Effects of a Potato Spindle Tuber Viroid Tomato Strain on the Symptoms, Biomass, and Yields of Classical Indicator and Currently Grown Potato and Tomato Cultivars

Alison E. Mackie,^{1,2} Martin J. Barbetti,^{1,3} Brendan Rodoni,⁴ Simon J. McKirdy,^{5,†} and Roger A. C. Jones^{2,3}

¹ University of Western Australia School of Agriculture and Environment, Faculty of Science, University of Western Australia, Crawley, Western Australia 6009, Australia

² Department of Primary Industries and Regional Development, Western Australia, Perth, Western Australia 6983, Australia

³ University of Western Australia Institute of Agriculture, University of Western Australia, Crawley, Western Australia 6009, Australia

⁴ AgriBio, Agriculture Victoria, La Trobe University, Bundoora, Victoria 3083, Australia

⁵ Harry Butler Institute, Murdoch University, Murdoch, Western Australia 6150, Australia

Abstract

The Chattering strain of potato spindle tuber viroid (PSTVd) infects solanaceous crops and wild plants in the subtropical Gascoyne Horticultural District of Western Australia. Classical PSTVd indicator hosts tomato cultivar Rutgers (R) and potato cultivar Russet Burbank (RB) and currently widely grown tomato cultivars Petula (P) and Swanson (S) and potato cultivars Nadine (N) and Atlantic (A) were inoculated with this strain to study its pathogenicity, quantify fruit or tuber yield losses, and establish whether tomato strains might threaten potato production. In potato foliage, infection caused spindly stems, an upright growth habit, leaves with ruffled margins and reduced size, and upward rolling and twisting of terminal leaflets (RB, A, and N); axillary shoot proliferation (A); severe plant stunting (N and RB); and necrotic spotting of petioles and stems (RB). Tubers from infected plants were tiny (N) or small and “spindle shaped” with (A) or without (RB) cracking. Potato foliage dry weight biomass was decreased by 30 to 44% in A and RB and 37% in N, whereas

tuber yield was diminished by 50 to 89% in A, 69 to 71% in RB, and 90% in N. In tomato foliage, infection caused epinasty and rugosity in apical leaves, leaf chlorosis, and plant stunting (S, P, and N); cupped leaves (S and P); and reduced leaf size, flower abortion, and necrosis of midribs, petioles, and stems (R). Mean tomato fruit size was greatly decreased in all three cultivars. Tomato foliage dry weight biomass was diminished by 40 to 53% (P), 42% (S), and 37 to 51% (R). Tomato fruit yield was decreased by 60 to 76% (P), 52% (S), and 64 to 89% (R), respectively. Thus, the tomato strain studied was highly pathogenic to classical indicator and representative current tomato and potato cultivars, causing major losses in fruit and tuber yields. Tomato PSTVd strains, therefore, pose a threat to tomato and potato industries worldwide.

Keywords: biological properties, biosecurity, economic impacts

Viroids are the smallest plant pathogens known. They vary widely in the host species that they infect, the types and severities of the diseases that they cause, and their epidemiology and pathogenicity (Garnsey and Randles 1987; Randles 2003). *Potato spindle tuber viroid* (PSTVd) is the type member of the *Pospiviroid* genus in the viroid family *Pospiviridae* (Diener 2001; Flores et al. 2000). PSTVd and three other species of the *Pospiviroid* genus infect tomato (*Solanum lycopersicum*) plants, but only PSTVd is known to infect potato (*Solanum tuberosum*) plants naturally (Diener 2001; Diener and Raymer 1971; EFSA Panel on Plant Health 2011; Gross et al. 1978; Randles 2003; Rocheleau and Pelchat 2006; Verhoeven et al. 2004). In addition, PSTVd naturally infects pepper and chili (*Capsicum annuum*), pepino (*Solanum muricatum*), eggplant (*Solanum melongena*), and several ornamental crop and wild plant species of the family Solanaceae. Moreover, hosts also occur in other plant families, including the cultivated plants avocado (*Lauraceae*), sweet potato

(*Convolvulaceae*), and *Dahlia* hybrids (*Asteraceae*) (Di Serio 2007; Luigi et al. 2011; Querci et al. 1995; Salazar 1989; Verhoeven et al. 2010a,b). Several additional weed or native plant hosts of PSTVd in the families *Asteraceae*, *Chenopodiaceae*, and *Solanaceae* were found in Australia (Mackie et al. 2016). PSTVd is stable outside infected plant cells, reaches high concentrations in infected plants, and is spread readily from plant to plant by contact transmission involving leaves brushing against each other naturally, plant handling by humans, and healthy plants rubbing against surface-contaminated farm and glasshouse equipment. It also passes readily from plant to plant via infected vegetatively propagated planting material and is seedborne in potato and tomato. New infections mostly result from planting infected seeds of tomato or seed tubers of potato (Benson and Singh 1964; Diener and Raymer 1971; Fernow et al. 1970; Hunter et al. 1969; Kryczyński et al. 1988; Mackie et al. 2015; Manzer and Merriam 1961; Owens and Verhoeven 2017; Salazar 1989; Verhoeven et al. 2010a).

