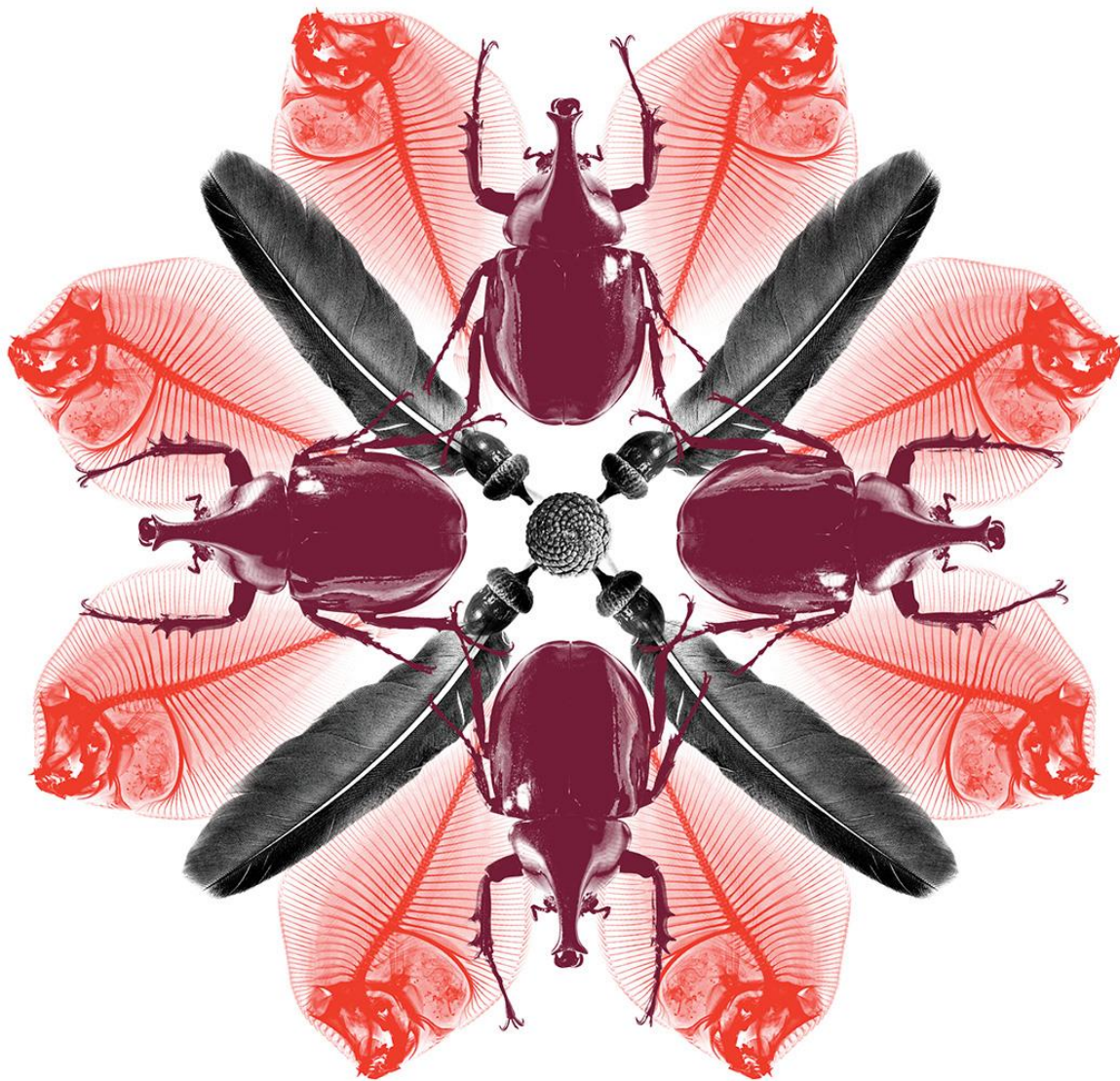


Draft pest risk analysis for *Pepino mosaic virus* and pospiviroids associated with tomato seed

August 2018



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Stakeholder submissions on draft reports

This draft report has been issued to give all interested parties an opportunity to comment on relevant technical biosecurity issues, with supporting rationale. A final report will then be produced taking into consideration any comments received.

Submissions should be sent to the Australian Government Department of Agriculture and Water Resources following the conditions specified within the related Biosecurity Advice, which is available at: <http://www.agriculture.gov.au/biosecurity/risk-analysis/memos>

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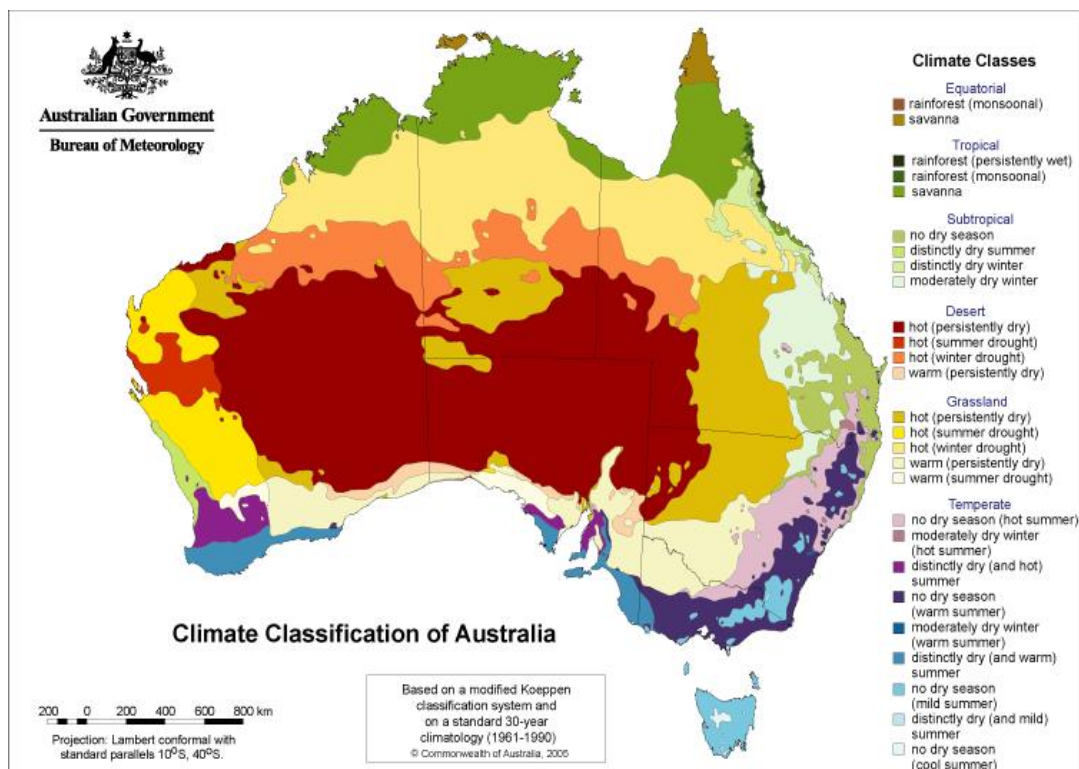
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Map 1 Map of Australia



Map 2 A guide to Australia's bio-climatic zones



Acronyms and abbreviations

Term or abbreviation	Definition
ALOP	Appropriate level of protection
AQIS	Australian Quarantine and Inspection Service
BICON	Australia's Biosecurity Import Condition System
BIRA	Biosecurity Import Risk Analysis
BA	Biosecurity Australia
CABI	CAB International, Wallingford, UK
CCEPP	Consultative Committee on Emergency Plant Pests
EPPRD	Emergency Plant Pest Response Deed
ICON	AQIS Import Conditions database, now superseded
IPPC	International Plant Protection Convention
IRA	Import Risk Analysis, a predecessor of the BIRA
ISF	International Seed Federation
ISPM	International Standard for Phytosanitary Measures
NAKT	Nederlandse Algemene Kwaliteitsdienst Tuinbouw (Netherlands Inspection Service for Horticulture – known as Naktuinbouw)
NPPO	National Plant Protection Organisation
NSW	New South Wales
NT	Northern Territory
OCPPPO	Office of the Chief Plant Protection Officer
PCR	Polymerase Chain Reaction
PFA	Pest Free Area
PHA	Plant Health Australia
PRA	Pest Risk Analysis
Qld	Queensland
RT-PCR	Reverse Transcription - PCR with the additional preparatory step of reverse transcription of target RNA to DNA
RT-qPCR	Reverse transcription PCR done by using a quantitative real-time method
SA	South Australia
SPS Agreement	WTO agreement on the Application of Sanitary and Phytosanitary Measures
Tas.	Tasmania
Vic.	Victoria
WA	Western Australia
WTO	World Trade Organization

