Potato spindle tuber viroid: Stability on Common Surfaces and Inactivation With Disinfectants

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Abstract

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The length of time *Potato spindle tuber viroid* (PSTVd) remained infective in extracted tomato leaf sap on common surfaces and the effectiveness of disinfectants against it were investigated. When sap from PSTVd-infected tomato leaves was applied to eight common surfaces (cotton, wood, rubber tire, leather, metal, plastic, human skin, and string) and left for various periods of time (5 min to 24 h) before rehydrating the surface and rubbing onto healthy tomato plants, PSTVd remained infective for 24 h on all surfaces except human skin. It survived best on leather, plastic, and string. It survived less well after 6 h on wood, cotton, and rubber and after 60 min on metal. On human skin, PSTVd remained infective for only 30 min. In general, rubbing surfaces contaminated with dried infective sap directly onto leaves caused less infection than when the sap was rehydrated with distilled water but overall results were similar. The effectiveness of five

disinfectant agents at inactivating PSTVd in sap extracts was investigated by adding them to sap from PSTVd-infected leaves before rubbing the treated sap onto leaves of healthy tomato plants. Of the disinfectants tested, 20% nonfat dried skim milk and a 1:4 dilution of household bleach (active ingredient sodium hypochlorite) were the most effective at inactivating PSTVd infectivity in infective sap. When reverse-transcription polymerase chain reaction was used to test the activity of the five disinfectants against PSTVd in infective sap, it detected PSTVd in all instances except in sap treated with 20% nonfat dried skim milk. This study highlights the stability of PSTVd in infective sap and the critical importance of utilizing hygiene practices such as decontamination of clothing, tools, and machinery, along with other control measures, to ensure effective management of PSTVd and, wherever possible, its elimination in solanaceous crops.

Potato spindle tuber viroid (PSTVd, family *Pospiviroidae*, genus *Pospiviroid*) was first described in North America in the early 1920s. It was found subsequently in 40 other countries and infects 18 crops naturally, including potato (*Solanum tuberosum*), tomato (*S. lycopersicum*), pepper (*capsicum*) (*Capsicum annuum*), eggplant (*S. melongena*), avocado (*Persea americana*), and sweet potato (*Ipomoea batatas*) (7,29,40). Depending upon the environmental conditions, PSTVd-infected tomato and potato plants may develop severe foliage symptoms and plant stunting. PSTVd can diminish tomato and potato production greatly, through reduced fruit or tuber yields and severe quality defects (4,7,31). PSTVd is transmitted readily through botanical seed and pollen of tomato and potato (5,17,39). Seed transmission occurs at levels of up to 11% in tomato and 33 to 67% in potato (7,42). Developing seed becomes infected with PSTVd through either the pollen or the ovule, and up to 100% of them may become infected (13,17,31,39). Plants grown from infected botanical seed of either species provide an important source of infection for further spread in tomato crops and potato breeding programs.

PSTVd is easily contact transmitted and the extent of such transmission depends on the stability of its nucleic acid, its concentration, and the source of inoculum (11,37). It spreads to healthy plants readily in infective sap when infected plants are handled, by natural plant-to-plant

leaf contact, and on glasshouse and farm equipment and machinery (e.g., knives, crates, and tractors) (16). Bonde and Merriam (6) found that transmission of PSTVd occurred when cutting knives contaminated with infectious sap were used to cut potato seed tubers before planting the crop. In particular, contamination of young tuber sprouts with infectious sap resulted in the greatest spread. Further, Merriam and Bonde (26) reported PSTVd transmission rates of 80 to 100% to healthy potato plants when tractor wheels became contaminated with infectious sap following bruising of diseased potato plants in the field. Although Manzer and Merriam (21) found nearly 100% PSTVd transmission via cultivating and hilling equipment, this was only when large plants were present; very little transmission occurred when plants were smaller or cultivation was undertaken earlier in the growing season. PSTVd spread on clothes was considered likely but remained to be demonstrated (28). In contrast, transmission following tomato fruit handling was considered unlikely unless the fruit has been squashed to release its sap (46). In the same way, PSTVd spread between infected and healthy plants by root contact was also considered unlikely (28,46), and no transmission occurred when PSTVd inocula were added to tomato roots (47). However, recent findings by Mehle et al. (24) suggested that PSTVd transmission via roots through water-mediated movement is of concern in hydroponic and intensive irrigation systems, with PSTVd infectivity surviving in water for up to 7 weeks.