PSTVd symptom expression in tomato and potato plants is influenced not only by its strain and environmental conditions but also, by host species and cultivar (Diener and Raymer 1971; Harris and Browning 1980; O'Brien and Raymer 1964; Pfannenstiel and Slack 1980; Singh et al. 1970). Severe PSTVd strains were first differentiated from mild strains based on symptoms in the classical indicator host tomato cultivar Rutgers (Fernow 1967) and subsequently, also in potato cultivar Russet Burbank (synonym = Netted Gem in Canada) (Singh 1983; Singh et al. 1970). In potato, plants infected with severe PSTVd strains have smaller leaves and an upright habit and are stunted, sometimes severely, whereas mild strains may be asymptomatic. Severe strains diminish tuber number and size and impair their quality through development of spindle-shaped or misshapen tubers, with deep eyes and growth cracks (Pfannenstiel and Slack 1980). Their eyes may be more pronounced and present on

†Corresponding author: S. J. McKirdy; S.McKirdy@murdoch.edu.au

Funding: A. E. Mackie acknowledges financial assistance from a scholarship and operating support from the Plant Biosecurity Cooperative Research Centre and Horticulture Australia Ltd.

*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary table is published online.

The author(s) declare no conflict of interest.

Accepted for publication 15 May 2019.

“knob-like” protuberances that sometimes develop into small tubers (Owens and Verhoeven 2009). In tomato, plants infected with severe strains develop leaf chlorosis, reddening or purpling (which may become severe), reduction in leaf size, and shedding of flowers and fruit, and they become stunted (Ling et al. 2013; Owens and Verhoeven 2009). As with potato, mild strains may be asymptomatic in tomato foliage (Owens and Verhoeven 2009). Severe strains diminish fruit size and sometimes impair their quality through irregular skin coloration (Kryczyński et al. 1988). Disease symptoms are more prevalent in warmer climates, because PSTVd replication and symptoms are enhanced as temperatures increase from 20°C to 37°C and light intensity increases (Grasmick and Slack 1985; Singh 1983; Singh et al. 1971). Under warm conditions, severe PSTVd strains cause major losses in yield and quality of potato tubers and tomato fruits. In field studies, PSTVd diminished potato tuber yields by 17 to 24% with mild strains and up to 64% with severe strains. However, the reduction in tuber weight per plant became more acute with each successive year of infection, causing losses of up to 46, 88, and 97% in the first, second, and third years, respectively (Singh et al. 1971, 1988). Depending on cultivar and strain, in a small-scale pot experiment, PSTVd was reported to decrease the yield of tomato fruit by up to 47% (Kryczyński et al. 1988).

In countries where PSTVd infection of potato has occurred for many years (e.g., in the United States and Canada), seed potatoes are grown under a PSTVd-free production system (Salazar 1989). The viroid is treated as a potato quarantine pest for all North American Plant Protection Organization (NAPPO) countries (NAPPO 2011), and after detailed surveys of their seed potato certification schemes, both Canada and the United States consider it to be eliminated from such schemes (De Boer and DeHaan 2005; Singh 2014; Sun et al. 2004), although PSTVd outbreaks still sometimes occur in tomato in this region (Ling et al. 2013). It is also considered a potato quarantine pest by the European Plant Protection Organization (EPPO) Department of Primary Industries and Regional Development, occurring in some parts of Eastern Europe, but phytosanitary regulations are in place to restrict its spread to others (EPPO 2011). Countries in other world regions also consider PSTVd to be a phytosanitary risk, and in addition to postentry regulations, certification of PSTVd-free status is required before potato importation (e.g., in Australia and New Zealand) (DAWR 2018a, b; MAF 2017; Salazar 1989). In Australia, PSTVd was detected infecting a potato breeding program in New South Wales (NSW) and later eradicated (Schwinghamer et al. 1983). Subsequently, several PSTVd outbreaks occurred in glasshouse- and field-grown tomato, pepper, and chili crops (Hailstones et al. 2003; Mackie et al. 2002, 2016; van Brunschot et al. 2014). Quarantine practices regulated by the Australian Federal (Commonwealth) (DAWR 2018a, b) and State (DPIRD 2018) governments are in place to help exclude it from healthy seed potato production programs. However, although these programs include testing potato plants used to produce the initial minituber generation for PSTVd, only the second of the subsequent generations is PSTVd tested in the Western Australian (WA) program, and such additional testing is lacking from the programs of some other Australian states.

Four PSTVd strains have been described in Australian tomato outbreaks: the Naaldwijk and Chittering strains, common European strain PTVCGA, and a strain previously found in North Carolina, United States (Geering 2017; Mackie et al. 2002, 2016; van Brunschot et al. 2014). After each detection, eradication programs were implemented to protect the potato industry. However, PSTVd is accepted as being established in the subtropical Gascoyne Horticultural District (GHD) in the arid central coastal region of WA (Mackie et al. 2016), which is remote from the nearest Australian potato seed production area in the southwest of WA. However, the GHD is a major tomato fruit supplier to the southwest WA market, especially in winter when it is too cold to grow tomato crops outside, and this market for GHD tomatoes includes WA seed potato production areas. In addition, the GHD sometimes supplies tomatoes to eastern Australian states where seed potatoes are grown.

Inadvertent planting of seed from PSTVd-infected tomato fruit in gardens seems likely to occur occasionally in potato seed-producing areas and risks establishment of local PSTVd infection reservoirs in solanaceous weeds and solanaceous crops (e.g., tomato, pepper, chili, and eggplant). Moreover, when Constable et al. (2019) tested samples from tomato seed lots imported to Australia via the international seed trade, PSTVd was sometimes detected. Inadvertent introduction of PSTVd by this means, therefore, constitutes an additional means by which PSTVd reservoirs could be initiated through sowing contaminated tomato seeds. Regardless of whether they are initiated by sowing contaminated tomato seed from fruit produced nationally or seed supplied by the international seed trade, such PSTVd reservoirs constitute a potential pathway by which tomato PSTVd strains could spread to nearby seed potato crops, including to early seed tuber propagation generations.