Summary

The Australian Government Department of Agriculture and Water Resources initiated this pest risk analysis in response to the introduction of emergency measures to manage pospiviroids and *Pepino mosaic virus* associated with traded seed of tomato (*Solanum lycopersicum*) and wild tomato (*Solanum chilense*, *S. chmielewskii*, *S. parviflorum*, *S. peruvianum* and *S. pimpinellifolium*). The pospiviroids managed under the emergency measures are *Columnea latent viroid*, *Pepper chat fruit viroid*, *Potato spindle tuber viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid* and *Tomato planta macho viroid*. With the exception of *Potato spindle tuber viroid*, these pathogens are not known to occur in Australia. Collectively, they are reported to cause serious damage to the avocado, capsicum, potato and tomato industries in other countries. Australia introduced emergency measures on imports of tomato seed and wild tomato seed in June 2008 and revised the measures in February, May and December 2012 and August 2013.

The International Plant Protection Convention and the World Trade Organisation Agreement on the Application of Sanitary and Phytosanitary Measures require that any phytosanitary measure applied against the introduction of a pest must be technically justified. The IPPC's International Standards for Phytosanitary Measures 1 (ISPM 1) indicates that countries may take emergency actions, including emergency measures, when a new or unexpected phytosanitary risk is identified; however, it also requires that an emergency measure should be evaluated as soon as possible to ensure the continuance of the measure is justified. The pest risk analysis presented in this draft report meets Australia's international obligations to review the emergency phytosanitary measures on the pospiviroids and *Pepino mosaic virus* associated with tomato seed.

This draft report presents pest risk assessments for *Pepino mosaic virus*, *Columnea latent viroid*, *Pepper chat fruit viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid* and *Tomato planta macho viroid* (the listed pathogens), but not for *Potato spindle tuber viroid*, as it is no longer categorised as a quarantine pest for Australia. Relevant information about tomato seed production and trade, the epidemiology of the pathogens and Australian testing for the pathogens during the period of application of emergency measures is also presented. The emergency measures on tomato seed imports, under which seed to be imported into Australia is tested for *Potato spindle tuber viroid*, will continue while the department examines the status and regulation of the viroid.

This draft report proposes a range of risk management measures that target the listed pathogens. The proposed measures include mandatory laboratory testing for the listed pathogens, or heat treatment and laboratory testing, combined with operational systems and verification of laboratory testing. Together these measures mitigate the risks posed by the listed pathogens associated with imports of tomato seed and thereby reduce the risks to achieve the appropriate level of protection for Australia.

The proposed measures are largely consistent with the emergency measures, but some amendments are indicated including a change to the sampling for *Pepino mosaic virus*, an option of heat treating seed lots to eliminate the virus and changes to the approval of laboratories for testing. The emergency measures will remain in place until the proposed measures are implemented, which is expected during 2019, depending on the finalisation of this report.

1 Introduction

1.1 Australia's biosecurity policy framework

Australia's biosecurity policies aim to protect Australia against the risks that may arise from exotic pests entering, establishing and spreading in Australia, thereby threatening Australia's unique flora and fauna, as well as those agricultural industries that are relatively free from serious pests.

The risk analysis process is an important part of Australia's biosecurity policies. It enables the Australian Government to formally consider the level of biosecurity risk that may be associated with proposals to import goods into Australia. If the biosecurity risks do not achieve the appropriate level of protection (ALOP) for Australia, risk management measures are proposed to reduce the risks to an acceptable level. If the risks cannot be reduced to an acceptable level, the goods will not be imported into Australia until suitable measures are identified or developed.

Successive Australian Governments have maintained a stringent, but not a zero risk, approach to the management of biosecurity risks. This approach is expressed in terms of the ALOP for Australia, which is defined in the *Biosecurity Act 2015* as providing a high level of protection aimed at reducing risk to a very low level, but not to zero. Australia's approach is consistent with the SPS Agreement, which acknowledges that there is some level of risk associated with international movement of goods.

Australia's risk analyses are undertaken by the Australian Government Department of Agriculture and Water Resources (the department) using technical and scientific experts in relevant fields, and involve consultation with stakeholders at various stages during the process.

Risk analyses may take the form of a biosecurity import risk analysis (BIRA) or a non-regulated risk analysis (such as scientific review of existing policy and import conditions, pest-specific assessments, weed risk assessments, biological control agent assessments or scientific advice).

Further information about Australia's biosecurity framework is provided in the *Biosecurity Import Risk Analysis Guidelines 2016* located on the [Australian Government Department of Agriculture and Water Resources website](#).

1.2 This risk analysis

The International Plant Protection Convention (IPPC) and the World Trade Organisation Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) requires that any phytosanitary measures against the introduction of new pests must be technically justified. The department undertook this pest risk analysis (PRA) to meet Australia's obligations under the IPPC and ISPM 1 to review the emergency phytosanitary measures, which were introduced to manage the risk of introducing into Australia seven pathogen species associated with tomato seed. The seven species regulated under the emergency measures were *Pepino mosaic virus* (PepMV), *Columnnea latent viroid* (CLVd), *Pepper chat fruit viroid* (PCFVd), *Potato spindle tuber viroid* (PSTVd), *Tomato apical stunt viroid* (TASVd), *Tomato chlorotic dwarf viroid* (TCDVd) and *Tomato planta macho viroid* (TPMVd). This draft report presents pest risk assessments for PepMV, CLVd, PCFVd, TASVd, TCDVd and TPMVd, but does not present a pest risk assessment for PSTVd. The department is evaluating the risks presented by PSTVd and the possibility of regulating PSTVd as a regulated non-quarantine pest. Australia introduced emergency measures for tomato seed and wild tomato seed imports and notified trading

partners of the emergency measures through World Trade Organization Sanitary and Phytosanitary (WTO SPS) notifications in June 2008, February 2012, May 2012 and November 2012 (G/SPS/N/AUS/225; G/SPS/N/AUS/225/Add.1; G/SPS/N/AUS/225/Add.2; G/SPS/N/AUS/225/Add.3).

1.2.1 Background

Australia's regulation of tomato seed

Prior to 1992, tomato (*Solanum lycopersicum* L.) seed was a restricted seed for which importation required an import permit, hot water treatment and immersion in trisodium phosphate solution under quarantine supervision.

In 1992, the former Australian Quarantine and Inspection Service (AQIS) reviewed the quarantine status of tomato seed following representations from seed importers. New import conditions that removed the treatment requirements were announced in Quarantine Circular Memorandum (Plants) 1992/95 on 2 December 1992. The requirement for seed treatment was removed on the basis that *Phoma lycopersici*, *Tomato mosaic virus* and *Clavibacter michiganensis* subsp. *michiganensis* occurred in Australia and were not under official control. Tomato seed was permitted entry after visual inspection by AQIS on arrival in Australia, following the same conditions as other permitted seeds.

Emergency Measures 2008

Following incursions of PSTVd in Australia in 2001, 2004, 2006 and 2007, the Consultative Committee on Emergency Plant Pests (CCEPP) requested that the former Biosecurity Australia undertake a PRA for PSTVd. The request was made because of concerns that the viroid was entering Australia in imported tomato seed. Biosecurity Australia was a prescribed agency of the Australian Government whose functions were subsumed into the department in 2012.

Work on a PRA for PSTVd in tomato seed commenced in September 2007. In light of that work, which recognised PSTVd as being seed-transmitted in tomato, Australia introduced emergency measures for PSTVd in tomato seed and wild tomato seed on 25 June 2008 to address the risk of the introduction of the viroid in imported seed.

