Cross-Protection with Strains of Potato Spindle Tuber Viroid in the Potato Plant and Other Solanaceous Hosts

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ABSTRACT

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When nucleic acids containing a mild and a severe strain of potato spindle tuber viroid (PSTV) were coinoculated to several potato cultivars, 19–37% of inoculated plants became infected with both strains. Variation of nucleic acids concentration favored dominance of one or other strain. However, both strains replicated in second generation plants grown from tubers from doubly inoculated plants. With potato cultivars of different resistances, cross-protection between strains of PSTV was determined by the absence of the challenge strain, which was detected by return-polyacrylamide gel electrophoresis. Cross-protection was complete in the highly susceptible Russet Burbank, but incomplete in tolerant BelRus. Second-generation potato plants infected with a single strain and grown from greenhouse or field-infected tubers were completely protected against reinoculation with another strain irrespective of the cultivar resistance, and no challenge strain was detected. However, protection was readily broken by changing the method of challenge inoculation. Studies of cross-protection with eight experimental host plants indicated that protection was more complete in those hosts in which the protecting strain multiplied rapidly and spread to the entire plant rather than in those where PSTV infection was difficult or the spread of the viroid was erratic. These studies point out that the extent of cross-protection depends highly on the host plant.

Additional keywords: complete cross-protection, coinoculation, host-range, return-electrophoresis, replication of viroids, secondary infection, viroid strains.

Cross-protection, by which plants systemically infected with one strain of virus or viroid are protected from the severe symptoms of a second related strain of the same virus or viroid, has been exploited as a disease control measure (7,8) or as an aid in the identification of viroid strains (5,15). Plant symptoms have played a dominant role in cross-protection studies. Consequently, cross-protection studies have been almost limited to those host plants that can display differential symptoms to various strains. Therefore, the potential of symptomless host plants has largely remained unexplored.

Studies on viral interference and cross-protection can be extended to additional host plants by using other criteria (2,4). Using the differences in electrophoretic mobility of related strains of potato spindle tuber viroid (PSTV), Khoury et al (10) studied cross-protection in tomato plant (*Lycopersicon esculentum* Mill. 'Sheyenne') (10). It was observed that a mild and a severe strain of PSTV replicated in doubly inoculated, cross-protected plants. The severe strain replicated faster than the mild strain and eventually replaced the latter in growing tissues of tomato (10). Additionally, increased duration between inoculation of protecting and challenge strains has increased protection of plants, but such protection is readily broken down by graft inoculation (16). These observations indicate that use of cross-protection for disease control may not be useful with PSTV when protection is not complete. The objective of this study was to compare cross-protection with PSTV using its natural host, the potato (*Solanum tuberosum* L.), and related experimental hosts. A preliminary report of this study has appeared elsewhere (12).

MATERIALS AND METHODS

Viroid cultures. Severe (S-PSTV), mild (MF-PSTV) (13), and intermediate strains of PSTV (14) were propagated in potato cultivar Russet Burbank. Sap inoculum (w/v, 1:1) was prepared from viroid infected leaves ground in buffer (0.05 M glycine and 0.03 M K_2HPO_4, pH 9.2). Partially purified viroid containing nucleic acids were prepared as described (13) and their concentration adjusted to 650–680 μg/ml for inoculation. The concentration was calculated with an extinction coefficient of 20 (mg ml^{-1}) cm^{-1} at a wavelength of 258 nm (11). Viroid concentration in partially purified nucleic acid samples ranged from 3 to 3.5 μg/ml. This was determined by comparing the intensity of viroid band from partially purified nucleic acids with those of known amounts of purified viroid bands (Fig. 1) using R-PAGE (see below).