We found no evidence that research has been done anywhere in the world to investigate the threat posed by infection of the potato crop with tomato PSTVd strains. Indeed, information was completely lacking on the effects of tomato isolates on potato foliage or its tuber production. Moreover, to the best of our knowledge, only one study has ever attempted to quantify fruit yield losses from PSTVd in tomato (Kryczyński et al. 1988) but without any statistical analysis. This situation needs to be rectified by research that establishes whether PSTVd strains from tomato have the potential to cause damaging losses in potato tuber and tomato fruit production. Such information is relevant not only to Australia but also, to other world regions, where PSTVd could spread from reservoirs initiated by inadvertent sowing of PSTVd-infected tomato seeds to potato crops in potato seed-producing areas.

The aims of this study were to determine the pathogenicity of a PSTVd strain from tomato by infecting (i) the classical PSTVd indicator hosts tomato Rutgers and potato Russet Burbank and (ii) representatives of newer commercial cultivars currently grown widely in Australia and elsewhere: cultivars Atlantic (potato), Nadine (potato), Petula (tomato), and Swanson (tomato). The effects of infection on plant growth (biomass) of both host species and yield and quality of tubers (potato) or fruit (tomato) were quantified, and they were shown to be considerable in both crop species.

Materials and Methods

PSTVd isolate and inoculations. The source of PSTVd inoculum was Chittering strain isolate WAC13195, which was first obtained from PSTVd-infected tomato in the GHD in 2006 (Mackie et al. 2016). This isolate was maintained by sap inoculation to tomato cultivar Grosse Lisse plants. For inoculation, PSTVd-infected Grosse Lisse sap was extracted in 0.1 M phosphate buffer, pH 7.2, by crushing infected tomato leaves with a leaf press (Pollahne). The extract was filtered through gauze and mixed with celite before being rubbed onto leaves (Hull 2009). Control plants were mock inoculated with healthy tomato sap.

Pathogenicity experiments. All four experiments were undertaken in the same insect-proof, air-conditioned, quarantine level QC2 standard glasshouse at the University of WA, which was maintained at 26 to 28°C. Experiments A and B were done in 2011, whereas experiments C and D were undertaken in 2013. They relied solely on natural light, and therefore, light intensity and day length varied depending on the time of year that each was done, the climate outside the glasshouse being Mediterranean type (i.e., hot dry summers and warm wet winters). Relative humidity also varied depending on the weather and closeness in time to watering of plants, but it generally ranged from 63 to 89%. The plants were grown in steam-sterilized soil, sand, and peat mix (1:1:1) in 45-liter “grow bags” placed on the floor (potato) and 20-liter pots (tomato) placed on the benches. The potato tubers planted came from pathogen-tested stock, which was virus and viroid free. Experiments A and B were conducted to establish the pathogenicity of Chittering strain PSTVd isolate WAC13195 toward potato Atlantic, Nadine, and Russet Burbank. Experiment C was similar but was with tomato Petula, Rutgers, and Swanson instead of potato. Experiment D resembled experiment C except that Swanson was absent. In experiments A, B, and C, five

plants of each cultivar were sap inoculated as described above. In experiment D, there were 10 plants of each cultivar. For controls, 5 (experiments A, B, and C) or 10 plants (experiment D) of each cultivar were mock inoculated with healthy tomato sap. Within each experiment, the four different treatments (i.e., mock or infected $\times$ cultivar) were arranged in a randomized block design on the floor (experiments A and B) or in the bench (experiments C and D). After 28 days postinoculation, tip leaf samples from each plant were tested individually for PSTVd presence by reverse transcription PCR (RT-PCR). The durations of the experiments were 10 (experiments B and D) and 12 weeks (experiments A and C).

Disease assessment. With experiments A and B, the potato foliage symptoms on each plant were assessed weekly for 10 weeks. On each assessment day, the plants were given a score of 0 to 4, where 0 = no symptoms, 1 = leaves darker in color, 2 = upright growth and twisted terminal leaflets, 3 = leaves with ruffled margins and reduced size, and 4 = necrotic spotting on petioles and main stems as well as plant stunting. The plants were then allowed to senesce and dry naturally. All of the dried leaves and stems from each senesced potato plant were removed, placed into brown paper bags (one per plant), and weighed. The tubers from each potato plant were then harvested, washed clean of soil, and left to dry. Tuber weight, shape, and number were recorded for each plant, and all tubers were assessed for visible PSTVd symptoms. With experiments C and D, the tomato foliage symptoms on each plant were also assessed weekly for 10 weeks. On each assessment day, they were given a score of 0 to 4, where 0 = no symptoms; 1 = epinasty and rugosity of apical leaves; 2 = growth reduction, chlorosis in upper leaves, and leaf cupping; 3 = necrosis of leaflet midribs, petioles, and stems and reddening or purpling of leaves; and 4 = stunted, shortened internode length and apical leaves small and compacted. Assessments of plant height and weight and fruit weight were done for each plant. The height above soil level of each tomato plant was measured at the seven truss stage, and its foliage (stems and leaves) and fruit were removed and weighed to obtain fresh weight values. The fruits were then assessed for visible signs of PSTVd symptoms, and the foliage was dried as described above before being reweighed to obtain dry weight values.

RT-PCR. To test for PSTVd infection, RNA was extracted from the leaf samples using a QIAGEN RNeasy Plant Mini Kit (QIAGEN) following the manufacturer's instructions. Sample extracts were then tested individually for PSTVd by RT-PCR using the CT20(R)/TG21(F) PSTVd-specific primers of Mackie et al. (2002). For RT-PCR, leaf samples from tomato plants infected with the WAC13195 isolate were used as the positive control, and leaf samples from a healthy tomato plant were used as a negative control. RT-PCR was done using a one-step RT-PCR kit (Invitrogen), and the reaction was in a final volume of 13 μ l containing 0.25 μ l of each primer (100 μ M stock solution) and 1.75 μ l of RNA extract. PCR amplification conditions were as follows: 48°C for 45 min and 94°C for 2 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s and a final elongation step of 72°C for 5 min. Temperature was held at 14°C after completion of PCR cycling. PCR amplicons were visualized by electrophoresis on 1.5% agarose gels stained with ethidium bromide.