The 2008 emergency measures for tomato seed required that consignments were accompanied by a Phytosanitary Certificate endorsed with one of the following additional declarations:

The tomato seed in lot(s) (numbers) in the consignment was grown in
... (Country) in an area that is free of *Potato spindle tuber viroid*, based on an official
survey covering the complete range of potato spindle tuber viroid hosts.

OR

The tomato seed in lot(s) (numbers) in the consignment was derived from
seed and pollen parent plants grown by (producer) in (country) that
were tested during the growing period and found free of *Potato spindle tuber
viroid*.

Following concerns raised by the Australian Seed Federation (ASF) and the National Plant Protection Organisations (NPPOs) of the Netherlands and Israel over the ability of tomato seed exporting countries to meet the requirements for seed already produced, Australia advised that

it would also accept the following additional declaration to allow the export of tomato seed to Australia:

No symptoms of diseases caused by potato spindle tuber viroid have been observed on the plants at the place of production during their complete cycle of vegetation.

The emergency measures, published at that time in the AQIS Import Conditions (ICON) database, were updated to reflect this change on 7 August 2008.

For tomato seed lines that were not certified free from PSTVd by one of the above declarations, Australia required a specific laboratory test for PSTVd on a sample of 20,000 seeds. Testing was done at the importer's risk and seed lines that tested positive for PSTVd were either re-exported or destroyed.

Biosecurity Australia considered that a sample size of 20,000 seeds was necessary to detect low levels of contamination of seed lines by PSTVd-infected tomato seed. This seed sample size was supported by an investigation in the United Kingdom of an outbreak in a greenhouse crop from which the contamination of a seed lot could be estimated (Mumford, Jarvis & Skelton 2004).

Revised emergency measures February 2012

Following an incursion of PSTVd in Queensland (Qld) in 2011, a CCEPP meeting in April of that year recommended that the emergency measures from 2008 be strengthened by removing the declaration based on freedom from symptoms. The department announced the revised emergency measures on 10 February 2012, removing the additional declaration and replacing it with one based on laboratory testing for PSTVd of a 20,000 seed sample from each seed lot. Samples were to be tested using a specific reverse-transcription polymerase chain reaction (RT-PCR). Acknowledging that there were several possible protocols to test for the viroid, the emergency measures did not stipulate a particular protocol for overseas laboratories. The option to use an additional declaration based on area freedom was retained.

When the emergency measures targeting PSTVd were amended on 10 February 2012, emergency measures were also introduced on tomato seed imports to prevent the entry of the potexvirus PepMV with the seed. An additional declaration for PepMV was required, with options for testing a 3,000 seed sample, area freedom or testing of parent plants.

Revised emergency measures May 2012

Following the introduction of the revised emergency measures on 10 February 2012, TCDVd was detected in tomato seed produced in a country in Africa and one in Europe through the program of testing on arrival. TCDVd is a quarantine pest for Australia that is transmitted through tomato seed (Chapter 6) and emergency measures were amended on 23 May 2012 to require testing and freedom from this viroid.

Revised emergency measures December 2012

Another unexpected detection occurred later in 2012 as a result of the testing on arrival. PCFVd was detected in seed produced in a country in the Middle East and one in Asia. PCFVd is also a quarantine pest for Australia and as a result, the emergency measures were revised again. The amended measures came into force on 3 December 2012 to require freedom from PCFVd, as well as freedom from the viroids CLVd, TASVd and TPMVd. CLVd, TASVd and TPMVd are quarantine

pests for Australia that pose a risk to Australia (Chapter 6). All of these pathogens are transmitted through tomato seed (Chapter 5).

Revised emergency measures 2013

The option for an additional declaration based on area freedom was removed on 28 August 2013. This option required an official survey to establish area freedom. No requests for this option were received by the department. Area freedom based on absence of records was not accepted by the department, as the Australian testing results showed that such claims were incorrect on several occasions. Furthermore, tomato seed is freely traded between countries and this appeared to be often done without testing for pospiviroids.

The option for an additional declaration based on parent plant testing was removed on 21 November 2013. This option required the testing of all parent plants and no requests for this option were received by the department. An option based on partial testing of parent plants was accepted for a period of time until additional testing detected pospiviroids in tomato seed imported under this option.

Current emergency measures

The emergency measures begun in 2008 and revised in 2012 and 2013 have been maintained to the present. Support for continuing the measures came from the findings of seed testing by Australian laboratories and from reviews of the literature done in preparation for this analysis.

Under the current emergency measures, tomato seed and wild tomato seed consignments must be accompanied by an official government Phytosanitary Certificate from the country of origin where the seed was grown. The Phytosanitary Certificate must be endorsed with additional declarations concerning testing for PepMV, CLVd, PCFVd, PSTVd, TASVd, TCDVd and TPMVd. A laboratory test report that supports the declarations must accompany the Certificate. The test report must include the name and address of the testing laboratory, the species tested and the number of seeds tested. Consignments not accompanied by a satisfactory official government Phytosanitary Certificate are tested on arrival in Australia at the importer's risk and expense. Consignments shown by testing to carry a quarantine pest are re-exported or destroyed at the importer's expense, as are consignments that otherwise do not meet the biosecurity requirements.

The current emergency import conditions for tomato seed can be viewed on the BICON database available at <http://www.agriculture.gov.au/import/online-services/bicon> and <https://bicon.agriculture.gov.au/BiconWeb4.0>.

Phytosanitary certification

Current phytosanitary certification requires an additional declaration indicating testing for CLVd, PCFVd, PSTVd, TASVd, TCDVd and TPMVd of the form:

The tomato seed in lot(s) (insert lot numbers), of name (insert name), grown in (insert name of country), was tested for *Columnnea latent viroid*, *Pepper chat fruit viroid*, *Potato spindle tuber viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid* and *Tomato planta macho viroid* and was found to be free of these viroids by using a reverse-transcription PCR test for the viroids on a

sample of 20,000 seeds drawn from the lot and divided and tested as sub-samples of no more than 400 seeds.'

In addition, one of the following declarations indicating testing for PepMV is required:

The tomato seed in the consignment in lot(s) (insert lot numbers), of name (insert name), grown in (insert name of country), was tested for *Pepino mosaic virus* and found to be free of the virus by using the ELISA test method of the Manual of Seed Health Testing Methods of the International Seed Federation (current version) on a sample of 3000 seeds drawn from the lot and divided and tested as sub-samples of no more than 250 seeds.

OR

The tomato seed in the consignment in lot(s) (insert lot numbers), of name (insert name), grown in (insert name of country), was tested for *Pepino mosaic virus* and found to be free of the virus by using a reverse-transcription PCR test for the virus on a sample of 3000 seeds drawn from the lot and divided and tested as sub-samples of no more than 400 seeds unless the testing laboratory is approved for sub-sample sizes greater than 400 seeds.

Testing on arrival

If an importer chooses to have a seed consignment tested on arrival, RT-PCR tests (laboratory tests using the reverse transcription polymerase chain reaction method) are done for the pospiviroids and PepMV. The tests for the pospiviroids are done on a sample of 20,000 seeds in one of the recognised Australian laboratories following an Australian protocol (Appendix A). The complete sample of 20,000 seeds is divided into subsamples of no more than 400 seeds, to give 50 subsamples. Each subsample is tested independently for the pospiviroids.

The RT-PCR test for PepMV uses a total sample of 3000 seeds divided into subsamples. This PepMV test may be done on RNA extracted from the subsamples taken for the pospiviroid testing, in which case RNA from at least eight subsamples is tested.

Imported seed lots of 300 grams or less are considered to be 'small lots' that may be sampled differently. For such a small seed lot, rather than requiring a test of 20,000 seeds, one fifth (20 per cent) of the lot, calculated by weight, may be tested on arrival. This option is currently only available for on-shore testing.

Currently imported seed lots comprising fewer than 100 seeds will be grown in a Post-Entry Quarantine (PEQ) greenhouse and after germination a leaf sample from every plant will be tested for PepMV and the pospiviroids at eight weeks.

Current status of *Pepino mosaic virus* and the tomato-infecting pospiviroids in Australia

Table 1.1 summarises the status of PepMV and the tomato-infecting pospiviroids in Australia. No report of PepMV in Australia was found during the drafting of this report. However, several pospiviroids have been detected in Australia and some are now established and endemic.