Viroid analysis. Nucleic acids were prepared (13) by grinding approximately 0.5 or 1.0 g of tissue (leaves and tubers) in extracting buffer (w/v, 1:3). The extraction buffer (0.53 M NH_4OH, 0.013 M disodium ethylenediaminetetraacetate [EDTA] adjusted to pH 7.0 with Tris, 4 M LiCl, and 0.1% purified bentonite [6]), and 2 or 4 ml of 0.05 M Tris-saturated phenol (containing 0.1 g of 8-hydroxyquinoline/100 ml) and samples were maintained at 4–5 C, homogenized with a polytron (PT-10-35 equipped with PT-20-ST microgenerator; Brinkman Instruments, Rexdale, Canada), and centrifuged (15 min, 7,710 *g* at 4 C). Nucleic acids were precipitated (−20 C, 30 min) by adding 2.5 vol of ethanol and 50–80 μl/sample of 4 M sodium acetate solution. The precipitate was collected by centrifugation as above, dried with a current of air, and dissolved in high salt buffer (89 mM Tris, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) containing 40% glycerol and 5–10 μl of a solution containing the dyes xylene cyanol FF and bromophenol blue (1%).

Return-polyacrylamide gel electrophoresis (R-PAGE) (13) was used to analyze the nucleic acids for the presence and identification of strains. The first electrophoretic separation of nucleic acids was at a constant current of 46 mA for 2.5 hr using an SE 600 series apparatus (Hoefer Scientific Instruments, San Francisco, CA) on 5% nondenaturing slab gels (5% polyacrylamide, 0.125% bisacrylamide, 14 × 16 × 0.15 cm) in high salt buffer using 6 μl of sample in each well. The buffer in both the upper and lower reservoirs for the second electrophoresis was replaced with a heated low salt buffer (1:8 dilution of high salt buffer) (13). The polarity was reversed and electrophoresis was performed at 70 C (46 mA constant current, 2.0 hr). Gels were stained using silver nitrate (13) and photographed with a Polaroid B/W-type 55 film using a Polaroid 4 × 5 Land Camera.

Potato cultivars and inoculation procedures. Potato plantlets in the form of test-tube cultures, free from common viruses and viroids were obtained from the Plant Propagation Centre (New Brunswick Department of Agriculture), Fredericton. The plantlets were transplanted to 15-cm clay pots containing soil:peat-moss:sand (2:1:1) and acclimatized in the greenhouse for 3 wk. Cultivars included were Atlantic, BelRus, Dĕsirĕe, Jemseg, Kennebec, Katahdin, Russet Burbank, Sebago, Shepody, and Yukon Gold.

For coinoculation experiments, plants of each cultivar were inoculated with 340 or 680 μg/ml of partially purified nucleic acids containing MF-PSTV, S-PSTV, or both. Three leaves previously dusted with Carborundum (400 mesh) were rubbed with viroid containing inoculum using a glass rod. The upper leaves (0.5 g) were sampled at 7, 9, 11, 13, 15, 29, and 43 days postinoculation (d.p.i.). Because of the scarcity of new growth, different leaves were sampled in the initial stages of the test. Forty-one tubers from doubly inoculated plants were grown and detection of both strains was followed for up to 9 wk.

Preliminary studies indicated that the BelRus plants did not develop diagnostic symptoms with MF-PSTV or S-PSTV strains. A severe symptom causing strain with intermediate mobility on R-PAGE (14) was used to compare the symptomology of various potato cultivars including BelRus. When it was established that BelRus is an almost symptomless carrier of PSTV, including intermediate strains, attempts were made to determine the relative susceptibility of BelRus and Russet Burbank to a more commonly encountered strain, MF-PSTV. Eight plants of each cultivar were inoculated with MF-PSTV containing nucleic acids (680 μg/ml) and a single leaflet from each leaf position from each plant was assayed for viroid weekly for up to 5 wk.