Data analysis. In all experiments, single-factor analysis of variance (ANOVA) by GenStat (18th Edition, GenStat Procedure Library Release PL23.2; <https://www.vsnl.co.uk/>) was applied to analyze the disease assessment, biomass, and yield data from PSTVd-infected and mock-inoculated healthy control plants for tomato and potato cultivars (i.e., to evaluate PSTVd-infected cultivar treatments). In experiments A and C, no PSTVd was ever detected in any samples from mock-inoculated control plants. However, in experiments B and D, it was detected. Any PSTVd-contaminated control plants removed from experiments B and D were managed appropriately as missing data within these particular ANOVAs. Means of separation using Fisher's least significant differences ($\alpha = 0.05$) were performed to establish the significance of treatment differences. We used Fisher's least significant differences, because these provide a statistical significance test that is valid for all sample

sizes and allows the significance of the deviation from a null hypothesis (e.g., P value) to be calculated exactly.

Results

Tests for PSTVd presence. In experiments A to D, PSTVd was detected in all samples collected from the plants inoculated. However, in experiments B and D, possible PSTVd symptoms were observed in 7 of 35 mock-inoculated control plants up to week 6, and these plants were removed. In experiments A and C, no symptoms were observed in mock-inoculated plants, and no PSTVd was ever detected.

Potato foliar symptoms. In experiments A and B, all three potato cultivars developed foliage symptoms owing to PSTVd infection. Compared with healthy plants, PSTVd-infected plants of Russet Burbank and Nadine were severely affected, and Atlantic plants moderately affected. Foliage symptoms of spindly stems, an upright growth habit, leaves with ruffled margins and reduced size, and upward rolling and twisting of terminal leaflets were common to all three cultivars (Fig. 1A to C). Atlantic, in which these symptoms were milder, also showed proliferation of axillary shoots. Infected plants of Nadine and Russet Burbank were severely stunted (Fig. 1D to H), and the Russet Burbank plants also developed necrotic spots on petioles and stems.

In experiment A, from the second week after inoculation onward, the weekly disease assessment ranking values for PSTVd-infected plant foliage were always significantly greater ($P < 0.001$) than those for the mock-inoculated control plants (Table 1). They were also always significantly greater in PSTVd-infected foliage than in control plant foliage of the same cultivar. The disease ranking values for infected plant foliage continued to increase in magnitude until 5 (Russet Burbank) and 7 weeks (Nadine) after inoculation with PSTVd before becoming relatively stable. With Atlantic, they continued to increase gradually throughout the 10-week assessment period. The disease assessment values for PSTVd-infected Nadine and Russet Burbank were not significantly different from each other on eight occasions, but they were significantly different on the two others (weeks 3 and 7), with Russet Burbank showing more severe symptoms in week 3 and Nadine showing more severe symptoms in week 7. With Atlantic, these values were always significantly smaller than those for the other two cultivars, except on week 10.

Overall, in experiment B, the foliage symptoms that developed in experiment B were slower to appear and less severe than in experiment A (Table 2). From the fourth week after inoculation onward, the overall disease assessment ranking values for PSTVd-infected plant foliage were always significantly greater ($P = 0.003$ or < 0.001) than those for the mock-inoculated control plants. Also, as in experiment A, they were always significantly greater for PSTVd-infected foliage than control plant foliage of the same cultivar. In addition, the disease assessment ranking values continued increasing in magnitude until 6 (Atlantic) or 7 weeks (Nadine and Russet Burbank) after inoculation, remaining relatively stable thereafter (Atlantic and Nadine). Between the sixth and ninth weeks, the score values for PSTVd-infected Atlantic were always significantly smaller than those for Russet Burbank and Nadine. In contrast, for weeks 5 to 9, those for PSTVd-infected Nadine and Russet Burbank were not significantly different from each other, only being significantly different on week 10 when the Nadine disease assessment value significantly exceeded that for Russet Burbank.

Potato tuber symptoms. Tubers from infected Russet Burbank plants were small, spindle shaped, and misshapen with deep eyes and knob-like protuberances; infected Atlantic plants produced small, spindle-shaped, severely cracked tubers with deep eyes and knob-like protuberances, and infected Nadine plants produced clusters of tiny "microtubers" (each microtuber ~ 3 mm in length) (Fig. 1G to I).

Potato foliage dry weight and tuber yields. In experiments A and B, the overall values for PSTVd-infected plant foliage and tuber weights were always significantly smaller than those for mock-inoculated control plants ($P = 0.04$ or < 0.001) (Table 3). Also, all comparisons between PSTVd-infected and mock-inoculated control plants of the same cultivar revealed statistically different mean values, except for one foliage dry weight comparison (Nadine in experiment A). In experiment A with both Atlantic and Russet Burbank,

the statistically significant foliage dry weight loss value from PSTVd infection was 30%. In experiment B, it was 44% for Russet Burbank and Atlantic and 37% for Nadine. PSTVd infection diminished tuber yield of Russet Burbank, Atlantic, and Nadine by 71, 89, and 90% in

experiment A, respectively, and 69, 50, and 90% in experiment B, respectively. Based on the tuber symptoms from the infected plants described above, none of these PSTVd-infected potato cultivars produced any marketable tuber yield.