PSTVd infectivity survives drying well (12). However, apart from a report by Spence and O'Neill (43), no research has been undertaken to determine its infection retention times on different types of common surfaces. This is in contrast to studies on retention time of viruses such as *Potato virus S* (PVS, genus *Carlavirus*), *Potato virus X* (PVX, genus *Potexvirus*), and *Zucchini yellow mosaic virus*

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(ZYMV, genus *Potyvirus*) on common surfaces (10,14,48). In those studies, PVX survived up to 3 h on iron, 6 h on painted wood, and 24 h in soil (49), while PVS was infective for 7 h on metal surfaces, 25 h on rubber surfaces, and 120 h on moist hessian (burlap) (14). When sap from ZYMV-infected leaves was applied to seven common surfaces (cotton, plastic, leather, metal, rubber vehicle tire, rubber soled footwear, and human skin) and left for up to 48 h before the ZYMV-contaminated surface was rubbed onto healthy zucchini plants, the virus was still infective after 48 h on tire, 24 h on plastic and leather, and up to 6 h on cotton, metal, and footwear (10). Given the stable nature of PSTVd, it seems likely it would also remain infective on these types of surfaces. Spence and O'Neill (43) examined the survival of PSTVd in dried sap on plastic, glass, aluminum, and concrete kept in the dark versus light. PSTVd survived for at least 4 weeks at 30°C on aluminum (in light and dark) and glass (in light), and proved more infectious from concrete when dried in the dark. However, on plastic, the viroid survived poorly in both light and dark, which they attributed to irreversible binding to the plastic.

Several commercially available products are recommended for decontaminating tools and surfaces from plant viruses and viroids (15,19,45). Their effectiveness and usefulness depends on the type and concentration of disinfectant, incubation time, availability, cost, and toxicity to humans or plants (19). Effective removal of PSTVd-infected plants and disinfection of contaminated tools, surfaces, and machinery are vital for successful control of PSTVd in glasshouse or field production systems. Several disinfectant chemicals such as sodium hypochlorite, sodium hydroxide + formaldehyde, and trisodium phosphate are potential disinfection agents for use with viroid-contaminated tools (3,15,22,23,33,34,41,44). However, sodium hypochlorite was the most effective in comparison with nine other products (citric acid, Hortisept, Horticide, Jet 5, TSOP®, Menno Florades, Panacide M, trisodium orthophosphate, and Virkon S), because only sodium hypochlorite at 0.5% (active chlorine at 5,000 ppm) eliminated PSTVd infectivity from both glass and concrete surfaces after 5 min (28). A 1% solution of sodium hypochlorite was effective at eliminating PSTVd infectivity on contaminated knives after a 5-s dip (28). Singh et al. (41) found that 1% sodium hypochlorite solution disinfected PSTVd contaminated tools but a 3% solution was necessary if PSTVd contamination levels were high. However, although it is effective, quick-acting, and relatively inexpensive, sodium hypochlorite is an irritant to humans, corrodes metal, and degrades when in contact with organic materials (19). Nonfat dried skim milk eliminates *Tobacco mosaic virus* (TMV) transmission to petunia plants (19) and *Hibiscus latent Fort Pierce virus* transmission from contaminated tools to hibiscus plants (18). Spray treatment with whole or skim milk was recommended to prevent the plant-to-plant transmission of TMV prior to handling tobacco plants (19). With ZYMV, Coutts et al. (10) found that nonfat dried skim milk and household bleach (42g/L sodium hypochlorite) were the only effective disinfectants of the nine they tested with ZYMV. Thus, nonfat dried skim milk treatments may have potential for PSTVd removal without the problems associated with sodium hypochlorite use.