Table 1.1 Status of PepMV and tomato-infecting pospiviroids in Australia

Pest	Status	Present in Australia	Distribution
<i>Pepino mosaic virus</i>	Quarantine pest	No	
<i>Citrus exocortis viroid</i>	Unregulated	Yes	All states and territories
<i>Columnea latent viroid</i>	Quarantine pest	No	
<i>Pepper chat fruit viroid</i>	Quarantine pest	No	
<i>Potato spindle tuber viroid</i>	Under evaluation	Yes	Qld, South Australia (under eradication), Victoria (under eradication) and Western Australia
<i>Tomato apical stunt viroid</i>	Quarantine pest	No	
<i>Tomato chlorotic dwarf viroid</i>	Quarantine pest	No	
<i>Tomato planta macho viroid</i>	Quarantine pest	No	

It has long been known that *Citrus exocortis viroid* (CEVd), a tomato-infecting pospiviroid that also infects citrus and grapevines, is present in Australia, although it is rare (Barkley & Büchen-Osmond 1988; Gillings, Broadbent & Gollnow 1991; Hardy et al. 2007).

In 2012, a national survey for PSTVd and other pospiviroids was undertaken in Australia that provided new information about pospiviroid infections of tomatoes and solanaceous ornamental plants. Tomato fruit production properties and ornamental nurseries were inspected and samples were tested for pospiviroids from more than 25,000 plants on 126 properties. Most of the samples were from tomato plants and through the survey, PSTVd was found in one greenhouse tomato crop in South Australia (SA). PSTVd was also detected in Qld in one line of an ornamental plant commonly called potato vine (*Solanum laxum* Spreng.). TCDVd was found in two *Calabrychoa* plants in SA and CEVd was detected in *Calabrychoa*, petunia and *Petchoa* plants in New South Wales (NSW), Qld, SA and Tasmania (Tas.).

The SA state government took action to eradicate PSTVd from the greenhouse tomato crop found in 2012. Surveys in 2013 and 2014 also detected PSTVd and PCFVd in greenhouse tomato crops in the same district of SA. However, only a single tomato plant was found infected with PCFVd and very few plants were found infected with PSTVd. The infected plants were destroyed and surveillance has continued to determine the status of the pospiviroids in SA. The SA state government also eradicated TCDVd by destroying the infected ornamentals found in 2012.

The *Solanum laxum* line found infected with PSTVd in Qld in 2012 was not destroyed because plants from the line had been sold to the public, and the same infected line was found at a second nursery at another location in Qld, which was a wholesale supplier. The Queensland state government judged that eradication was not technically feasible. Similarly, the ornamental plant lines found infected with CEVd in 2012 in NSW, Qld, SA and Tas. were not destroyed.

Currently, PepMV, CLVd, PCFVd, TASVd, TCDVd and TPMVd are considered to be absent from all Australian states and territories, whereas CEVd is believed to be present in all states and territories, and PSTVd is present in some areas in Qld and Western Australia (WA) but is otherwise absent.

Following the national survey for PSTVd in Australia in 2012, the CCEPP considered information from the surveys and detections of PSTVd. Whereas the CCEPP found that most areas of

Australia were free from PSTVd, the committee determined that the body of information showed the viroid to be present in some areas of Australia (Department of Agriculture 2015).

Australia notified the IPPC on 5 May 2015 that PSTVd had become established in the country (AUS-66/1). NSW, Victoria (Vic.), Tas. and the Northern Territory (NT) were reported to be free of PSTVd (Department of Agriculture and Water Resources, 2015). WA and Qld were reported to have cases of the viroid that had not been eradicated.

Domestic arrangements

The Australian Government is responsible for regulating the movement of goods such as plants and plant products into and out of Australia. However, the state and territory governments are responsible for plant health controls within their individual jurisdiction. Legislation relating to resource management or plant health may be used by state and territory government agencies to control interstate movement of plants and their products. Once plant and plant products have been cleared by Australian Government biosecurity officers, they may be subject to interstate movement conditions. It is the importer's responsibility to identify, and ensure compliance with all requirements.

1.2.2 Scope

This draft report presents an assessment of the risks of introducing into Australia the following pests with the importation of tomato seed and wild tomato seed from all sources for planting: PepMV, CLVd, PCFVd, TASVd, TCDVd and TPMVd. The draft report also presents a review of the existing emergency measures and proposes continuing phytosanitary measures. In this draft report, tomato (*Solanum lycopersicum* L.) is distinguished from wild tomato (*Solanum chilense*, *S. chmielewskii*, *S. parviflorum*, *S. peruvianum* and *S. pimpinellifolium*) for the purpose of regulation. Pathways for entry of these pests with seeds and nursery stock of other host plant species are not considered. Review work on other possible pathways for the listed pests, and other pests of tomato, falls outside the scope of this report, as does assessment of compliance with the emergency measures and with the requirements of any other regulatory and advisory body associated with importing commodities into Australia.

This draft report does not present a pest risk assessment for PSTVd, but a summary of the department's consideration of PSTVd is presented (Section 6.1).

The report proposes pest risk management measures to address the assessed risks and it reviews the existing emergency measures on tomato seed and wild tomato seed that target PepMV and the pospiviroids identified in previous sections.

1.2.3 Consultation

Between 2008 and 2013, the department communicated with tomato growers, importers and seed trading businesses, and with the National Plant Protection Organisations (NPPOs) of seed exporting countries about the introduction and revision of the emergency measures. Interested parties were provided with information by letter or email, and discussions were held by telephone, by teleconference or in face-to-face meetings.

Prior to their implementation and revision, the requirements of the emergency measures were published on the department's website and the department's Import Conditions system (ICON). Interested parties often identified themselves after one of the notifications. The department also

received comments from stakeholders when the emergency measures were implemented and revised.

Australia notified the World Trade Organisation of the emergency measures in May 2008 (WTO notification G/SPS/N/AUS/225) and notified the WTO and the International Plant Protection Convention (IPPC) of the amendments to the emergency measures in February, May and November 2012 (G/SPS/N/AUS/225/Add.1; G/SPS/N/AUS/225/Add.2; G/SPS/N/AUS/225/Add.3). Notifications were published on the WTO and IPPC websites.

The Australian Seed Federation (ASF), which is a peak body representing seed trading and importing businesses, was informed of the emergency measures prior to their introduction in 2008. In 2011, approximately seven months before the emergency measures were amended in 2012, the department wrote to a number of tomato seed importers and tomato fruit producers to explain the proposed measures and the reasons for the changes. Later in 2011 a number of teleconferences were held at which seed importers, including Australian representatives of major seed companies and other stakeholders commented and asked questions about the department's planned revisions. The ASF and seed businesses communicated directly with the department in response to the emergency measures on several occasions, as did representatives of seed businesses.

CCEPP meetings concerning PSTVd were held in 2008, 2011 and 2012, and CCEPP meetings concerning PCFVd were held in 2013. The Australian state and territory Chief Plant Health Managers were represented at these meetings, and the measures on tomato seed were discussed and supported. Teleconferences were also held with the Chief Plant Health Managers while the department was developing the proposal for revising the emergency measures in 2011 and 2012, and the managers supported the amendments to the measures.

In 2011 and 2012, the International Seed Federation (ISF) commented on the emergency measures. The ISF is based in Switzerland and represents major seed trading companies. The department responded formally, providing further information on the emergency measures and updating some references to ISF material in the documentation on the measures.

The emergency measures remain in place, and the requirements are now published on the department's BICON website.

1.2.4 Next Steps

Stakeholders are invited to comment on the outcomes of the review process and identify any scientific, technical, or other matters they believe require comment or attention.

The department will consider submissions received on the draft review and may consult informally with stakeholders. The final report will take into account relevant stakeholder comments.

The department will publish the final review on its website and notify registered stakeholders and the World Trade Organization (WTO) Secretariat. Recommendations made in the final report will form the basis of future import conditions.

2 Method for pest risk analysis

This chapter sets out the method used for the pest risk analysis (PRA) in this report. The Australian Government Department of Agriculture and Water Resources (the department) has conducted this PRA in accordance with the International Standards for Phytosanitary Measures (ISPMs), including ISPM 2: *Framework for pest risk analysis* (FAO 2007) and ISPM 11: *Pest risk analysis for quarantine pests* (FAO 2013) that have been developed under the SPS Agreement (WTO 1995).