Cross-protection with MF-PSTV protected plants. Three sets of 10 potato plantlets were used in each experiment. Plants in the first set were initially inoculated with MF-PSTV (protecting strain), then challenge inoculated with buffer 3 wk later; in the second set, plants were initially rubbed with buffer, then challenge inoculated 3 wk later with S-PSTV; in the third set, plants were initially inoculated with MF-PSTV, then challenge inoculated 3 wk later with S-PSTV. The first inoculation was carried out with nucleic acid concentration of 680 μg/ml and the second with 650 μg/ml. Challenge inocula were applied to the three terminal leaflets of each leaf (usually 6 to 9) on each plant. Combined samples for viroid assay were taken from each set biweekly for up to 12 wk post challenge inoculation (w.p.c.i.). Samples were collected from the upper third (top leaves), the middle third (middle leaves), and the lower third (bottom leaves) of the plants. Appropriate uninfected and infected controls consisting of tomato and *Scopolia sinensis* Hemsl. were always included. The experiment was carried out using BelRus (tolerant) and Russet Burbank (highly susceptible) potato cultivars.

Cross-protection with S-PSTV protected plants. These experiments were carried out in parallel with MF-PSTV protected plants, except that S-PSTV was the protecting strain and MF-PSTV was the challenge strain.

Cross-protection with second-generation infected plants. Six to eight plants from potato tubers of BelRus, Jemseg, Kennebec, Katahdin, and Sebago preinfected in the greenhouse with MF-PSTV, or S-PSTV were challenge inoculated with viroid containing nucleic acids (680 μg/ml). Upper leaves of these plants were analyzed for viroid strains up to 16 w.p.c.i.

In another test, plants were propagated from field tubers preinfected with MF-PSTV. One to five plants of Abnaki, Avon, Bintje, Caribe, Fundy, Green Mountain, Hindenberg, Hunter, Jemseg, Kennebec, Katahdin, Norchip, Norland, Red Pontiac, Saco, Sebago, USDA seedling 41956, and Warba were used and leaf samples for viroid strain detection were analyzed at 5, 9, and 12 w.p.c.i.

Cross-protection with other host plants. Five plants each of symptomatic hosts (*Solanum berthaultii* Hawkes, *S. demissum* Lindl., and *Scopolia sinensis*) and symptomless hosts (*Nicotiana debneyi* Domin., *N. tabacum* L. 'Samsun', *Physalis angulata* L., *P. floridana* Rydb., and *S. nigrum* L.) were first inoculated with MF-PSTV containing nucleic acids (680 μg/ml). Three weeks after inoculation, plants were assayed for the presence of MF-PSTV and challenge inoculated with S-PSTV containing nucleic acids (500 μg/ml). New growth of doubly inoculated plants was assayed for viroid strains biweekly up to 8 w.p.c.i.

Effect of challenge inoculation by grafting and pinpoint puncture methods. Healthy and MF-PSTV infected cuttings (11 each) of Kennebec were challenge inoculated by grafting scions containing S-PSTV. Upper leaves from scions and stocks (including axillary shoots from each node) were assayed for viroid strains at weekly intervals for 5 wk. Similarly, MF-PSTV or S-PSTV infected plants of Russet Burbank were challenge inoculated by dipping a needle in nucleic acids solution (680 μg/ml) containing S-PSTV or MF-PSTV and puncturing either the stem or petiole of the plants. About 10–15 punctures were made on each spot. Viroid analysis was done up to 5 w.p.c.i.

