where a very heat-stable strain of PVS occurred, eradication appeared to depend on bud size rather than on length of treatment. In one series of tests, plants that were developed from stem cuttings from a single Katahdin tuber were heat-treated and buds excised at 2-week intervals during treatment. The proportion of virus-free plantlets was approx the same regardless of length of treatment, but declined with increasing bud length (Table 3). Of the plantlets developed from buds 0.3-0.4 mm long, 73% were virus-free; from buds 0.5-0.6 mm long, 44% were virus-free; and from buds 0.7-0.8 mm long, only 18% were virus-free.

The influence of bud size on eradication of this strain of PVS was further shown when the heat-treated plants were derived from tuber pieces rather than from stem cuttings. From tuber pieces, shoots that developed during treatment were robust, with large, leafy axillary buds. From rooted stem cuttings, the shoots were slender; axillary buds were small; and the meristematic tip of each bud was noticeably smaller, probably half the wt of buds of similar length taken from sprouted tuber pieces. The results shown in Table 3 are for buds excised from heat-treated plants that were developed from stem cuttings. When the same Katahdin tuber was used as source material, but plants for heat treatment were developed from tuber pieces, the proportion of PVS-free plantlets was lower. From buds 0.5-0.6 mm long, 33% were virus-free; from buds 0.7-0.8 mm long, only 8% were virus-free.

Similar results were obtained when a single tuber of USDA seedling 41956 was used as source material from which to develop plants for heat treatment. Of plantlets derived from the slender buds taken from stem cuttings, 58/64 (91%) were free from PVS. Among plantlets derived from the stouter buds originating from tuber pieces, only 67/126 (53%) were free from PVS.

DISCUSSION.—A previous report on the eradication of PVX and PVS by nutrient culture of axillary buds excised from heat-treated plants was based on our

experience with a single cultivar (7). There we reported that PVS was the more difficult of the two viruses to eradicate, that size of the excised bud influenced eradication of both PVX and PVS, and that length of heat treatment had more effect on PVX than on PVS. The present series of tests confirmed some of our earlier observations, but qualified others. Although PVS was usually the more difficult to eradicate, there were exceptions. Within the limited size range of buds excised, length of bud had little influence on virus eradication except for the most heat-tolerant strains of PVS. Prolonged heat treatment eliminated many strains of PVX and some strains of PVS, but in certain cases increased the proportion of plantlets infected. Treating many different virus sources showed that both viruses have a wide range of heat tolerance, and that prolonged heat treatment may be less effective than relatively short treatment.

Increased incidence of virus after prolonged heat treatment has been observed before. Welsh & Nyland (9) eradicated several viruses from infected apple, but sometimes found the highest incidence of virus survival in buds propagated after the longest treatment period. Nyland (4) found that during heat treatment for vein yellows of pear, virus-free buds were taken after 5 weeks' heat, but not after 6-7 weeks. Posnette (5) observed a similar phenomenon in heat treatment of strawberry, apple, and quince, where he found short treatment to be more effective than long treatment. He suggested that during prolonged treatment the virus might mutate to more heat-tolerant strains, which might then invade growing tips which were previously virus-free. Our experience with Norland potato indicates that the heat tolerance of PVS is not necessarily a stable characteristic. It may be that heat-tolerant strains of the virus which survive prolonged treatment mutate after treatment, giving rise to more heat-labile strains which can be eradicated by subsequent treatment.

Another possible explanation is that some of the treated plants are infected with several strains of PVS, some more heat-tolerant than others, with the heat-labile ones predominating. During the first few weeks of heat treatment, when the concn of the heat-labile strains is reduced but before the heat-stable ones replace them, many of the buds excised are virus-free. Later, with the buildup of heat-stable strains, the probability of excised buds being free from PVS would be lessened. This theory would also explain the relatively small numbers of PVS-free plantlets of the Katahdin potato. Perhaps the source plant was infected only with a heat-stable strain of PVS which invaded the plant more extensively than would be possible in the presence of competing heat-labile strains. This would account for the large proportion of infected buds taken after any treatment period, and explain the fact that only the smallest of the excised buds were virus-free.

LITERATURE CITED

1. Mellor, F. C., & R. Stace-Smith. 1967. Eradication of potato virus X by thermotherapy. Phytopathology 57:674-678.
2. Mellor, F. C., & R. Stace-Smith. 1969. Development of excised potato buds in nutrient culture. Can. J. Bot. 47:1617-1621.
3. Murashige, T., & F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum 15:473-497.
4. Nyland, G. 1962. Thermotherapy of virus-infected fruit trees. 5th European Symposium Fruit Tree Virus Diseases Proc. Edizioni Agr., Bologna, Italy. p. 156-160.
5. Posnette, A. F. 1962. *In* 5th European Symposium Fruit Tree Virus Diseases Proc. Edizioni Agr., Bologna, Italy. p. 160.
6. Samson, P. J., & R. H. Taylor. 1968. A comparison of electron microscope, microprecipitin tests, and indicator plants for the detection of potato viruses S, X, and Y. Phytopathology 58:489-493.
7. Stace-Smith, R., & F. C. Mellor. 1968. Eradication of potato viruses X and S by thermotherapy and axillary bud culture. Phytopathology 58:199-203.
8. Vulic, M., & W. Hunnius. 1967. Die Reaktionen verschiedener Pflanzenarten auf Blattinfektionen mit S- und M-Virus der Kartoffel. Phytopathol. Z. 59: 225-248.
9. Welsh, M. F., & G. Nyland. 1965. Elimination and separation of viruses in apple clones by exposure to dry heat. Can. J. Plant Sci. 45:443-454.