



**Cooperative Research Centre
for National Plant Biosecurity**

Final Report

**CRC10164 - Phylogeny, pathogenicity
and epidemiology of potato spindle
tuber viroid (PSTVd) and related
pospiviroids in Australia**

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Table of contents

1. Executive Summary	4
2. Aims and objectives	5
3. Key findings	7
4. Implications for stakeholders.....	11
5. Recommendations.....	12
6. Abbreviations/glossary.....	13
7. Plain English website summary	13

1. Executive Summary

Potato growing areas of Australia are currently considered free of Potato Spindle Tuber Viroid (PSTVd) and this pathogen is classified as an emergency plant pest (Category 3) under the Emergency Plant Pest Response Deed (EPPRD). There have been 6 emergency responses for PSTVd in Western Australia (WA) and New South Wales (NSW) in the six years preceding commencement of this project, and the concern was that without any pathway control there would continue to be periodic detections of PSTVd in the future. The importation of tomato seed, which until June 2008 was unregulated, had been implicated as the likely source of PSTVd. However, research at that time from Europe identified alternative hosts of PSTVd as potentially the source of inoculum for PSTVd infected tomatoes. Moreover, research then in WA indicated that non-solanaceous species might be hosts.

Prior to this project, three strains of PSTVd had been detected in outbreaks of PSTVd in Australia in the preceding six years. The “Naaldwijk strain” was detected in tomatoes in 2001 and a survey of tomatoes in NSW detected a common European strain (PTVCGA). The last three outbreaks in WA since 2004 have been of the “Chittering strain” of PSTVd and the initial detection was in tomatoes. At that time, it was unclear as to what the source of inoculum for these outbreaks was, by what pathway the pathogen reached tomato crops and what the impact of these PSTVd strains could potentially have on tomato fruit yield and quality under Australian conditions. In a subsequent WA incursion of PSTVd in 2007, the pathogen was also detected in a host plant other than tomato. That finding raised the question as to whether Australia has a potential natural reservoir of PSTVd from which it is moving on to tomatoes when planted in close proximity.

There are six different pospiviroids known outside Australia that infect solanaceous plants naturally and all are closely related to PSTVd. Prior to this project, the concern was that these viroids had never been tested for in Australia and they might also be implicated in the PSTVd recent incursions. The pathogenicity of the PSTVd strains and/or related pospiviroids on potatoes in particular, and to a lesser extent on tomatoes, was not known prior to this project. Other aspects of the epidemiology of PSTVd were also poorly understood, such as how contact transmission occurred in tomato crops, the temporal and spatial dynamics of epidemics in the field, the stability of PSTVd inoculum infectivity on different surfaces and the role of seed in spreading the pathogen. A better understanding of such parameters would greatly influence containment and eradication strategies for this Emergency Plant Pest (EPP). This project has addressed these above concerns and the main findings from this project are as follows:

i. Finding 1

Surveys in WA detected PSTVd in Blackberry Nightshade, Annual Saltbush and volunteer Tomato, Capsicum and Thornapple plants confirmed for the first time that diverse solanaceous hosts and even non-solanaceous hosts can harbour PSTVd. An undetermined Pospiviroid was detected in both Thorny Saltbush and Annual Saltbush. These surveys have extended the known range of potential host species for PSTVd and established a host range for the undetermined Pospiviroid.

ii. Finding 2

This project has provided the critical knowledge of genetic relationships between the strains of PSTVd that occur in Australia, with five of the first 12

samples testing positive for PSTVd. PSTVd specific sequences for these isolates (181, 189, 209, 212 and 217) have now been obtained. A further two isolates were weakly positive for the Pospiviroid RT-PCR test and negative for both the PSTVd specific RT-PCR tests. Of a subsequent eight additional samples screened for PSTVd and pospoviroids, while all were negative for PSTVd for both RT-PCR tests, four samples (viz. 259, 269, 280 and 283) produced a faint band with the Pospiviroid PCR and cloning and sequencing yielded Pospiviroid specific sequence for one isolate (269) and this sequence was most closely related to PSTVd. These results confirm, for the first time, the likely presence of an additional Pospiviroid species in Australia.