Reverse-transcription polymerase chain reaction (RT-PCR) is used routinely to detect PSTVd in potato tubers, leaves, pollen, and botanical seed (36); find newly discovered PSTVd strains (4,32); and, experimentally, to detect PSTVd in environmental samples (e.g., swabs from concrete and glasshouse structures and plants) (43). However, it cannot differentiate between infectious and inactivated viroids, particularly in environmental samples where RT-PCR could amplify viroids inactivated by disinfectants. Thus, wherever possible, effective infectivity tests should also be used in addition to RT-PCR.

In Australia, PSTVd was found infecting a wild *Solanum* sp. in the Northern Territory (4); cultivated potato in Victoria, New South Wales, South Australia, and Tasmania (8,9,35); glasshouse-grown tomato in Queensland, New South Wales, South Australia (27,45), and Western Australia (20); and field-grown tomato and chili in 2008 in the Carnarvon Horticultural District in coastal central Western Australia (2). Following each PSTVd detection, major

eradication programs were undertaken in which sodium hypochlorite, Virkon S, and Farmcleanse were used for disinfection without attempts to demonstrate their efficacies beforehand. In this study, we report the results of experiments undertaken to determine (i) the stability over time of PSTVd infectivity in sap on eight different types of common surfaces likely to be encountered in commercial plant-growing systems and (ii) the effectiveness of five commonly used chemical disinfectants at eliminating PSTVd and degrading its nucleic acid sufficiently to prevent its detection by RT-PCR. This information is critical for inclusion in protocols to ensure success from future PSTVd management programs worldwide.

Material and Methods

For the purposes of this investigation, “contact transmission” refers to infected plant material coming into contact with healthy plants in the absence of an abrasive and without causing visible damage, while “mechanical transmission” refers only to experimental inoculation with sap from PSTVd-infected leaves containing an abrasive such as diatomaceous earths.

Viroid isolates, plants, and inoculations. Tomato ‘Grosse Lisse’ plants were grown in pots in a steam-sterilized soil, sand, and peat mix (1:1:1). All plants were grown in insect-proof, air-conditioned glasshouses maintained at 18 and 30°C, night and day, respectively. Western Australian PSTVd isolates WAC13195 (from Carnarvon in 2007) and WAC13122 (from Carnarvon in 2006) were maintained in tomato plants by sap inoculation. This was done by grinding PSTVd-infected tomato leaves in 0.1 M phosphate buffer maintained at pH 7.2 and mixing the sap with celite before rubbing onto the leaves of plants.

RT-PCR. Following sap inoculation, samples from newly expanded tip leaves from all plants were tested individually to confirm PSTVd infection. For this, leaf samples were extracted using a Qiagen RNeasy Plant Mini Kit (QIAGEN, USA) following the manufacturer’s instructions. Sample extracts were then tested for PSTVd by RT-PCR using CT20(R)/TG21(F) PSTVd-specific primers (9). RT-PCR was done using an Invitrogen One-step RT-PCR kit. The RT-PCR reaction was in a final volume of 13 µl containing 0.25 µl of each primer (100 µM stock solution) and 1.75 µl of RNA extract. PCR amplification conditions were as follows: 48°C for 45 min; 94°C for 2 min; followed by 35 cycles of 94°C for 30 s, 55°C for 30 s (for PSTVd) or 62°C for 30 s (for Pospiviroids in general), and 72°C for 30 s; and a final elongation step of 72°C for 5 min. The temperature was then held at 14°C at the completion of PCR cycling. PCR amplicons were visualized by electrophoresis on 1.5% agarose gels stained with ethidium bromide. Extracts from leaf samples from tomato culture plants infected with PSTVd isolate WAC 13122 were used as positive controls and leaf samples from a healthy tomato plant as negative control comparisons.