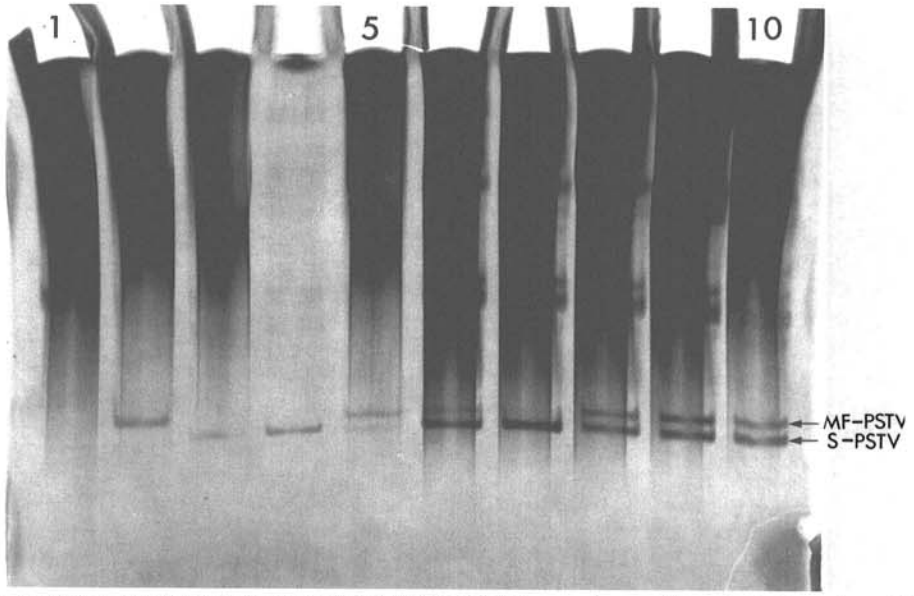

Fig. 1. Return-polyacrylamide gel electrophoretic analysis of MF + S-PSTV infected second-generation plants. Lane 1, nucleic acids from healthy plant; lane 2, partially purified nucleic acids (680 μg/ml) containing MF-PSTV; lane 3, similar to lane 2 but S-PSTV; lane 4, purified S-PSTV (33 ng) as a concentration standard; lane 5, crude nucleic acid containing a mixture of MF + S-PSTV (340 μg/ml each); lanes 6–10, nucleic acids from doubly infected second-generation potato plants at 3, 5, 7, 10, and 11 wk after germination, respectively. First electrophoresis was on 5% gels under nondenaturing conditions at 46 mA for 2.5 hr and the second was under denaturing conditions at 46 mA for 2 hr.

RESULTS

Coinoculation of potato plants with mild and severe strains. When concentration of nucleic acids inoculum containing both strains was low (340 μg/ml), S-PSTV detection from coinoculated plants dominated. At 43 d.p.i. S-PSTV was detected from 25 coinoculated plants and MF-PSTV alone from none (Table 1). Both strains together were detected initially (7 d.p.i.) from one or two plants of the five coinoculated cultivars. However, the detection of MF-PSTV was erratic, and at 43 d.p.i. both strains together were detected only from 19% of plants (Table 1). When the nucleic acids inoculum was doubled in concentration (680 μg/ml), the amount of MF-PSTV in the mixture became slightly higher than the amount of S-PSTV (Fig. 1, lane 2 vs. lane 3). From plants coinoculated at the doubled concentration, the detection of MF-PSTV increased. At 43 d.p.i., MF-PSTV was detected from 11 plants and S-PSTV from two plants only. The detection of both strains together increased to 37% of the inoculated plants (Table 1).

When tubers from MF + S-PSTV infected plants were tested, the presence of MF- and S-PSTV together was detected in 66% (27/41) of the tubers. All second-generation plants resulting from the 27 doubly infected tubers showed the presence of both strains in leaves throughout the 11-wk growing period (Fig. 1). The concentration of S-PSTV in doubly infected plants was not higher than that of MF-PSTV, a result previously observed in doubly infected tomato plants (10).

Comparative susceptibility of four potato cultivars. The plants of BelRus did not develop diagnostic symptoms and grew normally compared to Kennebec, Red Pontiac, and Russet Burbank, which were severely stunted and had reduced leaf size. Based on symptomology, BelRus was the most tolerant cultivar to PSTV and Russet Burbank was the most susceptible to PSTV. These two cultivars were selected for further studies.