iii. *Finding 3*

This project has, for the first time, provided an understanding of the pathogenicity of Australian PSTVd isolates and the first clarity in relation to the role infected alternative hosts likely play in the epidemiology of PSTVd incursions on tomatoes and in relation to the potential impact of this EPP on the Australian potato and tomato industries.

iv. *Finding 4*

This project has demonstrated a clear and differential role for PSTVd surviving on contaminated materials (e.g., contaminated metal remained infective after 1 hour; contaminated cotton remained infective after 6 hours; and contaminated wood, plastic and leather remained infective after 24 hours). Importantly, sodium hypochlorite and milk powder were demonstrated to be effective disinfectants in suppressing PSTVd infection, whilst also disrupting the viroid RNA so that it could not be detected by RT-PCR.

v. *Finding 5*

This project has made substantial contributions towards the development of International Plant Protection Convention (IPPC) PSTVd diagnostic protocol currently under review by the IPPC. Upon approval by the IPPC, this diagnostic protocol will be submitted to the Australian Sub-committee for Plant Health Diagnostic Standards Australian (SPHDS) for acceptance as the national diagnostic protocol for PSTVd detection across Australia.

2. Aims and objectives

- i. Investigate the relationships between PSTVd isolates detected in solanaceous hosts in Australia over the previous 7 years.
- ii. Determine the pathogenicity of selected isolates of PSTVd, and related pospiviroids (if detected).
- iii. Identify alternative hosts of pospiviroids in Australia.
- iv. Understand the dynamics of PSTVd epidemics in the field, how contact transmission occurs, the survival of its infectivity on different surfaces and the role of nurseries as potential reservoirs.
- v. Provision of scientific data to support import risk assessments of solanaceous plant products into Australia.
- vi. Train a post graduate student in Plant Virology (viroids) and Plant Biosecurity.

This project represented a strong contribution to CRC Strategic Plan in relation to:

- i. CRC Outcome 6: Provide specific training for PhD candidates in plant biosecurity
- ii. CRC Outcome 2: The effective delivery and validation of a suite of new diagnostic technology, novel tools and innovative enabling technology.

The major beneficiaries from this research are the horticulture industries and biosecurity agencies across Australia as this project addresses a major Biosecurity Issue for Australia. Since 2001 there have been six reported incursions of PSTVd in tomatoes in Australia. In each instance, an intense eradication program successfully removed the source of inoculum and, as a consequence, PSTVd remains a quarantine pest of significance in Australia. At a Consultative Committee of Emergency Plant Pests (CCEPP) in 2008, it was agreed that the current incursion of PSTVd was able to be eradicated. However, due to the frequency and associated costs of the eradication programs, the CCEPP indicated that undertaking future eradication programs for PSTVd could only be justified if the pathway for the recent PSTVd incursions was determined. A critical component of this project is the delivery of this essential information relating to understanding pathways for incursion. This project targeted the critical information needed for Australian horticulture, particularly the potato industry, such that Australia could potentially be in a position to remain free of this destructive pathogen. In particular, the component of this project in relation to investigation into the potential entry pathways of PSTVd in tomatoes is vitally important in this respect.

Of particular concern when this project was commencing, was the fact that research in Europe at that time suggested that PSTVd infection could be widespread in certain wild solanaceous hosts, and that these infection reservoirs rather than seed transmissions in tomato may be responsible for outbreaks in tomato in different parts of the world. Further, in WA at that time, there was also a suggestion that certain wild non-solanaceous hosts could potentially be involved.

It was evident at the beginning of this project that there was an urgent need to provide information on and address the following:

- i. The phylogenetic relationships between strains of PSTVd and related pospiviroids occurring in Australia.
- ii. The pathogenicity of selected strains of PSTVd and related pospiviroids.
- iii. The potential role of alternative hosts and nurseries as PSTVd reservoirs within the tomato crop production system.
- iv. Other key aspects of PSTVd epidemiology, including how contact transmission occurs, the temporal and spatial dynamics of epidemics in the field and the stability of inoculum on different types of surfaces.