A PRA is ‘the process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it’ (FAO 2015). A pest is ‘any species, strain or biotype of plant, animal, or pathogenic agent injurious to plants or plant products’ (FAO 2015). This definition is also applied in the *Biosecurity Act 2015*.

Biosecurity risk consists of two major components: the likelihood of a pest entering, establishing and spreading in Australia from imports and the consequences should this happen. These two components are combined to give an overall estimate of the risk.

Unrestricted risk is estimated taking into account the existing commercial production practices of the exporting country and that, on arrival in Australia, the department will verify that the consignment received is as described on the commercial documents and its integrity has been maintained.

Restricted risk is estimated with phytosanitary measure(s) applied. A phytosanitary measure is ‘any legislation, regulation or official procedure having the purpose to prevent the introduction and/or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests’ (FAO 2015).

A glossary of the terms used in the risk analysis is provided at the end of this report.

The PRAs are conducted in the following three consecutive stages: initiation, pest risk assessment and pest risk management.

2.1 Stage 1 Initiation

The initiation of a risk analysis involves identifying the pest(s) and pathway(s) that should be considered for risk analysis in relation to the identified PRA area. According to ISPM No. 2 (FAO 2016b), a PRA process may be initiated as a result of:

- identification of a pathway that presents a potential pest risk (a means of pest introduction or spread);
- identification of a pest that may require phytosanitary measures (a pest may have been detected or intercepted, a request made to import it or it may have been reported elsewhere);
- review or revision of existing phytosanitary policies and priorities; or
- identification of an organism not previously known to be a pest.

This PRA was initiated by the department to review and, if appropriate, revise the emergency measures introduced in 2012 and 2013 (see section 1.2.1). In Australia, PepMV and TCDVd have

been regulated as quarantine pests since February 2012 and May 2012, respectively, and CLVd, PCFVd, TASVd and TPMVd have been regulated as quarantine pests since December 2012. For this PRA, the 'PRA area' is defined as Australia for PepMV, CLVd, PCFVd, TASVd, TCDVd and TPMVd.

2.2 Stage 2 Pest risk assessment

A pest risk assessment (for quarantine pests) is the 'evaluation of the probability of the introduction and spread of a pest and of the magnitude of the associated potential economic consequences' (FAO 2015).

The following three, consecutive steps were used in pest risk assessment:

2.2.1 Pest categorisation

Pest categorisation identifies which of the pests with the potential to be on the commodity are quarantine pests for Australia and require pest risk assessment. A 'quarantine pest' is a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled (FAO 2015).

The process of a pest categorisation is summarized by ISPM No. 11 (FAO 2016e) as a screening procedure based on the following criteria:

- identity of the pest
- presence or absence in the PRA area
- regulatory status
- potential for establishment and spread in the PRA area
- potential for economic consequences (including environmental consequences) in the PRA area.

The quarantine pests were carried forward for pest risk assessment.

2.2.2 Assessment of the probability of entry, establishment and spread

Details of how to assess the 'probability of entry', 'probability of establishment' and 'probability of spread' of a pest are given in ISPM 11 (FAO 2013). The SPS Agreement (WTO 1995) uses the term 'likelihood' rather than 'probability' for these estimates. In qualitative PRAs, the department uses the term 'likelihood' for the descriptors it uses for its estimates of likelihood of entry, establishment and spread. The use of the term 'probability' is limited to the direct quotation of ISPM definitions.

A summary of this process is given here, followed by a description of the qualitative methodology used in this risk analysis.

Likelihood of entry

The likelihood of entry describes the likelihood that a quarantine pest will enter Australia as a result of trade in a given commodity, be distributed in a viable state in the PRA area and subsequently be transferred to a host. It is based on pathway scenarios depicting necessary steps in the sourcing of the commodity for export, its processing, transport and storage, its use in Australia and the generation and disposal of waste. In particular, the ability of the pest to survive is considered for each of these various stages.

The likelihood of entry estimates for the quarantine pests for a commodity are based on the use of the existing commercial production, packaging and shipping practices of the exporting country. Details of the existing commercial production practices for the commodity are set out in Chapter 3. These practices are taken into consideration by the department when estimating the likelihood of entry.

For the purpose of considering the likelihood of entry, the department divides this step into two components:

- **Likelihood of importation**—the likelihood that a pest will arrive in Australia when a given commodity is imported.
- **Likelihood of distribution**— the likelihood that the pest will be distributed, as a result of the processing, sale or disposal of the commodity, in the PRA area and subsequently transfer to a susceptible part of a host.

Factors to be considered in the likelihood of importation may include:

- distribution and incidence of the pest in the source area
- occurrence of the pest in a life-stage that would be associated with the commodity
- mode of trade (for example, bulk, packed)
- volume and frequency of movement of the commodity along each pathway
- seasonal timing of imports
- pest management, cultural and commercial procedures applied at the place of origin
- speed of transport and conditions of storage compared with the duration of the lifecycle of the pest
- vulnerability of the life-stages of the pest during transport or storage
- incidence of the pest likely to be associated with a consignment
- commercial procedures (for example, refrigeration) applied to consignments during transport and storage in the country of origin, and during transport to Australia.

Factors to be considered in the likelihood of distribution may include:

- commercial procedures (for example, refrigeration) applied to consignments during distribution in Australia
- dispersal mechanisms of the pest, including vectors, to allow movement from the pathway to a host
- whether the imported commodity is to be sent to a few or many destination points in the PRA area
- proximity of entry, transit and destination points to hosts
- time of year at which import takes place
- intended use of the commodity (for example, for planting, processing or consumption)
- risks from by-products and waste.

Likelihood of establishment

Establishment is defined as the ‘perpetuation for the foreseeable future, of a pest within an area after entry’ (FAO 2015). In order to estimate the likelihood of establishment of a pest, reliable

biological information (for example, lifecycle, host range, epidemiology and survival) is obtained from the areas where the pest currently occurs. The situation in the PRA area can then be compared with that in the areas where it currently occurs and expert judgement used to assess the likelihood of establishment.

Factors to be considered in the likelihood of establishment in the PRA area may include:

- availability of hosts, alternative hosts and vectors
- suitability of the environment
- reproductive strategy and potential for adaptation
- minimum population needed for establishment
- cultural practices and control measures.

Likelihood of spread

Spread is defined as ‘the expansion of the geographical distribution of a pest within an area’ (FAO 2015). The likelihood of spread considers the factors relevant to the movement of the pest, after establishment on a host plant or plants, to other susceptible host plants of the same or different species in other areas. In order to estimate the likelihood of spread of the pest, reliable biological information is obtained from areas where the pest currently occurs. The situation in the PRA area is then carefully compared with that in the areas where the pest currently occurs and expert judgement used to assess the likelihood of spread.

Factors to be considered in the likelihood of spread may include:

- suitability of the natural and/or managed environment for natural spread of the pest
- presence of natural barriers
- potential for movement with commodities, conveyances or by vectors
- intended use of the commodity
- potential vectors of the pest in the PRA area
- potential natural enemies of the pest in the PRA area.

Assigning likelihoods for entry, establishment and spread

Likelihoods are assigned to each step of entry, establishment and spread. Six descriptors are used: high; moderate; low; very low; extremely low; and negligible (Table 2.1). Definitions for these descriptors and their indicative probability ranges are given in Table 2.1. The indicative probability ranges are only provided to illustrate the boundaries of the descriptors and are not used beyond this purpose in qualitative PRAs. These indicative probability ranges provide guidance to the risk analyst and promote consistency between different pest risk assessments.

Table 2.1 Nomenclature of likelihoods

Likelihood	Descriptive definition	Indicative range
High	The event would be very likely to occur	$0.7 < \text{to} \leq 1$
Moderate	The event would occur with an even likelihood	$0.3 < \text{to} \leq 0.7$
Low	The event would be unlikely to occur	$0.05 < \text{to} \leq 0.3$
Very low	The event would be very unlikely to occur	$0.001 < \text{to} \leq 0.05$
Extremely low	The event would be extremely unlikely to occur	$0.000001 < \text{to} \leq 0.001$
Negligible	The event would almost certainly not occur	$0 < \text{to} \leq 0.000001$

Combining likelihoods

The likelihood of entry is determined by combining the likelihood that the pest will be imported into the PRA area and the likelihood that the pest will be distributed within the PRA area, using a matrix of rules (Table 2.2). This matrix is then used to combine the likelihood of entry and the likelihood of establishment, and the likelihood of entry and establishment is then combined with the likelihood of spread to determine the overall likelihood of entry, establishment and spread.