In Russet Burbank plants, the viroid (MF-PSTV) was detected in each leaf position after 2 w.p.i., but was not detected in BelRus plants (Table 2). All plants and most of the leaves on each Russet Burbank plant contained MF-PSTV (3 w.p.i.), while it was detected from only five of the eight BelRus plants. MF-PSTV was detected in all except one bottom leaf of Russet Burbank at 5 w.p.i., but about one-third of the bottom leaf contained no detectable MF-PSTV in BelRus (Table 2).

Cross-protection with MF-protected potato plants. In plants of BelRus protected with MF-PSTV and challenged with S-PSTV, the latter was detected only once (Table 3). Individual testing of doubly inoculated plants indicated that S-PSTV was detected from only two of the 10 plants and that the concentration of S-PSTV was very low. All Russet Burbank plants protected with MF-PSTV and challenged with S-PSTV remained free of S-PSTV (Table 3). Control plants inoculated with only MF-PSTV or S-PSTV were infected as expected, irrespective of cultivars (Table 3). The concentration of MF-PSTV in protected and challenged plants was similar to plants protected but not challenged (data not shown).

Cross-protection with S-PSTV protected potato plants. With one exception only S-PSTV was detected in BelRus plants inoculated first with S-PSTV and 3 wk later with MF-PSTV (Table 4). Individual testing of doubly inoculated plants showed that five plants out of 10 were infected with both strains and infection by MF-PSTV was confined to top leaves. All plants of Russet Burbank were only infected with S-PSTV (Table 4). All the control plants were infected with S-PSTV or MF-PSTV as expected, indicating that no cross-contamination of the strains had occurred.

Cross-protection of second-generation infected potato cultivars. Plants of cultivars Jemseg, Kennebec, Katahdin, and Sebago, grown from tubers that were preinfected with MF-PSTV in the greenhouse in previous generation were challenge inoculated with S-PSTV (680 μg/ml) or buffer and new growth was analyzed for viroid strains for 16 w.p.c.i. No challenge strain was detected in any plant during this period. In another test, five second-generation plants of Russet Burbank were challenge inoculated with 10 times higher concentration (6 mg/ml). No challenge strain was detected during 9 w.p.c.i. from doubly inoculated plants.

In parallel tests, Jemseg and Kennebec plants preinfected with S-PSTV in the first generation (originating from infected tubers) were challenged with MF-PSTV or buffer. These plants failed to show the presence of the challenge strain during the 14 w.p.c.i. testing.

To extend this study to field-grown plants, tubers of 20 cultivars preinfected with MF-PSTV in the field were planted and the resulting plants were challenge inoculated with S-PSTV. Viroid analysis at 5, 9, and 12 w.p.c.i. failed to detect the presence of S-PSTV in these plants.

TABLE 1. Detection of different strains of potato spindle tuber viroid (PSTV) in coinoculated potato cultivars at different intervals after inoculation

	Detection of PSTV[a] (days postinoculation)						
Cultivars	7	9	11	13	15	29	43
340 μg/ml of each strain[b]							
Atlantic	2/5 M + S[c]	0	0	5/5 S	3/5 S	5/5 S	5/5 S
BelRus	0	0	1/3 S	1/3 S	3/3 S	2/3 S	
				1/3 M + S		1/3 M + S	3/3 M + S
Dĕsirĕe	2/5 M + S	0	0	5/5 M + S	5/5 S	5/5 S	3/5 S
							2/5 M + S
Kennebec	2/5 M + S	0	0	1/5 S	5/5 S	5/5 S	5/5 S
				4/5 M + S			
Russet Burbank	3/3 S	NT	NT	1/3 S	1/3 S	3/3 S	2/3 S
							1/3 M + S
Shepody	2/5 S	0	1/5 S	4/5 S	3/5 S	5/5 S	5/5 S
	1/5 M + S						
Yukon Gold	1/5 S	0	0	2/5 S	3/5 S	5/5 S	5/5 S
	1/5 M + S			2/5 M + S			
680 μg/ml of each strain[b]							
BelRus	0	3/10 M	0	0	2/10 M	1/10 M	2/10 M
					1/10 S	2/10 S	1/10 S
						2/10 M + S	4/10 M + S
Jemseg	0	4/10 M	1/10 M	5/10 M	4/10 M	4/10 M	4/10 M
				2/10 S	2/10 S	3/10 S	1/10 S
					1/10 M + S	2/10 M + S	5/10 M + S
Kennebec	0	1/10 M	0	0	5/10 M	5/10 M	5/10 M
						4/10 M + S	4/10 M + S