3. Key findings

Please note that the key findings outlined below under individual headings frequently address several of the aims despite being listed under one particular aim

3.1. Aims (i) – (iii): Investigate the relationships between PSTVd isolates detected in solanaceous hosts in Australia over the last 7 years; Determine the pathogenicity of selected isolates of PSTVd, and related pospiviroids (if detected); Identify alternative hosts of pospiviroids in Australia

To address Aims (i) to (iv), research undertaken in this project included weed and alternative host surveys in Carnarvon, WA, and sequencing of positive samples.

For each of two surveys in the Carnarvon area, the first during the 2009/2010 summer and the second in 2011 following the Carnarvon floods, samples of volunteer solanaceous hosts [such as tomato, capsicum, eggplant, chilli or Cape Gooseberry (*Physalis peruviana*)] and weeds, [including Apple of Peru (*Nicandra physalodes*), thornapple (*Datura stramonium*) blackberry nightshade (*Solanum nigrum*) and flaxleaf fleabane (*Conyza bonariensis*)], growing alongside or near commercial host crops were collected. Also, weeds and native plants, [such as annual saltbush (*Atriplex semilunaris*), Thorny saltbush (*Rhagodia eremeae*) and various Malvaceae species] were collected along roadside verges and on common land in areas prone to flooding or adjacent to commercial host crops. All samples collected were tested by RT-PCR using universal Pospiviroid primers and PSTVd specific primers. Subsequently, to confirm the presence of Pospiviroids, nucleic acid samples were cloned and sequenced by the Department of Primary Industries Victoria (DPIVic).

In the first survey, PSTVd was detected in Blackberry Nightshade, Annual Saltbush and volunteer Tomato and Capsicum plants. In the second survey the previous flooding appeared to have spread Thornapple plants to areas previously free of this host. PSTVd was detected in Thornapple, Blackberry Nightshade and Annual Saltbush. An undetermined Pospiviroid was also detected in Thorny saltbush and Annual saltbush. PSTVd specific nucleotide sequences were obtained for 21 separate isolates, confirming that diverse solanaceous hosts and even non-solanaceous hosts can harbour PSTVd.

For studies in relation PSTVd sequencing/Phylogeny, a total of 24 survey samples were forwarded to the DPIVic at Knoxfield for PSTVD testing and sequencing of PSTVd specific PCR products as follows:

- Twelve samples supplied from WA in December 2010 were screened for the presence of PSTVd and related pospoviroids. Two PSTVd specific RT-PCR tests and one Pospoviroid-generic RT-PCR tests were conducted on each sample. Five of the 12 samples tested positive for PSTVd and amplicons from these samples were cloned and sequenced. PSTVd specific sequences for these isolates (181, 189, 209, 212 and 217) have been obtained.
- A further two isolates were weakly positive for the Pospiviroid RT-PCR test and negative for both the PSTVd specific RT-PCR tests. This result suggests the presence of a different viroid species. Unfortunately, to date the cloning of these weak PCR products has been unsuccessful and their pospoviroid sequence has not been obtained.

- In July 2011, a further eight samples were screened for PSTVd and pospoviroids. All eight samples were negative for PSTVd for both RT-PCR tests. Four of the samples (259, 269, 280 and 283) produced a faint band with the Pospiviroid PCR. Cloning and sequencing only yielded Pospiviroid specific sequence for one isolate (269) and this sequence was most closely related to PSTVd.
- An additional four isolates from Wild Gooseberry (Wild Gooseberry 1–4) in April 2012 tested negative to both the PSTVd specific RT-PCR tests and to the pospiviroid test.
- Cloning and sequencing yielded Pospiviroid specific sequence for one isolate (269) and this sequence was most closely related to PSTVd.
- These results confirm, for the first time, the likely presence of an additional Pospiviroid species in Australia.