Stability of PSTVd infectivity in sap. Two identical experiments were conducted to establish the stability of PSTVd infectivity in sap. Sap was extracted from tomato leaves infected with PSTVd isolate WAC13195 using a leaf press (Pollahne, Germany) and filtered through gauze. Sap extracts (2 ml) were incubated in individual glass beakers at about 20°C in an air-conditioned glasshouse and subjected to natural light during the day. They were allowed to dry for 5 min and 1, 6, and 24 h before 2 ml of distilled water was added to resuspend each sample. After each time period, once the sap was resuspended, it was immediately inoculated onto leaves of five tomato plants, each without the use of buffer or abrasive. Five replicate plants were left uninoculated as controls. A single newly expanded tip leaf was sampled from each plant 28 days after inoculation and tested individually for PSTVd by RT-PCR.

Stability of PSTVd infectivity in sap on different surfaces. Four separate experiments were conducted to establish the stability of PSTVd infectivity on different common surfaces. Sap was extracted from tomato leaves infected with PSTVd isolate WAC13195 using a leaf press, filtered through gauze, and pipetted onto each surface. The test surfaces were metal (steel), cotton (washed and worn), tire (inner rubber tube), leather (worn boot), wood (unpainted wooden

stake), plastic (black horticultural plastic mulch), string (horticultural twine-fiber), and skin (human). For each surface, 0.5 ml of infective sap was applied to a 2-by-2-cm area and left at room temperature for 5 min and then 1, 6, and 24 h. The only exception was with skin, where it was left for 5 and 30 min and then for 1 h. Five tomato plants were inoculated as described above for each surface for each time period. In experiments 1 and 2, the surface contaminated with infective sap was rubbed directly onto the leaves of plants. In experiments 3 and 4, the surface contaminated with infective sap was rehydrated with 0.5 ml of distilled water prior to rubbing directly onto leaves of plants. For control comparisons, an additional two plants were left uninoculated for each time period while five plants were mock inoculated with fresh, uninfected sap extract. A single newly expanded tip leaf was sampled from each plant 28 days after inoculation and tested individually for PSTVd by RT-PCR.

Inactivation of PSTVd infectivity. Two identical experiments were done to establish the efficacy of six disinfectant treatment solutions at inactivating PSTVd. Sap was extracted from tomato leaves infected with PSTVd isolate WAC13195 using a leaf press and filtered through gauze. Extract (1 ml) was diluted with 1 ml each of the following substrates: nonfat dried skim milk (20% [wt/vol]), household bleach (sodium hypochlorite at 42 g/liter, 4% available chlorine, diluted 1:4 with water), Virkon S (potassium peroxydisulfate, 50% [wt/wt], diluted to 0.5 or 1% [wt/wt]), Farmcleanse (alkylbenzene sulfonic acid 5 to 10%, diluted 1:10 with water), denatured ethanol (70%), or distilled water. All disinfectants were used at the manufacturer's recommended rate by diluting in distilled water immediately prior to application. The mixture of infective sap and disinfectant was left for 1 min prior to being used to rub without buffer or abrasive onto leaves of tomato plants. For mock-inoculated controls, tomato plants were each similarly rubbed with sap from healthy or PSTVd-infected plants (10 plants each). A single newly expanded tip leaf was sampled from each plant 28 days after inoculation and tested individually for PSTVd by RT-PCR.

Degradation of PSTVd RNA by disinfectants. In two further experiments, the same six disinfectant treatment solutions were tested for their abilities to disrupt PSTVd RNA such that it can no longer be detected by RT-PCR. Sap was extracted from tomato leaves infected with PSTVd isolate WAC 13122 using a leaf press and filtered through gauze. Fifteen 0.1-ml samples (A to E) were processed simultaneously. Samples A and B were negative and positive controls, respectively, and contained healthy (A) or PSTVd-infective (B) sap in 0.4 ml of RLT buffer (Catalogue no. 79216, QIAGEN, USA) without any disinfectant or neutralizing agent being added. In sample C, 0.1 ml of neutralizing agent (sodium thiosulfate 0.5% [wt/vol]) was added to the infective sap immediately prior to extraction with 0.3 ml of RLT buffer. This control treatment tested whether, when present alone, the neutralizing agent would influence PSTVd detection. In samples D1 to 6, infective sap was mixed with