Fig. 1. The effects of infection with potato spindle tuber viroid Chattering strain on foliage and tubers of potato plants. **A**, Symptoms of upright growth habit, leaves with reduced size, and leaflets with upward rolling and twisting in cultivar Russet Burbank. **B**, Apical shoot symptoms of upright growth and upcurled leaves in Russet Burbank. **C**, Close up of upcurled leaf lamina symptoms in apical leaves of cultivar Nadine. **D**, Severe stunting in the plant of Russet Burbank (left) compared with the healthy plant (right). **E**, Moderate stunting in the plant of cultivar Atlantic (left) compared with the healthy plant (right). **F**, Severe stunting in the plant of Nadine (left) compared with the healthy plant (right). **G**, Small, spindly, misshapen Russet Burbank tubers with deep eyes and “knob-like protuberances” (right) and a healthy tuber (left). **H**, Small, spindly, misshapen, and severely cracked Atlantic tubers with deep eyes and knob-like protuberances (right) and a healthy tuber (left). **I**, Clusters of tiny Nadine “microtubers” about 3 mm in length (right) and a healthy tuber (left).

Table 1. Mean disease assessment rankings for foliage of potato cultivars Russet Burbank, Atlantic, and Nadine infected with potato spindle tuber viroid in experiment A

Cultivar	Week ^a								
	2	3	4	5	6	7	8	9	10
Atlantic infected ^b	0.8	0.6	1.8	2.8	2.6	2.0	3.0	3.0	3.4
Atlantic healthy	0	0	0	0.6	0.6	0	0	0	0
Nadine infected	1.0	1.4	3.0	3.8	3.8	4.0	3.6	4.0	4.0
Nadine healthy	0	0	0	0	0	0	0	0	0
Russet Burbank infected	1.0	2.0	3.2	3.8	3.6	3.0	3.6	3.8	3.6
Russet Burbank healthy	0.2	0.2	0.4	1.6	0	0	0	0	0
Significance <i>P</i> value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>L.s.d.</i> ^c	0.33	0.52	0.70	0.87	0.88	0.50	0.41	0.24	0.89
<i>df</i> ^c	20	20	20	20	20	20	20	20	20

^a The data were collected as mean weekly foliage disease assessment rankings.
^b Disease assessment rankings: 0 = no symptoms, 1 = leaves darker in color, 2 = upright growth and twisted terminal leaflets, 3 = leaves with ruffled margins and reduced size, and 4 = necrotic spotting on petioles and main stems and severe plant stunting.
^c *L.s.d.* indicates least significance difference at *P* < 0.05; *df* indicates degrees of freedom. Means separation was conducted using Fisher’s *L.s.d.* (α = 0.05) to establish the significance of treatment differences.

Tomato foliar symptoms. In experiment C, Rutgers showed the most severe foliar symptoms, Petula was moderately to severely affected, and Swanson was moderately affected. When compared with healthy plants of the same cultivar, infection caused symptoms of epinasty and rugosity in apical leaves, leaf chlorosis, and plant stunting in all three cultivars; cupped leaves in Swanson and Petula; and reduced leaf size, flower abortion, and necrosis of midribs, petioles, and stems in Rutgers (Fig. 2A to F). In experiment D, symptoms were milder than those observed in experiment C. Both Petula and Rutgers developed leaf epinasty and rugosity. Symptoms were most severe in

Rutgers, which also developed chlorosis in upper leaves and cupping of apical leaves.

In experiment C, maximum disease assessment values were reached between weeks 4 and 10 depending on tomato cultivar (Table 4). From week 3 onward, all PSTVd-infected cultivar treatments had weekly disease assessment values significantly greater than those of mock-inoculated control plants of the same cultivar ($P < 0.001$). In addition, the disease assessment ranking values continued increasing in magnitude until 4 (Rutgers), 8 (Swanson), or 10 weeks (Petula) after inoculation. In weeks 4 to 9, the values for

Table 2. Mean disease assessment rankings for foliage of potato cultivars Russet Burbank, Atlantic, and Nadine infected with potato spindle tuber viroid in experiment B

Cultivar	Week ^a							
	3	4	5	6	7	8	9	10
Atlantic infected ^b	0.4	1.4	1.6	2.2	2	2.2	2.2	2.2
Atlantic healthy	0	0	0	0	0	0	0	0
Nadine infected	0.8	1.4	2.0	2.75	3.0	2.8	2.8	2.8
Nadine healthy	0	0	0	0	0	0	0	0
Russet Burbank infected	0.6	1.4	2.4	2.8	3.0	2.6	2.8	2.0
Russet Burbank healthy	0	0	0	0	0	0	0	0
Significance <i>P</i> value	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.001
<i>L.s.d.</i> ^c	0.46	0.59	0.56	0.38	0.40	0.42	0.38	0.52
<i>df</i> ^c	18	18	18	18	18	18	18	18

^a The data were collected as mean weekly foliage disease assessment rankings.

^b Disease assessment rankings: 0 = no symptoms, 1 = leaves darker in color, 2 = upright growth and twisted terminal leaflets, 3 = leaves with ruffled margins and reduced size, and 4 = necrotic spotting on petioles and main stems and severe plant stunting.

^c *L.s.d.* indicates least significance difference at $P < 0.05$; *df* indicates degrees of freedom. Means separation was conducted using Fisher's *L.s.d.* ($\alpha = 0.05$) to establish the significance of treatment differences.