For example, if the likelihood of importation is assigned a descriptor of 'low' and the likelihood of distribution is assigned a descriptor of 'moderate', then they are combined to give a likelihood of 'low' for entry. The likelihood for entry is then combined with the likelihood assigned for establishment of 'high' to give a likelihood for entry and establishment of 'low'. The likelihood for entry and establishment is then combined with the likelihood assigned for spread of 'very low' to give the overall likelihood for entry, establishment and spread of 'very low'. This can be summarised as:

importation x distribution = entry [E]

low x moderate = low

entry x establishment = [EE]

low x high = low

[EE] x spread = [EES]

low x very low = very low

Table 2.2 Decision rules for determining the consequence impact score based on the magnitude of consequences at four geographic scales

	High	Moderate	Low	Very low	Extremely low	Negligible
High	High	Moderate	Low	Very low	Extremely low	Negligible
Moderate		Low	Low	Very low	Extremely low	Negligible
Low			Very low	Very low	Extremely low	Negligible
Very low				Extremely low	Extremely low	Negligible
Extremely low					Negligible	Negligible
Negligible						Negligible

Time and volume of trade

One factor affecting the likelihood of entry is the volume and duration of trade. If all other conditions remain the same, the overall likelihood of entry will increase as time passes and the overall volume of trade increases.

The department normally considers the likelihood of entry on the basis of the estimated volume of one year's trade. This is a convenient value for the analysis that is relatively easy to estimate and allows for expert consideration of seasonal variations in pest presence, incidence and behaviour to be taken into account. The consideration of the likelihood of entry, establishment and spread and subsequent consequences takes into account events that might happen over a number of years even though only one year's volume of trade is being considered. This difference reflects biological and ecological facts, for example where a pest or disease may establish in the year of import but spread may take many years.

The use of a one year volume of trade has been taken into account when setting up the matrix that is used to estimate the risk and therefore any policy based on this analysis does not simply apply to one year of trade. Policy decisions that are based on the department's method that uses the estimated volume of one year's trade are consistent with Australia's policy on appropriate level of protection and meet the Australian Government's requirement for ongoing quarantine protection. If there are substantial changes in the volume and nature of the trade in specific commodities then the department will review the risk analysis and, if necessary, provide updated policy advice.

2.2.3 Assessment of potential consequences

The objective of the consequence assessment is to provide a structured and transparent analysis of the potential consequences if the pests or disease agents were to enter, establish and spread in Australia. The assessment considers direct and indirect pest effects and their economic and environmental consequences. The requirements for assessing potential consequences are given in Article 5.3 of the SPS Agreement (WTO 1995), ISPM 5 (FAO 2015) and ISPM 11 (FAO 2013).

Direct pest effects are considered in the context of the effects on:

- plant life or health

- other aspects of the environment.

Indirect pest effects are considered in the context of the effects on:

- eradication, control
- domestic trade
- international trade
- non-commercial and environmental.

For each of these six criteria, the consequences were estimated over four geographic levels, defined as:

Local—an aggregate of households or enterprises (a rural community, a town or a local government area).

District—a geographically or geopolitically associated collection of aggregates (generally a recognised section of a state or territory, such as ‘Far North Queensland’).

Regional—a geographically or geopolitically associated collection of districts in a geographic area (generally a state or territory, although there may be exceptions with larger states such as Western Australia).

National—Australia wide (Australian mainland states and territories and Tasmania).

For each criterion, the magnitude of the potential consequence at each of these levels was described using four categories, defined as:

Indiscernible—pest impact unlikely to be noticeable.

Minor significance—expected to lead to a minor increase in mortality/morbidity of hosts or a minor decrease in production but not expected to threaten the economic viability of production. Expected to decrease the value of non-commercial criteria but not threaten the criterion’s intrinsic value. Effects would generally be reversible.

Significant—expected to threaten the economic viability of production through a moderate increase in mortality/morbidity of hosts, or a moderate decrease in production. Expected to significantly diminish or threaten the intrinsic value of non-commercial criteria. Effects may not be reversible.

Major significance—expected to threaten the economic viability through a large increase in mortality/morbidity of hosts, or a large decrease in production. Expected to severely or irreversibly damage the intrinsic ‘value’ of non-commercial criteria.

The estimates of the magnitude of the potential consequences over the four geographic levels were translated into a qualitative impact score (A-G) using Table 2.3. For example, a consequence with a magnitude of ‘significant’ at the ‘district’ level will have a consequence impact score of D.

Table 2.3 Decision rules for determining the consequence impact score based on the magnitude of consequences at four geographic scales

Magnitude	Geographic scale			
	Local	District	Region	Nation
Indiscernible	A	A	A	A
Minor significance	B	C	D	E
Significant	C	D	E	F
Major significance	D	E	F	G

Note: In earlier qualitative PRAs, the scale for the impact scores went from A to F and did not explicitly allow for the rating 'indiscernible' at all four levels. This combination might be applicable for some criteria. In this report, the impact scale of A to F has been changed to become B-G and a new lowest category A ('indiscernible' at all four levels) was added. The rules for combining impacts were adjusted accordingly.

The overall consequence for each pest is achieved by combining the qualitative impact scores (A–G) for each direct and indirect consequence using a series of decision rules. These rules are mutually exclusive, and are assessed in numerical order until one applies.

Table 2.4 Decision rules for determining the overall consequence rating for each pest

Rule	The impact scores for consequences of direct and indirect criteria	Overall consequence rating
1	Any criterion has an impact of 'G'; or more than one criterion has an impact of 'F'; or a single criterion has an impact of 'F' and each remaining criterion an 'E'.	Extreme
2	A single criterion has an impact of 'F'; or all criteria have an impact of 'E'.	High
3	One or more criteria have an impact of 'E'; or all criteria have an impact of 'D'.	Moderate
4	One or more criteria have an impact of 'D'; or all criteria have an impact of 'C'.	Low
5	One or more criteria have an impact of 'C'; or all criteria have an impact of 'B'.	Very Low
6	One or more but not all criteria have an impact of 'B', and all remaining criteria have an impact of 'A'.	Negligible

2.2.4 Estimation of the unrestricted risk

Once the assessment of the likelihood of entry, establishment and spread and for potential consequences are completed, the unrestricted risk can be determined for each pest or groups of pests. This is determined by using a risk estimation matrix to combine the estimates of the likelihood of entry, establishment and spread and the overall consequences of pest establishment and spread. Therefore, risk is the combination of likelihood and consequence.

When interpreting the risk estimation matrix, note the descriptors for each axis are similar (for example, low, moderate, high) but the vertical axis refers to likelihood and the horizontal axis refers to consequences. Accordingly, a 'low' likelihood combined with 'high' consequences, is not the same as a 'high' likelihood combined with 'low' consequences—the matrix is not symmetrical. For example, the former combination would give an unrestricted risk rating of 'moderate', whereas, the latter would be rated as a 'low' unrestricted risk.

Table 2.5 Risk estimation matrix

Likelihood of pest entry, establishment and spread	Consequences of pest entry, establishment and spread					
	Negligible	Very low	Low	Moderate	High	Extreme
High	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Moderate	Negligible risk	Very low risk	Low risk	Moderate risk	High risk	Extreme risk
Low	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk	High risk
Very low	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk	Moderate risk
Extremely low	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk	Low risk
Negligible	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Negligible risk	Very low risk

2.2.5 The appropriate level of protection (ALOP) for Australia

The SPS Agreement defines the concept of an ‘appropriate level of sanitary or phytosanitary protection (ALOP)’ as the level of protection deemed appropriate by the WTO Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory.