0.05 ml of each disinfectant and 0.05 ml of neutralizing agent simultaneously, and extracted after 30 s of incubation. This tested whether making the neutralizing agent part of the reaction mixture to stop the reaction would influence PSTVd detection. In samples E1 to 6, after 30 s of incubation of infective sap with 0.05 ml of each disinfectant, the disinfectant was neutralized by adding 0.05 ml of the same neutralizing agent prior to extraction. This tested whether adding the neutralizing agent subsequently would influence PSTVd detection. For each of samples D and E, RLT buffer was added to make a total of 0.5 ml. With all samples, the RNA in the combined solutions was extracted as described above before being tested for PSTVd by RT-PCR. In an additional experiment, internal RNA-specific control primers (nad5) were included as described Menzel et al. (25) to establish whether sample exposure to nonfat dried skim milk inhibits the PCR reaction.

Results

Stability of PSTVd infectivity in sap. In two experiments, infective sap from tomato leaves remained infective on glass for at least 24 h after extraction, with no sign of PSTVd infectivity declining. In experiment 1, at 5 min, three to five of five plants became infected; at 1 h, five of five; at 6 h, four of five; and at 24 h, five of five. Similarly in experiment 2, the numbers of plants infected were five of five after 5 min and 1 and 24 h, and three of five at 6 h. No control plants became infected.

Stability of PSTVd infectivity in sap on different surfaces. On cotton, leather, string, and wood surfaces, infective sap was completely absorbed within 60 min and fully dried by 6 h. With tire, metal, and plastic, sap started to dry out within 60 min and had also dried completely by 6 h. In general, survival of infectivity was greater in experiments 3 and 4 than in experiments 1 and 2, reflecting rehydration with distilled water before inoculation by rubbing onto leaves in the former versus direct rubbing onto leaves without rehydration in the latter (Table 1). In experiments 1 and 2, infectivity was still detected after 24 h in at least one of the two experiments on tire, leather, metal, plastic, and string but only survived up to 6 h on wood and cotton and up to 30 min on human skin. In experiments 3 and 4, it was still detected after 24 h with all surfaces apart from human skin, on which it only persisted up to 30 min. Greatest infectivity retention occurred with string and leather, with three of five plants infected in all instances in experiments 3 and 4. Survival was also higher with plastic, with four of five plants still infected after 24 h in experiment 2. At the other extreme, on human skin, no infectivity was ever detected at 60 min in any experiment. With metal, infectivity survived poorly after 60 min and on wood, cotton, and tire after 6 h. All plants rubbed with freshly extracted infective sap after 5 min became infected and no plants became infected when healthy plants were left uninoculated for the duration of each experiment.

Inactivation of PSTVd infectivity by disinfectants. In the two experiments to test the efficacy of disinfectants at inactivating

Table 1. Infectivity of *Potato spindle tuber viroid* (PSTVd) in dried or rehydrated tomato sap after incubation for different time periods on eight common surfaces^a

Surface	5 min		30 min		60 min		6 h		24 h	
	Exp 1, 2	Exp 3, 4	Exp 1, 2	Exp 3, 4	Exp 1, 2	Exp 3, 4	Exp 1, 2	Exp 3, 4	Exp 1, 2	Exp 3, 4
Fresh infective sap	5,5	5,5	...	...	...	...	...	...	...	...
Cotton	3,2	4,5	...	...	0,0	4,4	1,0	2,2	0,0	1,0
Wood	0,0	5,4	...	...	1,0	5,4	0,1	2,3	0,0	1,1
Tire (rubber - inner tube)	3,3	5,3	...	...	0,1	3,1	0,1	3,2	1,1	1,0
Leather	1,0	5,5	...	...	0,1	5,4	2,0	4,3	1,1	3,3
Metal	1,2	5,4	...	...	1,0	4,5	0,1	0,2	1,1	1,1
Plastic	2,4	5,4	...	...	1,5	4,4	0,5	3,2	0,4	1,1
Human skin (living)	0,2	2,3	1,1	0,1	0,0	0,0	nt,nt	nt,nt	nt,nt	nt,nt
String	3,3	5,3	...	...	3,4	4,5	2,2	3,4	1,2	3,3
Uninoculated plants	0,0	0,0	...	...	0,0	0,0	0,0	0,0	0,0	0,0