Publications:

Mackie, A., Barbetti, M., Rodoni, B., McKirdy, S. and Jones, R. (2010) Pospiviroids in the West Australian sub-tropics: incidence and new hosts. *Poster Abstract for the Global Biosecurity Conference, Brisbane Queensland 2010.*

3.2 Aim iv: Understand the dynamics of PSTVd epidemics in the field, how contact transmission occurs, the survival of its infectivity on different surfaces and the role of nurseries as potential reservoirs.

To address Aim (iv), two areas of study were investigated as follows:

Firstly, studies were undertaken to determine the infectivity retention of PSTVd on various surfaces. A trial was conducted to determine the length of time PSTVd remains infective on various surfaces (viz. metal, cotton, glass, string, plastic, rubber, leather and wood). Details of this work undertaken included extracting infected sap from known infected tomato plants held in the PC2 glasshouse at The University of Western Australia (UWA) and pipetted onto different materials and, then after four specified time periods (viz. 5 minutes, 1 hour, 6 hours and 1 day) the sap on the different materials was used to inoculate healthy tomato plants. The inoculated plants were tested by RT-PCR after 4-6 weeks to confirm infection or its absence.

Across the eight different surfaces tested for their infectivity retention of PSTVd, PSTVd-contaminated metal was still infective after 1 hour while PSTVd-contaminated cotton and skin was still infective after 6 hours. Results for wood, plastic, glass, rubber, string and leather revealed that sap was still infective after 24 hours. These results have implications regarding PSTVd transmission when contaminated surfaces brush against healthy tomato plants.

Publications:

Mackie, A., Barbetti, M., Rodoni, B., McKirdy, S. and Jones, R. (2010) Retention time of infectious Potato spindle tuber viroid (PSTVd) on common surfaces. *Poster Abstract for the Australasian Plant Virology Workshop 2010.*

Secondly, additional studies were undertaken to determine the efficacy of disinfectants against PSTVd and, in particular, whether RT-PCR is a suitable method for identifying whether "decontaminated" surfaces still harbor residual

infectious viroids and to evaluate commonly used disinfectants for their ability to both inactivate PSTVd and disrupt viroid RNA so that they cannot be detected by RT-PCR. Nine treatments have been tested and include: water, healthy sap, infected sap, ethanol, milk powder, sodium hypochlorite, VirKonS 1:100, VirKonS 1:200, and Farmcleans.

Ten plants were inoculated for each treatment, with sap applied to a watch glass followed by the disinfectant. The mixture was allowed to sit for one minute then used to inoculate 10 plants and tested by RT-PCR. Results indicate that sodium hypochlorite and milk powder are effective in suppressing PSTVd infection, whilst also disrupting the viroid RNA so that it could not be detected by RT-PCR.

3.3 Aim (v): Provision of scientific data to support import risk assessments of solanaceous plant products into Australia

Provision of scientific data to support import risk assessments of solanaceous plant products into Australia has been a critical component of this project. To meet this aim, this has focused on developing two protocols as follows:

IPPC PSTVd diagnostic protocol:

Dr Rodoni was nominated in July 2009 as a member of the Expert Working Group of the Technical Panel on Diagnostic Protocols (TPDP) for drafting the Protocol for Potato spindle tuber viroid and has provided input into developing the most appropriate tests for PSTVd detection and also involved in editing the document for publication. In August 2010, Dr Rodoni visited Dr Colin Jefferies at the Scottish Science and Advice Centre for Scottish Agriculture (SASA, Edinburgh, Scotland), who is the team leader of the PSTVd TPDP. A final version of the PSTVd protocol was submitted in October 2011 to the International Plant Protection Convention (IPPC) for review and approval (see Appendix 1). Once accepted by the IPPC this document will be submitted to the Australian Subcommittee for Plant Health Diagnostic Standards (SPHDS) for acceptance as the national diagnostic protocol for PSTVd detection in Australia.