Table 3. Effects of potato spindle tuber viroid infection on foliage dry weight and tuber yield of potato cultivars Russet Burbank, Atlantic, and Nadine in experiments A and B

Cultivar	Mean foliage dry weight per plant		Mean tuber weight per plant	
	Experiment A	Experiment B	Experiment A	Experiment B
Russet Burbank infected	87.4 (30%)	55.1 (44%)	112 (71%)	58.3 (69%)
Russet Burbank healthy	124.8 ^a	98.5	388	241.2
Atlantic infected	90.8 (30%)	69.7 (44%)	74 (89%)	109.2 (50%)
Atlantic healthy	130.4	120.3	677	219.0
Nadine infected	81.8 (21%)	64.0 (37%)	65 (90%)	16.1 (90%)
Nadine healthy	103.5	101.6	652	158.8
Significance <i>P</i> value	0.04	<0.001	<0.001	<0.001
<i>L.s.d.</i> ^b	35.29	19.11	235.9	34.87
<i>df</i> ^b	20	18	20	18

^a Data are mean weight per plant in grams. Numbers in parentheses are percentage yield losses.

^b *L.s.d.* indicates least significance difference at $P < 0.05$; *df* indicates degrees of freedom. Means separation was conducted using Fisher's *L.s.d.* ($\alpha = 0.05$) to establish the significance of treatment differences.

Table 4. Mean disease assessment rankings for foliage of tomato cultivars Rutgers, Swanson, and Petula infected with potato spindle tuber viroid in experiment C

Cultivar	Week ^a							
	3	4	5	6	7	8	9	10
Petula infected ^b	2.0	3.0	3.0	3.0	2.8	3.0	2.8	3.2
Petula healthy	0	0	0	0	0	0	0	0
Swanson infected	1.6	2.0	2.4	2.4	2.4	2.8	2.6	2.4
Swanson healthy	0	0	0.4	0.2	0	0	0	0
Rutgers infected	1.8	4.0	4.0	4.0	4.0	4.0	4.0	3.2
Rutgers healthy	0	0	0.4	0	0	0.4	0	0.6
Significance <i>P</i> value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
<i>L.s.d.</i> ^c	0.58	0.54	0.75	0.40	0.37	0.58	0.40	0.95
<i>df</i> ^c	20	20	20	20	20	20	20	20

^a The data were collected as mean weekly foliage disease assessment rankings.

^b Disease assessment rankings: 0 = no symptoms; 1 = epinasty and rugosity of apical leaves; 2 = growth reduction, chlorosis in upper leaves, and leaf cupping; 3 = necrosis of leaflet midribs, petioles, and stems and reddening or purpling of leaves; and 4 = stunted, shortened internode length and apical leaves small and compacted.

^c *L.s.d.* indicates least significance difference at $P < 0.05$; *df* indicates degrees of freedom. Means separation was conducted using Fisher's *L.s.d.* ($\alpha = 0.05$) to establish the significance of treatment differences.

PSTVd-infected Swanson were always significantly smaller than those for infected Rutgers. Those for infected Petula were significantly smaller than those of Rutgers in weeks 4 to 9 and significantly greater than those of Swanson on weeks 4 to 7. Overall, in experiment D, symptoms were slower to appear and milder than in experiment C, with maximum mean disease assessment values for each cultivar being reached in weeks 7 (Petula) and 8 (Rutgers) (Supplementary Table S1). In weeks 4 to 7, there were significant differences between overall mean symptom assessment values ($P = 0.01$ to < 0.001). In addition with Rutgers, in weeks 4 to 7, the assessment values for PSTVd-infected plants were always significantly greater than those for control plants. With Petula, this was so only for weeks 6 and 7.

Tomato fruit symptoms. Average fruit size was smaller with infected plants of Petula and Swanson, whereas fruit from infected Rutgers was both smaller and malformed (Fig. 2G to L). No other fruit symptoms were recorded.

Tomato foliar dry and fresh weights and fruit yields. In experiments C and D, the overall foliage fresh and dry weights and fruit weight comparisons between PSTVd-infected and mock-inoculated control plants always provided statistically different mean values ($P < 0.001$) (Table 5). In all instances, for each cultivar, the PSTVd-infected weight values were significantly smaller than those for the control plants. In experiment C, the foliage fresh weight losses owing to PSTVd infection were 53% for Petula and 42% for Swanson and Rutgers, whereas in experiment D, they were 19% for Petula

Fig. 2. The effects of infection with potato spindle tuber viroid Chattering strain on foliage and fruit of tomato plants. **A**, Symptoms of veinal necrosis in a leaf of cultivar Rutgers. **B**, Leaf curling symptoms. **C**, Reduction of leaf size symptoms in apical leaves. **D**, Stunting in the plant of cultivar Petula (left) compared with a healthy plant (right). **E**, Stunting in the plant of cultivar Swanson (left) compared with a healthy plant (right). **F**, Stunting in the plant of Rutgers (left) compared with a healthy plant (right). **G**, Mostly small-sized fruits harvested from an infected plant of Petula. **H**, Mostly small-sized fruits harvested from an infected Swanson plant. **I**, Mostly small-sized and malformed fruits harvested from an infected Rutgers plant. **J**, Fruits harvested from a healthy plant of Petula. **K**, Fruits harvested from a healthy plant of Swanson. **L**, Fruits harvested from a healthy plant of Rutgers.