^a Sap extracted from PSTVd isolate WAC13195-infected leaves was applied to each surface and left at room temperature for each specified incubation period. Experiments 1 and 2 and experiments 3 and 4 differed in that, in the former, the surface contaminated with infective sap was wiped directly onto the leaves but, in the latter, it was rehydrated with 0.5 ml of distilled water before rubbing directly onto the leaves of healthy plants. Extracts from each surface were inoculated individually to five plants of tomato. Newly emerged leaves from all plants were tested individually 28 days after inoculation by reverse-transcription polymerase chain reaction. Values for numbers of plants infected in each identical experiment are separated by a comma; nt = not tested.

PSTVd infectivity, no plants became infected when nonfat dried skim milk or household bleach (1:4) were added to infectious sap prior to inoculation (Table 2). With the other four disinfectant treatments (viz., denatured ethanol, Farmcleanse, and Virkon S 1% and 0.5%), more than half of the plants inoculated always became infected. With the distilled water treatment, 9 to 10 of 10 plants became infected in each experiment and, when infective sap was used without any disinfectant, all 10 plants became infected. No plants inoculated with healthy sap developed PSTVd infection.

PSTVd RNA degradation by disinfectants. In two experiments to determine whether disinfectants could disrupt PSTVd RNA, only the nonfat dried skim milk treatment in the absence of the neutralizing agent ever degraded and eliminated its RNA (sample E2, Table 3). In the control treatments, no PCR amplification occurred (sample A) or amplification was detected (samples B and C). Thus, adding neutralizing agent to infected sap did not prevent amplification of PSTVd. When tested with the internal control primers, PCR amplification was detected in all samples, indicating that the disinfectants tested did not inhibit the PCR reaction. When each disinfectant was mixed simultaneously with infective sap and neutralizing agent, this resulted in amplification of PSTVd in all instances, including with nonfat dried skim milk (samples D1 to 6).

In an additional experiment, in which internal RNA-specific control primers (nad5) were included, these failed to detect any RNA after sample exposure to nonfat dried skim milk treatment.

Discussion

This study found that PSTVd remained infective at room temperature in sap on seven types of common surfaces for at least 24 h. The only exception to this was on human skin. It also confirmed that PSTVd could be inactivated reliably by household bleach and demonstrated that this also occurs with the “user-friendly” disinfectant nonfat dried skim milk. The nonfat dried skim milk degraded PSTVd RNA but the household bleach did not. These findings are important because they highlight the stability of PSTVd in infective sap and reproduce common PSTVd contact transmission scenarios during plant handling or when machinery is operating in tomato or potato production systems. They also provide a solution to contact spread of PSTVd through employing disinfectants that destroy its infectivity. Moreover, they emphasize the importance of using phytosanitary measures such as deploying healthy planting material previously tested for viroid presence and strict hygiene or sanitation practices before, during, and after plant cultivation. Such measures help to minimize both the initial reservoir of PSTVd infection and its subsequent spread, thereby ensuring effective management of PSTVd in susceptible solanaceous crops and, wherever feasible, its complete elimination.

Table 2. Inactivation of *Potato spindle tuber viroid* (PSTVd) in infective sap with five disinfectants

Treatment	Experiment ^a	
	1	2
Nonfat dried skim milk (20% [wt/vol])	0	0
Household bleach (1:4) (sodium hypochlorite at 42 g/liter and 4% chlorine)	0	0
Virkon S (0.5% [wt/vol])	8	6
Virkon S (1% [wt/vol])	10	10
Farmcleanse (1:10)	10	10
Denatured ethanol (70%)	7	5
Undiluted infective sap	10	10
Distilled water	9	10
Healthy sap	0	0

^a Sap was extracted from PSTVd isolate WAC13195-infected tomato leaves and diluted (1:1) with disinfectant or water, then used immediately to inoculate tomato plants (10 plants/treatment) without buffer or abrasive. Undiluted infective sap and healthy controls were also included. Samples from newly expanded tip leaves from all plants were tested individually by reverse-transcription polymerase chain reaction 28 days after inoculation. Values are numbers of plants out of 10 infected for each treatment.