Seed testing protocol:

At the commencement of this project the European Union (EU) funded research to develop a tomato seed testing protocol for the detection of PSTVd was almost completed and it was hoped that this protocol would be available for adoption and validation in Australia. The ultimate goal was to submit the finalised protocol to SPHDS for ratification at a national level. This protocol would then be used to screen commercial tomato seed for the presence of PSTVd. Unfortunately, the EU project never completed the diagnostic protocol for the detection of PSTVd in tomato seed as they could not obtain a reliable source of PSTVd tomato seed and hence were both unable to validate the sensitivity of the protocol and unable to provide a genuine positive control to use on a routine basis. As such this EU protocol was not available for inclusion in either the Australian diagnostic protocol or the IPPC diagnostic protocol for the detection of PSTVd. However, in the absence of the EU protocol, the Department of Agriculture Fisheries and Forestry (DAFF) in Australia recommended a protocol published in Japan (Hoshino et al., 2006) to be used to screen tomato seed for PSTVd, screening batches of 400 seed per RT-PCR test. This protocol was adapted for this purpose and is currently being used by DAFF to screen commercial tomato seed lots entering Australia. A detailed description of the Hoshino et al (2006) protocol is provided in Appendix 2. The absolute sensitivity of this protocol has not been determined due to a lack of

a reliable source of PSTVd infected tomato seed, as was the situation with the EU project. Despite this, the protocol is still used by DAFF to screen 20,000 seeds per seed lot in batches of 400 seed per RT-PCR test. This protocol, using this sampling regime, has detected PSTVd in several batches of tomato seed.

Further to the above, critical to provision of the relevant scientific data needed to support import risk assessments of solanaceous plant products into Australia, also required determining the pathogenicity of PSTVd on the most high risk host species for PSTVd, especially potato and tomato. To address this, pathogenicity studies were undertaken using commonly grown potato and tomato cultivars in the PC2 glasshouse at UWA to establish what pathogenic effects the Oceania isolates of PSTVd from WA have on commonly grown potato and tomato cultivars. This work focused on the pathogenicity of WA isolate of PSTVd (Carnarvon strain) on potato and tomato cultivars. Ten plants of each cultivar (potato cultivars: Russet Burbank, Atlantic and Nadine; tomato cultivars: Rutgers, Petula and Swanson) were grown in pots in a PC2 glasshouse at UWA. The plants and harvested tubers and fruit were assessed for disease symptoms and quality parameters (yield and fresh and dry weights) in comparison to uninfected controls. Quite early on in this trial, all three potato cultivars showed symptoms of PSTVd infection with Russet Burbank the most severely affected variety, Nadine was moderately to severely affected and Atlantic was mildly to moderately affected. All three tomato cultivars showed symptoms of PSTVd infection. Rutgers showed the most severe symptoms. Petula is moderately to severely affected and Swanson was only mildly so.

The final harvest of tomato and potato plants was conducted at the end of February 2012. Petula plants were moderately to severely affected and were stunted with cupped leaves and chlorosis in the plant tops and infected plants weighed on average 52% less than healthy plants. Swanson was mildly affected with infected plants being stunted with cupped leaves and weighing 40% less than healthy plants. Rutgers showed severe symptoms of PSTVd infection of shortened, thickened internodes and stems, necrotic midribs and reduced apical leaf size, and infected plants weighed 42% less than healthy plants. Russet Burbank was the most severely affected variety with infected plants being stunted, with small leaves and with necrotic spots on stems. Infected plants weighed on average 30% less than healthy plants. Atlantic was moderately affected, with infected plants being smaller in size and showing apical bunching, and infected plants weighed 34% less than healthy plants. Nadine was moderately to severely affected and infected plants weighed an average 21% less than healthy plants.

Infected tomato cultivars failed to produce any fruit of marketable yield (based on size) under glasshouse conditions. Infected potato cultivars failed to produce any tubers of marketable yield under glasshouse conditions. Tubers from infected Russet Burbank plants were small and spindle shaped, infected Atlantic plants produced small, severely cracked tubers and infected Nadine plants produced clusters of "micro-tubers" (each "micro-tuber" approximately 3mm in length).