Table 5. Effects of potato spindle tuber viroid on fresh and dry foliage weights and fruit yields of tomato cultivars Rutgers, Petula, and Swanson in experiments C and D^a

Cultivar	Mean foliage fresh weight per plant		Mean foliage dry weight per plant		Mean fruit weight per plant	
	Experiment C	Experiment D	Experiment C	Experiment D	Experiment C	Experiment D
Petula infected	424 (53%)	500 (19%)	137 (53%)	185 (40%)	157 (76%)	350 (60%)
Petula healthy	899	616	283	307	655	882
Swanson infected	425 (42%)	—	147 (42%)	—	214 (52%)	—
Swanson healthy	727	—	252	—	628	—
Rutgers infected	483 (42%)	369 (40%)	162 (37%)	133 (51%)	48 (89%)	352 (64%)
Rutgers healthy	833	614	257	269	443	969
Significance P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
l.s.d. ^b	243.4	114.4	86.4	58.5	143.0	150.5
df ^c	20	24	20	24	20	24

^a Data are mean weight per plant in grams. Numbers in parentheses are percentage yield losses. — = Swanson absent from Experiment D.

^b l.s.d. indicates least significance difference at $P < 0.05$. Means separation was conducted using Fisher's l.s.d. ($\alpha = 0.05$) to establish the significance of treatment differences.

^c df indicates degrees of freedom.

and 40% for Rutgers. Foliage dry weight losses owing to PSTVd infection were 53 (Petula), 42 (Swanson), and 37% (Rutgers) in experiment C, and in experiment D, they were 40 (Petula) and 51% (Rutgers). Fruit yield losses owing to PSTVd infection were 76 (Petula), 52 (Swanson), and 89% (Rutgers) in experiment C and 60 (Petula) and 64% (Rutgers) in experiment D. Based on their small size, none of the infected tomato cultivars produced any marketable fruit yield in experiments C and D.

Discussion

This study showed for the first time that a tomato strain of PSTVd can be highly pathogenic not only to a classical PSTVd indicator cultivar of potato but also, to representatives of widely grown potato cultivars. Also, for the first time, it quantified PSTVd-induced losses in tomato biomass (dry weight) and fruit yields using statistical analysis and demonstrated that a tomato PSTVd strain can significantly decrease potato plant biomass and tuber yields. The magnitude of the PSTVd-induced yield losses was considerable regardless of cultivar or crop: 50 to 90% for potato tubers and 52 to 89% for tomato fruit. Moreover, PSTVd infection considerably diminished the size of tomato fruits and potato tubers, and potato tuber quality was severely compromised. In tomato, the classic PSTVd indicator host used (Rutgers) developed more severe foliage symptoms than the more commonly grown cultivars. In potato, the classic indicator host used (Russet Burbank) and cultivar Nadine both developed severe PSTVd foliage symptoms. However, more severe foliage symptoms did not necessarily translate into greater losses in biomass or yields in either crop. Because PSTVd outbreaks arising from sowing infected tomato seeds are occurring at an increasing frequency globally, these findings are cause for concern for warmer world regions first where tomato crops are widely grown and second where these crops are planted in close proximity to potato crops.

Spread of virus or viroid infection to control plants constitutes a hazard in studies examining their yield-limiting potential (Bos 1982), and this is especially so when the pathogen involved is readily contact transmitted and highly contagious as with PSTVd (Diener and Raymer 1971; Mackie et al. 2015; Owens and Verhoeven 2017). By maintaining wide separation between individual plants, always mock inoculating control plants before inoculating plants with PSTVd, using disposable gloves, and otherwise, minimizing plant handling, it was possible to avoid PSTVd contamination of control plants in experiments A (with potato) and C (with tomato). However, some control plants became contaminated in experiments B (with potato) and D (with tomato). Nevertheless, when the contaminated plants were accounted for in the statistical analysis of foliage dry weight and yield data from these two experiments, statistically significant differences ($P \leq 0.05$) were still obtained with all combinations of cultivar and crop plant. Moreover, the magnitude of these losses was similar to those obtained in experiments A and C. The only exception to this was in experiment B with dry weight biomass, which PSTVd infection did not diminish significantly in potato Nadine. The source of the PSTVd contamination could only have been the PSTVd-infected plants within each experiment, and the most likely reason for contamination was contact transmission resulting from touching infected plants before healthy control plants within the same replicate during daily watering or regular application of liquid fertilizer. In all other routine operations during the experiments, such as staking and tying up plants, the mock-inoculated plants were always attended to before the PSTVd-inoculated plants. Because there was PSTVd contamination in both of the 2013 experiments but neither of the 2011 experiments, there was apparently less touching of plants during watering and liquid fertilizer application in the earlier year. Those conducting future PSTVd experiments of this type are advised to pay special attention to avoiding any occurrence of such contaminations.

For each experiment, using weekly disease assessment rankings of 0 to 4 for foliage symptom expression after PSTVd infection of different potato and tomato cultivars and analyzing these data by ANOVA proved an effective way to establish the timing of symptom appearance and compare the relative extents of symptom severity expressed by different cultivars. The weekly disease assessment

ranking values were significantly greater than those for the mock-inoculated control plants by weeks 2 and 3 in experiments A and B with potato and weeks 2 and 4 in experiments C and D with tomato. Thus, symptom appearance occurred fastest in experiments A and C and slowest in experiment D. In potato Nadine and Russet Burbank, symptoms reached maximum rankings of 3.8 to 4.0 and 3.0 in experiments A and B, respectively, but the corresponding figures for Atlantic were 3.4 (experiment A) and 2.2 (experiment B). In tomato Rutgers, they reached 4.0 in experiment C but only 2.9 in experiment D, and the corresponding figures for tomato Petula and Swanson were 2.8 to 3.2 in experiment C, and 2.0 for Petula in experiment D. Thus, potato Nadine and Russet Burbank expressed more severe symptoms than Atlantic, and symptoms in tomato were most severe in Rutgers and least severe in Swanson. The poorer PSTVd symptom expression in the experiments done in 2013 (experiments B and D) than in those in 2011 (experiments A and C) seems most likely because of different environmental conditions related to the season of the year when each experiment was performed. Because the glasshouse temperature remained much the same all year because of the air conditioning, variation in day length and light intensity constitute the most likely reasons for these symptom differences. Thus, because the poorest symptom expression was in experiment D, this experiment most likely experienced the shortest day lengths and lowest light intensities, whereas experiments A and C experienced the opposite, and experiment B experienced intermediate conditions.