Like many other countries, Australia expresses its ALOP in qualitative terms. The ALOP for Australia, which reflects community expectations through government policy, is currently expressed as providing a high level of sanitary or phytosanitary protection aimed at reducing risk to a very low level, but not to zero. The band of cells in Table 2.5 marked ‘very low risk’ represents the ALOP for Australia.

2.3 Stage 3 Pest risk management

Pest risk management describes the process of identifying and implementing phytosanitary measures to manage risks to achieve the ALOP for Australia, while ensuring that any negative effects on trade are minimised.

The conclusions from pest risk assessment are used to decide whether risk management is required and if so, the appropriate measures to be used. Where the unrestricted risk estimate does not achieve the ALOP for Australia, risk management measures are required to reduce this risk to a very low level. The guiding principle for risk management is to manage risk to achieve the ALOP for Australia. The effectiveness of any proposed phytosanitary measures (or combination of measures) is evaluated, using the same approach as used to evaluate the unrestricted risk, to ensure the restricted risk for the relevant pest or pests achieves the ALOP for Australia.

ISPM 11 (FAO 2013) provides details on the identification and selection of appropriate risk management options and notes that the choice of measures should be based on their effectiveness in reducing the likelihood of entry of the pest.

Examples given of measures commonly applied to traded commodities include:

- options for consignments—for example, inspection or testing for freedom from pests, prohibition of parts of the host, a pre-entry or post-entry quarantine system, specified conditions on preparation of the consignment, specified treatment of the consignment, restrictions on end-use, distribution and periods of entry of the commodity
- options preventing or reducing infestation in the crop—for example, treatment of the crop, restriction on the composition of a consignment so it is composed of plants belonging to resistant or less susceptible species, harvesting of plants at a certain age or specified time of the year, production in a certification scheme
- options ensuring that the area, place or site of production or crop is free from the pest—for example, pest-free area, pest-free place of production or pest-free production site
- options for other types of pathways—for example, consider natural spread, measures for human travellers and their baggage, cleaning or disinfestations of contaminated machinery
- options within the importing country—for example, surveillance and eradication programs
- prohibition of commodities—if no satisfactory measure can be found.

Risk management measures are identified for each quarantine pest where the level of biosecurity risk does not achieve the ALOP for Australia. These are presented in Chapter 7 of this draft report.

3 **Commercial tomato seed production and trade**

3.1 **Tomato seed production and trade**

This section on commercial tomato seed production and trade draws on published and unpublished sources. These include documents that accompanied seed imports monitored by the department during the period of application of emergency measures, and an audit report by the Interim Inspector General Biosecurity (IIGB) 'Effectiveness of biosecurity controls for importation of tomato and carrot seeds', which detailed substantial information on the international production of tomato seed (IIGB 2016).

Tomato fruit is one of the most important vegetable crops globally. The 'scale of production, trade and distribution has increased tremendously' in recent years (GSPP 2013), and it is believed that tomato seed production and trade has increased concomitantly. Tomato seed importers support Australian agriculture, as most tomato fruit production crops in Australia are grown from imported seed, and almost all tomato breeding is done overseas.

Tomato fruit growers usually order seed from Australian representatives of international seed trading companies or from Australian businesses with links to international seed trading companies. On average 760 kilograms of tomato seed is imported into Australia annually, and is grown into more than 200 million tomato plants.

Almost all of the imported seed is thought to be first generation (F1) hybrid seed produced by cross pollination (hybridisation) of parental lines. Tomato hybrids are reported to have better vigour, uniformity, disease resistance and stress tolerance, and to have desirable horticultural traits including early fruiting, longer shelf life and consistent yield (IIGB 2016).

Tomato seed production is relatively complex. The production process begins with plant breeding and involves the production of parent lines which are usually hybridised to produce the seed. After harvesting the fruit, the seed is extracted and separated from the pulp using processes that clean the seed. Typically the pulp is fermented for several hours, washed with an acidic solution, and then washed with water several times. This extraction process may be done on site by the farm workers. Before packaging, the seed may be further treated with chemicals including fungicides, and it may be primed for germination or pelleted. The seed is often treated after shipment to a facility in another country. After extraction and treatment, seed lots may be stored for several years and portions of a lot are sold to fruit production growers and nurseries in many countries.

3.2 **International tomato seed production**

The hybridisation step is important (section 3.1) as it is very labour intensive, and for this reason hybrid tomato seed is commonly produced in countries where labour costs are low (IIGB 2016; ISF 2017).

The IIGB noted that:

Seeds present significant biosecurity risks due to the numerous complex, variable international production pathways, including contracted farms in countries where biosecurity might not always be consistent with Australian standards (IIGB 2016).

Hybrid tomato seed sent to Australia is produced from crops grown in countries in Asia, Europe, Africa, the Middle East and the Americas.

The production of some hybrid tomato seed lots typically involves activities in several countries (IIGB 2016; ISF 2017). Plant lines used to produce hybrid seed are often grown, selected and multiplied in two or three countries successively (IIGB 2016; ISF 2017; Werkman & Sansford 2008). As an example, two parental lines may be bred in the Netherlands, then larger quantities of seed of these lines (basic seed) may be produced in France or Spain, and this basic seed may then be grown in Thailand or China where the tomato flowers are cross-pollinated to produce the hybrid seed (IIGB 2016; ISF 2017). In another example observed by the department, parental lines bred and selected in a country in the Northern Hemisphere were sent to a country in the Southern Hemisphere where breeding and selection continued so that two seasons of breeding were achieved in a single year.

Furthermore, after production some tomato seed is trans-shipped by airfreight through other countries. Some tomato seed sent to Australia is trans-shipped through France, Israel, Japan, the Netherlands or the USA.

Lot numbers are usually used to identify seed production lots produced on one farm or field in one season. The IIGB was informed that in Thailand seed lot numbers are retained unaltered from the field production site through to the sale of the seed (IIGB 2016).

During processing and shipment tomato seed from one lot is commonly divided up into a series of batches. Batches may be treated differently and are commonly re-packaged. Each time a batch is divided, treated or repackaged, the batch and its derivatives are usually assigned new batch numbers.

3.3 Production supervision

When considering biosecurity, it is notable that seed trading businesses selling tomato seed to Australian growers do not usually produce the seed, nor do they fully supervise the production. Instead the supervision is sub-contracted to businesses that work in the countries where the seed is produced (Tay 2002; Venkateswarlu 2007). Furthermore, subcontractors that organise production (organisers), commonly contract out production of seed to many different growers (Tay 2002; Venkateswarlu 2007).

The IIGB observed these business relationships in Thailand and noted that:

Major international seed companies contract out vegetable seed production (including tomato and carrot seeds) in one or more of around 25 countries... (IIGB 2016).

Larger seed companies typically contract out the production and multiplication processes to farmers, farmers' associations or private firms, often in countries with low production costs (IIGB 2016).

The IIGB (2016) reviewed the production of hybrid tomato seed by one company in Thailand and noted a range of phytosanitary and traceability measures. It is not clear whether the same phytosanitary measures, or similar ones, are practiced in other countries or practised by other seed production businesses.

The measures in Thailand noted by the IIGB included:

- inspections by field supervisors, who are employees of the subcontracting business, to monitor crop production and sanitation practices and ensure records of pest and disease incidents and the use of chemicals are maintained.
- inspection by a visiting plant pathologist, who is an employee of the parent seed trading company, to ensure that pest and disease incidents are managed as early as possible, and ensure that production practices meet agreed protocols and requirements.
- collection of leaf samples from diseased plants by the plant pathologist which are sent to the parent company laboratory for testing.
- inspections by quarantine inspectors of the Thailand Department of Agriculture.

3.4 The Good Seed and Plant Practices System

The IIGB visited tomato seed production facilities in Thailand and the Netherlands that operate under the Good Seed and Plant Practices (GSPP) system. The GSPP system sets a number of standards for production (GSPP 2013) with the aim of minimising the risk posed by the seed-transmitted bacterial pathogen *Clavibacter michiganensis* subsp. *michiganensis*, which causes bacterial canker of tomatoes. The GSPP system incorporates phytosanitary measures including containment of plants in controlled facilities, monitoring inputs, and laboratory tests of seed and plant samples.