[a]Top leaves (0.5 g) were used for nucleic acid extraction and assayed by return-polyacrylamide gel electrophoresis.
[b]Partially purified nucleic acid containing either strain was adjusted to 340 or 680 μg/ml concentration, when mixed in equal volumes.
[c]M = MF-PSTV, S = S-PSTV, number of plants with viroid/number of plants indexed; 0 = viroid was not detected; NT = not tested.

As controls, potato plants originating from healthy tubers were inoculated with MF- or S-PSTV at the time of challenge inoculation. Detection of the respective viroid strain in control plants was as expected.

Cross-protection of other solanaceous species. Almost complete cross-protection was observed in MF-PSTV or S-PSTV protected potato plants as opposed to tomato (10); it was of interest to determine the nature of cross-protection with other experimental hosts of PSTV. Eight host species with different symptoms (Table 5) were used, including plants of tuber-producing (*S. demissum*) and non-tuber bearing (*S. nigrum*) species. Except for the *Nicotiana* species, cross-protection was complete and no challenge strain (S-PSTV) was detected up to 8 w.p.c.i. (Table 5). Plants of *N. debneyi* from which MF-PSTV was detected at 2 w.p.c.i., prevented the establishment of S-PSTV after challenge inoculation. One plant, from which MF-PSTV was not detected at 2 w.p.c.i., was the one that became infected with both strains (Table 5). *N. tabacum* 'Samsun' was difficult to preinfect with MF-PSTV and, consequently, only the challenge strain was detected at later stages of infection. In a subsequent test, it was established that while *N. debneyi* was readily infected with three strains of mild PSTV, *N. tabacum* 'Samsun' was not.

Breakdown of the cross-protection by inoculation methods. Preinfection of potato plants with MF-PSTV by mechanical inoculation provided complete protection against S-PSTV in the second-generation plants of 20 potato cultivars. To determine whether this resistance to superinfection by S-PSTV was similar to immunity, the MF-PSTV protected plants were challenge inoculated by grafting S-PSTV infected scions. Four of the 11 Kennebec plants grafted with S-PSTV-infected scions became infected with this strain within 15 days, and the remaining plants within 30 days after graft inoculation. Grafted scions provide a continuous source of challenge inoculum. To differentiate between inoculum pressure and inoculum placement, the pinpoint puncture method was used for the challenge inoculation. This method places the inoculum below the epidermal layer in contrast

TABLE 2. Progression of mild potato spindle tuber viroid infection in various leaflets over a period of 5 wk postinoculation in singly inoculated plant

Leaf position	Weeks postinoculation							
	2		3		4		5	
	RB[a]	B	RB	B	RB	B	RB	B
1 Bottom	1/7[b]	0/8	5/8	1/8	3/8	3/8	7/8	3/8
2	3/7	0/8	8/8	4/8	7/8	3/8	8/8	5/8
3	2/7	0/8	8/8	4/8	8/8	5/8	8/8	5/8
4	1/2	0/7	8/8	5/8	8/8	5/8	8/8	5/8
5	···	0/4	7/7	5/8	8/8	6/8	8/8	6/8
6	···	0/2	3/3	5/8	8/8	6/8	8/8	6/8
7	···	0/1	···	3/6	7/7	6/8	7/7	8/8
8	···	···	···	1/4	3/3	6/8	8/8	7/8
9	···	···	···	0/2	···	5/6	7/7	8/8
10	···	···	···	0/1	···	4/4	3/3	8/8
11	···	···	···	···	···	2/2	···	6/6
12	···	···	···	···	···	1/1	···	4/4
13	···	···	···	···	···	···	···	2/2
14 Top	···	···	···	···	···	···	···	1/1

[a]RB = Russet Burbank; B = BelRus.
[b]Number of leaflets infected/number of plants tested. Eight plants of each cultivar were inoculated. One leaflet from each leaf of each plant was tested by return-polyacrylamide gel electrophoresis at various periods after inoculation. ··· = Not tested.