Air temperature has a profound effect on the severity of foliage symptoms caused by PSTVd (Harris and Browning 1980; Morris and Smith 1977). This is because PSTVd multiplies very rapidly at high temperatures in PSTVd-infected host plants, causing more severe symptoms, whereas its rate of multiplication at low temperatures can be so low that it becomes eliminated from infected plants (Lizarraga et al. 1980). PSTVd symptom severity and rate of spread in tomato plants in Australian commercial glasshouses, where temperatures are maintained between 20 and 30°C, differed from those in the open field in the GHD, where average temperatures vary between 11 and 32°C (BOM 2018). Under commercial glasshouse conditions in NSW, infected tomato plant foliage showed symptoms of epinasty, rugosity and lateral twisting of new leaflets, necrosis, and stunting (Hailstones et al. 2003). PSTVd-infected plants growing in glasshouses in the great southern region of WA were also stunted and spindly with severe leaf yellowing and in some cases, a purple tinge; their leaflets were puckered, and smaller basal leaflets were yellow and curled (Mackie et al. 2002). In comparison, tomato plants infected with PSTVd under field conditions in the GHD were occasionally stunted with mild leaf chlorosis but otherwise, asymptomatic. This suggested that either the Chittering PSTVd strain present in the GHD was mild or that symptom expression was being modified by climatic or cultural factors. In our study under glasshouse conditions, all three tomato cultivars infected with the Chittering strain showed symptoms of PSTVd infection, the most severe developing in Rutgers. These foliage symptoms expressed in tomato plants were, therefore, similar to those seen in previous PSTVd detections in glasshouse-grown tomatoes in WA. The diminished fruit size symptom caused by infection with Chittering PSTVd strain in tomato was also similar to those seen in previous detections in WA, with the exception of the 2001 detection, where uneven ripening of fruit was also present.

The effects of infection of potato cultivar Saco with mild or severe PSTVd strains were studied by Singh et al. (1971), who found in a field experiment that three mild strains reduced tuber yield by 17 to 25% and that a severe strain reduced yield tuber by 64%. Pfannenstiel and Slack (1980) and Verhoeven et al. (2004) found that disease severity in potatoes increased with each successive year of infection. Their studies also found that foliar symptoms appeared in all cultivars tested, regardless of PSTVd strain. In Russet Burbank, tuber weight decreased and tuber symptoms increased when infected with PSTVd severe strain, and although infection with PSTVd mild strain did not decrease tuber weight, tuber symptoms were more pronounced (Pfannenstiel and Slack 1980). In our study, the PSTVd Chittering strain from tomato infected Russet Burbank, Nadine,

and Atlantic, and all three potato cultivars developed foliar symptoms. As with previous studies (Pfannenstiel and Slack 1980; Verhoeven et al. 2004), the decrease in tuber weight after infection resulted primarily from reduction in size. In our study, Atlantic and Nadine infected with PSTVd Chittering strain developed even more severe tuber symptoms than those that occurred with Russet Burbank. Russet Burbank produced typical spindle-shaped tubers, but in Atlantic, in addition to these symptoms, a high frequency of growth cracks was present. By contrast, a large number of tiny tubers formed in Nadine. Previously, infection with a severe strain of PSTVd caused the same tiny tuber symptom in cultivar Norgold Russet that we obtained with the Chittering strain in Nadine (Pfannenstiel and Slack 1980). PSTVd symptom expression is likely to be more obvious under heated glasshouse conditions, because the 24-h temperature average is more likely to be above 20°C than in the field. Therefore, under field conditions, PSTVd symptoms are likely to be less severe, especially in the cooler potato-growing areas of temperate world regions. As mentioned above, in our study, symptoms were less severe in experiments B and D than in experiments A and C. Because experiments A and C were undertaken in a different year from earlier experiments A and C, cooler glasshouse conditions may account for the milder symptom expression in the latter, especially in experiment D.

Globally, potato production is increasing in subtropical and tropical regions of the world. This increase is being driven by global warming and the need to provide a year-round supply of staple food crops, such as potato, to address food insecurity in developing countries arising from rapid population increase and climate change (Godfray et al. 2010; Hammond 2014; Tschamtkke et al. 2012; von Braun 2009). As potato and tomato production in world regions with warmer climates continues to increase, it is likely that the detrimental impact of PSTVd infection on tomato fruit yields and potato tuber yield and quality will increase in such countries. Therefore, driven by the threat that it poses to both seed and ware potato and tomato production, the worldwide quarantine status of PSTVd should be maintained. For example, biosecurity regulations in Australia still maintain PSTVd as a significant quarantine pest, although it is accepted as being established in one remote region of the country, the GHD. In addition, our research provides scientific data that support the need for future biosecurity import risk assessments involving solanaceous plant products crossing regional, state, or international borders.

Acknowledgments

The University of Western Australia and Department of Primary Industries and Regional Development for Western Australia provided operational support and both controlled environment glasshouse and laboratory support. We thank Stuart Vincent, Robert Creasy, and Bill Piasini for assisting with plant propagation and other routine glasshouse activities; Brenda Coutts for assistance with statistical analysis; and Ian McPharlin, Peter Dawson, and Andrew Taylor for supplying healthy potato tubers and providing potato management advice.

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