Publications:

Mackie, A., Barbetti, M., Rodoni, B., McKirdy, S. and Jones, R. (2012) Studies on Pospiviroid isolates from solanaceous and wild plant hosts in Western Australia. *Abstract for the CRC for National Plant Biosecurity Science Exchange, Perth Western Australia, 2012.*

3.4. Aim (vi): Train a post graduate student in Plant Virology (viroids) and Plant Biosecurity

Alison Mackie came to this project with excellent background skills from having completed a Bachelor of Agricultural Science with honours in Plant Pathology at the University of Tasmania in 1997. Subsequent periods working in the Department of Agriculture and Food Western Australia as a Quarantine Research Officer provided her with additional knowledge and experience of Australia's quarantine regulations as relating to plants and plant pathogens. However, this project has greatly increased her technical skills capacity, as she has developed a wide range of additional skills and expertise in relation to both plant virology (particularly viroids) and also in dealing with plant biosecurity issues with highly transmissible viroids. Through this project, she has undertaken specialist training and developed expert skills and understanding in relation to the identification, characterisation and phylogenetic analysis of viroids; in plant virus/viroid epidemiology; and in determination of plant virus/viroid pathogenicity. Further, this project has helped her develop a wider network of potential scientific collaborators across the University, Plant Biosecurity and state Department of Agriculture sectors across Australia.

Further, a unique opportunity arose when Dr Ko Verhoeven from the Plant Protection Service, Wageningen, The Netherlands, visited Victoria in November 2010 to meet CRC10164 project members and to present a keynote address on pospoviroids ("Identification and epidemiology of pospoviroids") at the Australasian Plant Virology Conference. Dr Ko Verhoeven is a world expert on the detection and characterisation of pospoviroids, including PSTVd and related pospoviroids that infect solanaceous hosts. While in Melbourne, Dr Verhoeven held a meeting with Ms Mackie, Dr Roger Jones and Dr Brendan Rodoni to discuss project goals and progress. Dr Verhoeven offered input with interpretation of data and other assistance where possible, providing additional opportunities for Ms Mackie to learn further new skills.

4. Implications for stakeholders

Prior to this project, the role of alternative hosts in PSTVd epidemiology in and around tomato crops in Australia had never been investigated. Similarly, other aspects of the epidemiology of PSTVd, such as how contact transmission occurs, the dynamics of how PSTVd epidemics could occur in the field, the stability of PSTVd inoculum infectivity on different surfaces and the role of seed and/or nurseries in spreading the pathogen had never been studied anywhere. This project has provided the critical knowledge of genetic relationships between the strains of PSTVd that occur in Australia by showing that five of the 12 samples tested positive for PSTVd and that a further 2 isolates were weakly positive for the Pospiviroid RT-PCR test and negative for both the PSTVd specific RT-PCR tests. This is the first indication that different viroid species are likely present in Australia. This project has provided the first understanding of the pathogenicity of Australian PSTVd isolates and the first clarity in relation to the role infected alternative hosts likely play in the epidemiology of PSTVd incursions on tomatoes. Together, these findings have provided unique insights into the potential impact of this EPP on the Australian potato and tomato industries for the future.

Further, stakeholders will benefit from now having, for the first time, some understanding of the origins of the PSTVd outbreaks that have been detected in Australia in recent years. In addition, for the first time, stakeholders now have

understanding of the dynamics of PSTVd epidemics in the field, how contact transmission occurs, how the survival of infectivity is affected by different surfaces, and what measures need to be taken at a nursery level (e.g., to ensure seed is free of PSTVd contamination) to minimise future spread the pathogen via nurseries into commercial tomato and/or potato crops.

End users and the main beneficiaries of this work include Office of the Chief Plant Protection Officer (Australia) (OCPPO), Biosecurity Australia, Plant Health Australia, and the tomato and potato industries. In particular, this project has provided a much greater capacity towards safeguarding Australia's potato and tomato industries via by provision of an understanding of PSTVd epidemiology. Further, border security can and is now being enhanced following identification of the most likely sources of infection and will be further enhanced once possible measures for disinfestation of materials have been defined for managing contaminated plant, seed or other materials. Together, this research has not only significantly improved the effectiveness future import risk assessments of solanaceous plant products into Australia, but now enables development of effective containment and eradication strategies for this EPP.