TABLE 3. Detection of potato spindle tuber viroid (PSTV) strains in BelRus and Russet Burbank potato plants protected with mild strain and challenge inoculated with severe strain

Treatment[a]	Leaf sample	Weeks post challenge inoculation					
		2	4	6	8	10	12
BelRus							
MF-PSTV followed by PSTV	Top	M[b]	···[c]	···	M	M + S	M
	Middle	M	M	M	M	M + S	M
	Bottom	···	M	M	M	M	M
MF-PSTV followed by Buffer	Top	M	M	M	M	M	M
	Middle	M	M	M	M	M	M
	Bottom	···	M	M	M	M	M
Buffer followed by S-PSTV	Top	···	···	S	S	S	S
	Middle	···	S	S	S	S	S
	Bottom	···	S	S	S	S	S
Russet Burbank							
MF-PSTV followed by S-PSTV	Top	M	M	M	M	M	M
MF-PSTV followed by Buffer	Top	M	M	M	M	M	M
Buffer followed by S-PSTV	Top	S	S	S	S	S	S

[a]Combined leaf samples from 5 to 10 plants with each cultivar were used for nucleic acid extraction. First inoculation was protecting strain, followed by challenge inoculation 21 days after.
[b]M = MF-PSTV; S = S-PSTV.
[c]··· = Viroid not detected.

TABLE 4. Detection of potato spindle tuber viroid (PSTV) strains in BelRus and Russet Burbank potato plants protected with severe strain and challenge inoculated with mild strain

Treatment[a]	Leaf position	Weeks post challenge inoculation					
		2	4	6	8	10	12
BelRus							
S-PSTV followed by MF-PSTV	Top	S[b]	S	S	S	S + M	S + M
	Middle	S	S	S	S	S + M	S
	Bottom	···[c]	···	S	S	S	S
S-PSTV followed by Buffer	Top	···	S	S	S	S	S
	Middle	···	S	S	S	S	S
	Bottom	···	···	S	S	S	S
Buffer followed by MF-PSTV	Top	···	···	M	M	M	M
	Middle	···	M	M	M	M	M
	Bottom	···	M	···	M	M	M
Russet Burbank							
S-PSTV followed by MF-PSTV	Top	S	S	S	S	S	S
S-PSTV followed by Buffer	Top	S	S	S	S	S	S
Buffer followed by MF-PSTV	Top	M	M	M	M	M	M

[a]Combined leaf samples from 5 to 10 plants with each cultivar were used for nucleic acid extraction. First inoculation was protecting strain, followed by challenge inoculation 21 days after.
[b]M = MF-PSTV; S = S-PSTV.
[c]··· = Viroid not detected.

TABLE 5. Detection of severe potato tuber spindle viroid (PSTV) strain in various Solanaceous plants protected with mild PSTV strain

Plant species	Type of symptoms[a]	No. of plants	Weeks post challenge inoculation with severe PSTV			
			2	4	6	8
Nicotiana debneyi	Latent	4/5[b]	M[c]	M	M	M
		1/5	···	M + S	M + S	M + S
N. tabacum 'Samsun'	Latent	4/5	···	···	S	S
Physalis angulata	Latent	5/5	M	M	M	M
P. floridana	Latent	5/5	M	M	M	M
Scopolia sinensis	Necrotic leaves	5/5	M	M	M	M
Solanum berthaultii	Necrotic leaves	5/5	M	M	M	M
S. demissum	Stunted leaves	5/5	M	M	M	M
S. nigrum	Latent	5/5	M	M	M	M