This study has added practical significance because the stability of PSTVd infectivity was determined by inoculation of plants without use of abrasives and without dilution of infective tomato sap with phosphate buffer during extraction or inoculation. When infective sap was incubated in glass beakers, there was no evidence of infectivity diminishing quickly because all plants inoculated still became infected after 24 h. Its infectivity only survived for up to 30 min on human skin but for 24 h on all the other surfaces tested, with greatest infectivity survival occurring on string, plastic, and leather but still significant survival on wood, cotton, and metal. All surfaces evaluated in the present study are found commonly in tomato production systems. Therefore, these findings highlight the likelihood that considerable PSTVd spread via contact transmission will occur on clothing, machinery, and farm equipment and when plants are tied up on wires. Thus, spread can occur when machinery moves through crops (e.g., during spraying, fertilizing, or plant tying activities), on weeding implements (e.g., hoes), and during crop monitoring and harvesting (e.g., footwear or clothing). Verhoeven et al. (47) previously reported a somewhat longer survival period for PSTVd infectivity in infective sap on human skin, with sap from PSTVd-infected plants of *Brugmansia suaveolens* and *S. jasminoides* remaining infective on fingertips for up to 2 h at 25°C. The differences in PSTVd survival periods between the two studies may be due to differences in viroid titer, source plant for the infective sap, or ambient temperature and air moisture content where the studies were undertaken. Coutts et al. (10) reported similar results for surface contamination by ZYMV. They suggested that ZYMV might be inactivated by skincare products or rapid drying of sap when applied to the skin. However, in our study with PSTVd, skincare products were not applied to the area of skin contaminated. Possibly, the presence of RNAases secreted by living skin might have degraded the PSTVd RNA.

PSTVd is transmitted along rows during normal tomato production practices such as pruning, supporting, and harvesting. Such transmission occurs mainly via hands and pruning shears contaminated with

Table 3. Detection of *Potato spindle tuber viroid* (PSTVd) RNA in infective sap after treatment with five disinfectants^a

Treatment ^b	Experiment	
	1	2
A Healthy sap control	–	–
B Infected sap control	+	+
C Neutralizing Agent (0.5% [wt/vol] sodium thiosulfate)	+	+
D1 Denatured ethanol (70%)	+	+
D2 Nonfat dried skim milk (20% [wt/vol])	+	+
D3 Bleach (1:4) (sodium hypochlorite at 42 g/liter and 4% chlorine)	+	+
D4 Farmcleanse (1:10)	+	+
D5 Virkon S (1% [wt/vol])	+	+
D6 Virkon S (0.5% [wt/vol])	+	+
E1 Denatured ethanol (70%)	+	+
E2 Nonfat dried skim milk (20% [wt/vol])	–	–
E3 Bleach (1:4) (sodium hypochlorite at 42 g/liter and 4% chlorine)	+	+
E4 Farmcleanse (1:10)	+	+
E5 Virkon S (1% [wt/vol])	+	+
E6 Virkon S (0.5% [wt/vol])	+	+

^a Sap was extracted from PSTVd isolate WAC13122-infected tomato leaves; 0.1 ml of infective sap was used in each RNA extraction and tested individually by reverse-transcription polymerase chain reaction.