The national benefit of training of a PhD student in the highly specialised area of plant virology (viroids), along with associated plant biosecurity and plant viroid disease epidemiology, cannot be underestimated and provides security to the tomato, potato and other horticultural industries involved with solanaceous plants or plant products.

5. Recommendations

Studies undertaken in this project have highlighted the need for further work and to address this need the following critical issues need to be addressed:

- i. Wider and more extensive surveys of wild and cultivated solanaceous and non-solanaceous plant species across Australia for pospoviroids, particularly PSTVd and related pospoviroids that infect solanaceous hosts. Characterizing the exact nature and identity of related pospoviroids remains an urgent priority.
- ii. While commercial tomato seed imported into or produced in Australia is currently tested for PSTVd using the DAFF-Hishino protocol, there is an urgent need to, firstly, determine the absolute sensitivity of this protocol by locating and utilising a reliable source of PSTVd infected tomato seed to confirm the sensitivity and adequacy of this test and to improve it as required; and, secondly, to develop and deploy a test that is effective and reliable in detecting related pospoviroids. This is the only way all tomato seed imported into and produced in Australia can be accurately tested such that all seed supplied to and utilised in nurseries across Australia is free of PSTVd and related pospoviroids.
- iii. Further to (i) and (ii) above, it is critical to establish and put in place the required protocols to protect Australian exports of seed potatoes, tomatoes, other solanaceous cultivated species, and products of these solanaceous hosts.

6. Abbreviations/glossary

ABBREVIATION	FULL TITLE
CCEPP	Consultative Committee of Emergency Plant Pests
CRCNPB	Cooperative Research Centre for National Plant Biosecurity
DAFF	Department of Agriculture, Fisheries and Forestry
EPP	Emergency Plant Pest
EPPRD	Emergency Plant Pest Response Deed
EU	European Union
IPPC	International Plant Protection Convention
OCPP	Office of the Chief Plant Protection Officer (Australia)
PSTVd	Potato spindle tuber viroid
RT-PCR	Reverse transcription polymerase chain reaction
SPHDS	Sub-committee for Plant Health Diagnostic Standards
TPDP	Technical Panel on Diagnostic Protocols
Vic DPI	Department of Primary Industries, Victoria

7. Plain English website summary

CRC project no:	CRC10164
Project title:	Phylogeny, pathogenicity and epidemiology of potato spindle tuber viroid (PSTVd) and related pospiviroids in Australia
Project leader:	Dr Martin Barbetti
Project team:	Ms Alison Mackie, Dr Roger Jones, Dr Brendan Radoni, Dr Martin Barbetti
Research outcomes:	This project has determined the extent of Potato spindle tuber viroid (PSTVd) in solanaceous and non-solanaceous weeds in Western Australia; defined the genetic relationships between the strains of PSTVd that occur in Australia; demonstrated how this pathogen is able to survive and spread on contaminated surfaces; identified effective disinfectants; and has provided key information to allow instigation of appropriate quarantine and management protocols to prevent its further importation into and spread within Australia.
Research implications:	The horticultural industries in Australia now have, for the first time, an understanding of the origins of PSTVd outbreaks that have been detected in Australia over recent years. Further, also for the first time, they have an understanding of the dynamics of PSTVd epidemics in the field, the sources of infection, how contact transmission occurs, how the survival and infectivity is affected by different material surfaces, how effective disinfestation can be undertaken, and what measures need to be taken at a national, state and industry level (e.g., protocols to ensure seed is free of PSTVd contamination) to minimise future spread the pathogen within Australia. The findings of this project have significantly advances Australia's capacity safeguard its potato, tomato

	and other solanaceous plant-based industries. Highlighting of effective measures for disinfestation of contaminated materials enhances Australia's border security while also securing the future of valuable horticultural exports from Australia.
Research publications:	None finalised to date.
Acknowledgements:	Horticulture Australia Limited for matching funding, Department of Agriculture and Food Western Australia for half salary of Martin Barbetti during this project.