[a]Type of symptoms caused by both mild and severe strains on these plants.
[b]Number of plants with viroid bands/number of plants tested.
[c]M = mild MF strain; S = severe strain; and M + S = both mild and severe strains of potato spindle tuber viroid.

to mechanical inoculation, where the inoculum is mainly confined to the epidermal cells. Of the 11 plants challenge inoculated with S-PSTV by this method, only one became infected within 4 wk of inoculation. In a subsequent test, 25 plants were challenge inoculated with S-PSTV by pinpoint puncture method and no challenged strain was detected within 8 w.p.c.i. Control plants inoculated by this method became infected.

DISCUSSION

In contrast to studies with tomato plants in which coinoculation with mild and severe strains of PSTV resulted in infection and multiplication of both strains in each plant (10), coinoculation of potato plants resulted in differential infection, some plants becoming infected with one strain, others with the second strain (Table 1). A relatively low concentration of the mild strain in inoculum failed to establish a single infection of mild strain in potato plants, providing an estimate of the threshold concentration of this strain when in a mixed inoculum. Doubling the concentration in the inoculum mixture but maintaining the ratio of strains resulted in infection of a third of coinoculated plants by the mild strain alone, indicating no interference with mild strain replication by the severe strain. This suggestion is further supported by the continued detection of both strains in secondarily infected plants, grown from doubly infected tubers (Fig. 1). Prevention of the accumulation of one strain by another has been attributed to interference between viroid strains (1). It was proposed that if the viroid replication complex is a persistent structure, its formation around one viroid could cause a competing viroid to be excluded (1). Although the separate occurrence of mild and severe strains in some plants (Table 1) supports such an interpretation, there is no information about the existence of a persistent viroid replication complex.

Cross-protection, defined by the absence of replication of the challenge strain, was complete in Russet Burbank plants and incomplete in BelRus (Tables 3 and 4). Incomplete cross-protection in BelRus could have resulted from slow infection, and the slow spread of MF-PSTV in the plant (Table 2). This hypothesis is strengthened by the observation that secondarily infected plants of BelRus, which should be fully infected, prevented the establishment of the challenge strain throughout the growing season. Thus, cross-protection in potato differs from cross-protection in tomato, where both strains were detected early in the growing season (10). Studies with other hosts indicated that tuber-bearing or non-tuber-bearing *Solanum* species or plants of other species that develop symptoms on infection with PSTV or carry PSTV infection symptomlessly did not affect the cross-protection (Table 5). It appears that in host plants that are difficult to infect or in which MF-PSTV is slow to spread, cross-protection is incomplete. Although tomato is very susceptible to MF-PSTV (5,15), it is not as susceptible as tissue-culture propagated potato plantlets used in this study (Singh, *unpublished data*).

Cross-protection is generally studied by mechanical inoculation of the protecting and challenge strain in the plant. Occasionally, challenge strains have been inoculated by aphids (3,9). This method satisfactorily protected plants from symptoms of the challenge strain, but the accumulation of challenge infection is unknown. Introduction of the challenge strain by grafting (tissue union) resulted in the failure of symptomatic cross-protection (3). The placement or the quantity of challenge strain inoculum available through grafting is possibly different than through mechanical inoculation. Although a 10 times higher amount of inoculum on secondarily infected potato plants failed to break the cross-protection, in one case a pinpoint puncture application of inoculum resulted in the breakdown of cross-protection. This suggests that factors associated with the epidermal layer of the host plants may be involved in the cross-protection.

On the basis of the cross-protection reaction of the second-generation potato plants, one could classify reaction of PSTV in potato cultivars as "immunity" using Hamilton's suggestion (8). However, inoculation methods can destroy the cross-protection totally and host species can affect the reaction pattern (Table 5). These findings point out the need for additional studies before a generalized pattern of cross-protection phenomenon can be made.

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