^b Samples A and B were negative and positive controls, respectively, and contained 0.4 ml of RLT buffer and healthy (A) or infective (B) sap without added disinfectant or neutralizing agent. Sample C was a control in which 0.1 ml of the neutralizing agent (0.5% [wt/vol]) sodium thiosulfate was mixed with the infective sap and 0.3 ml of RLT buffer. With samples D1 to 6, infective sap in 0.3 ml of RLT buffer was mixed with 0.05 ml of disinfectant and 0.05 ml of neutralizing agent, and incubated for 30 s before testing. With samples E1 to 6, infective sap in 0.3 ml of RLT buffer was mixed with 0.05 ml of disinfectant and incubated for 30 s before addition of 0.05 ml of the neutralizing agent and testing.

infective leaf sap from damaged foliage. When PSTVd spreads within tomato and potato plants, the viroid can infect all or most plant parts (47), reaching its greatest concentration in leaf hairs (7). Further, within tomato plants, it moves rapidly from an inoculated leaf to actively growing tissue via the phloem (29). Within potato plants, it also causes rapid systemic invasion, being detected most readily in the upper leaves and tubers (47). Hence, there are ample opportunities for its rapid transfer in both crops during standard production practices. During PSTVd detections in Western Australia in 2001 and the United Kingdom in 2003, monitoring of plants in protected cropping situations revealed local spread down rows, and this was generally in the direction of working. It was associated with clusters of infected tomato plants (20,38). In our experiments, PSTVd infectivity survived well on both dry and rehydrated string. String is used in supporting tomato plants on wires or stakes and its use requires considerable handling activity in both glasshouse and field production systems, thus favoring the transfer of PSTVd to plants. Although use of disposable gloves is often recommended to avoid PSTVd spread on contaminated hands (27), PSTVd transmission was greater when disposable gloves were worn than when they were not used (45); and, as mentioned above, we found only a 30-min infectivity retention time on human skin. Thus, ease of contact transmission, survivability on disposable gloves, and ability to survive storage on some common surfaces are all cause for concern over the effectiveness of traditional hygiene practices used to implement effective crop disease management strategies to prevent PSTVd spread.

Unexplained long-distance spread sometimes occurs in tomato crops, with isolated infected plants appearing many rows distant from the original PSTVd infection focus (20,38). This highlights the need to disinfect contaminated tools and materials effectively to prevent PSTVd spread using disinfectants. In this study, both household bleach and nonfat dry skim milk were equally effective in preventing PSTVd infection. This is an important finding which agrees with those obtained in similar studies with several plant viruses (10,18,19). However, as mentioned above, the active ingredient in bleach, sodium hypochlorite, is corrosive and its efficacy is reduced by organic matter. In contrast, milk is inexpensive, widely available, and safe to use for humans, plants, equipment, and the environment. It is an ideal disinfectant, particularly for situations involving extensive human contact with sprayed materials. Moreover, in the present study, nonfat dried skim milk powder was the only disinfectant tested that degraded PSTVd RNA, the possibility of PCR inhibition by this treatment being eliminated by use of internal RNA-specific control primers (nad5). Another advantage of nonfat dried skim milk powder is that testing environmental samples by RT-PCR following a PSTVd management program involving other disinfectants may result in false positives. It is particularly suitable for reducing infection in organic farming situations (10). Although nonfat skim milk is thought to contain proteins that encapsulate plant virions, thereby preventing virus transmission (1), how milk inhibits or decreases infection by viroids remains unknown.

In this study, Virkon S (1:100 and 1:200) was ineffective at inactivating PSTVd in infective sap. This differs from the findings of O'Neill and Mumford (28) described earlier. Possibly, the different outcomes between these two studies can be explained by the different contact times between the disinfectants with the contaminated surface. O'Neill and Mumford (28) left the disinfectants for 5 min in contrast to the briefer 1-min duration used in our study, which was selected as most likely to reflect commercial conditions. Although ethanol is routinely used in laboratories to sterilize tools and surfaces such as bench tops, it was ineffective against PSTVd infectivity in this study.

During outbreaks of PSTVd, growers spread the viroid from infected to healthy plants inadvertently during normal production activities. This emphasizes the importance of including measures that address contact transmission such as washing down machinery, disinfecting cutting tools and surfaces, and limiting handling and movement within crops in disease management and containment strategies for PSTVd. Management strategies that minimize the risk of introducing PSTVd via human activities include using healthy planting

material and disposable gloves and cleaning clothing and equipment (2). Thus, effective management of PSTVd has the greatest chance of succeeding through a combination of viroid containment and preventing spread.

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