When considering the GSPP seed production system the IIGB noted that:

In Thailand, GSPP facilities consisted of a number of low-cost net houses and were spread out in a relatively large area. In the Netherlands, sophisticated, automated glasshouse facilities employed, state-of-the-art technology and follow strict hygiene and safety protocols that significantly reduce the risk of *Clavibacter michiganensis* spp. *michiganensis* infection in tomato seed [sic] (IIGB (2016)).

Only a small proportion of internationally traded tomato seed is produced under the GSPP system, because of the associated costs.

3.5 Biosecurity concerns related to production systems

The level of biosecurity practised in tomato seed production systems varies considerably. The phytosanitary measures noted above by the IIGB may reduce certain phytosanitary risks. Other aspects of tomato seed production may introduce or increase phytosanitary risks.

The parental plants are commonly grown in open fields (IIGB 2016) and so the plants are exposed to invertebrates that may transmit seed-transmitted plant pathogens to the plants. Equally importantly, because hybridisation involves emasculation of flowers and pollination by hand (Cheema & Dhaliwal 2005) it has the potential to mechanically transmit and spread certain plant pathogens.

By growing plant lines in several different countries successively, the plant lines may be exposed to a greater range of pathogens than present in a single country. Furthermore, the places where seed production crops are grown change relatively often, as tomato crops are typically rotated every year in response to pest and pathogen pressures (Gould 2013). Moreover, the location of crops will change as the organisers and farm businesses, who are independent of the seed trading businesses, make decisions about subcontracted seed production work every year (Venkateswarlu et al. 2015).

Blending of seed may also introduce infected seeds to healthy seed lots, and may produce seed lots that include very small numbers of infected seeds, which are difficult to detect because of their small numbers. Blending of vegetable seed lots is a standard commercial practice (Bello & Bradford 2016; ISF 2017) but no report was found during the preparation of this draft report that indicated how often blending is done.

The IIGB noted that:

Except under certain circumstances (such as, to meet demand of one buyer for large quantities of seeds of a particular variety), seeds produced in different countries are not bulked up, and are sold as separate lots/batches (IIGB (2016).

In describing the tomato seed production process the IIGB (2016) provided evidence that:

- workers in the tomato seed crops become contaminated by plant sap;
- the identities of suspected bacterial infections of tomato plants were investigated, but tomato plants suspected to be infected by viruses were disposed of, and the infections were not necessarily investigated, and
- seed infected with PepMV might be packaged before it is disinfected.

This evidence is significant because the virus species and viroids reviewed in this draft report are easily transmitted when worker's hands and equipment are contaminated. Under these circumstances, the pathogens are transmitted through plant sap and by minor accidental abrasions of plants, as discussed in other sections of this draft report.

The evidence provided by the IIGB (2016) is also significant because when the causes of plant disease symptoms are not investigated in a seed production crop, as indicated for suspected viral infections, the business and the country concerned may not be aware of the health status of the exported seed. Furthermore, the packaging of seed from plants infected with PepMV that has not been decontaminated may contaminate packaging materials, equipment and other seed lots.

Seed lots infected with the listed pospiviroids have been detected many times by Australian laboratory testing, and seed lots infected with PepMV have been detected many times by European testing (Sections 4.6, 4.7 and 5.8). In light of this evidence, it appears that the standard phytosanitary measures used by the tomato seed production industry do not ensure that exported seed is free of these pathogens. In many instances infecting pathogens are not detected or identified until the seed reaches the Australian border.

Exporting countries usually manage the phytosanitary risks of exported seed by certifying the phytosanitary condition of seed lots. Exporting countries commonly visually inspect seed crops to assure themselves that certain pests are not present in the crop. However many infections cannot be detected by visual inspection, it is difficult to identify PepMV-infected and pospiviroid-infected plants (sections 4.4 and 5.2), and sometimes these plants are asymptomatic. It is also difficult to comprehensively inspect large crops and many small farms. Therefore, visual inspection of crops is not a suitable method for detecting these pathogens.

The ISF has also recognised that phytosanitary certification of seed can be challenging because the final destination of the seed may not be known when the seed is produced (ISF 2017). Failing to retain the seed lot number, which distinguishes the place and season of production, and re-

packaging and re-labelling of seed lots may also make phytosanitary certification difficult or unreliable.

Another element of the phytosanitary risk relates to the distribution and cultivation of trial lines or trial lots of tomato seed. Fruit production businesses and seed businesses collaborate to grow trial lines to determine their suitability for Australian conditions. Tomato trial lines are usually grown at the same time and in the same place as larger fruit production crops. Significantly, trial lines are sometimes a source of pospiviroid outbreaks (van Brunschot et al. 2014b) and trial lines of seeds have been found by Australian seed testing to be contaminated with infected seeds (section 5.8).

4 Pepino mosaic virus

4.1 Pepino mosaic virus biology

Pepino mosaic virus (PepMV) belongs to the genus *Potexvirus*, family Alphaflexiviridae. Like other potexviruses it has filamentous particles and a small monopartite, positive-strand RNA genome (Verchot-Lubicz, Ye & Bamunusinghe 2007). Currently five main genetic variants of PepMV are recognised as strains. The North American strains (US genotypes), PepMV-US1 and PepMV-US2, are closely related to each other but differ from the European (EU genotype), Chilean (CH2 genotype) and Peruvian (LP genotype) strains (Hanssen et al. 2009; Moreno-Pérez et al. 2014; van der Vlugt 2009).

PepMV was originally detected on pepino plants (*Solanum muricatum*) in Peru in 1974 (Jones, Koenig & Lesemann 1980). It was first reported affecting major crops when it was found infecting tomato in Germany, the Netherlands and United Kingdom in 1999 (van der Vlugt et al. 2002; van der Vlugt et al. 2000; Wright & Mumford 1999). PepMV has since been detected in many countries in Europe and in countries in Africa, Asia and Americas, with this transcontinental distribution probably produced by recent rapid spread (Gómez, Sempere & Aranda 2012)(section 4.6; Table 4.1).

PepMV has a moderately wide host range. It has been found infecting tomato and pepino crops and cultivated capsicum, potato and basil (*Ocimum basilicum*) (Blystad et al. 2015; Davino et al. 2009a; Hanssen & Thomma 2010). Experiments have shown the virus may also infect eggplant (*Solanum melongena*), but as yet no natural infections have been observed in eggplant crops (EPPO 2014d; Hanssen & Thomma 2010).

In addition to cultivated plants, PepMV infects a range of weed species and species of wild plants (Blystad et al. 2015; CSL 2005; Soler et al. 2002). Several of the weedy and wild alternative hosts are from the family Solanaceae, whereas others are from the Amaranthaceae, Asteraceae, Boraginaceae, Brassicaceae, Chenopodiaceae, Convolvulaceae, Malvaceae, Plantaginaceae and Polygonaceae (Córdoba, Martinez-Priego & Jordá 2004; Papayiannis, Kokkinos & Alfaro-Fernández 2012; Soler et al. 2002).

4.2 Presence in seeds, fruits and floral parts

PepMV spreads through plants systemically, and has been detected in leaves, fruits, roots, flower parts and on seeds (Alfaro-Fernández et al. 2009; Ling 2008; Mehle et al. 2014; Minicka et al. 2016; Özdemir 2010; Schwarz et al. 2010; Shipp et al. 2008). Within leaves, the virus has been detected in phloem and xylem vessels, sieve elements and spongy and palisade mesophyll (Alfaro-Fernández et al. 2009; Minicka et al. 2016).

In tomato flowers, PepMV particles have been detected in the stigma, petals, anthers and anther filaments (Ling 2008; Özdemir 2010; Shipp et al. 2008). No report was found indicating PepMV particles are present in pollen, but the virus has been detected on bumble bees (*Bombus impatiens*) that have been foraging on infected plants (Shipp et al. 2008) suggesting that pollen may carry the virus and may be contaminated with virus particles.

PepMV has been detected many times in commercially traded tomato seed lots (Table 4.2). One significant experiment suggested the virus is present in the seed coat (testa), as well as on seed surfaces (Ling 2008). The virus was not detected in embryonic tissue or in seed membranes (Ling 2008).

Fruit from infected plants are reported to contain high concentrations of the virus and this is so even of fruit that do not show symptoms (van der Vlugt 2009).

4.3 Transmission of pepino mosaic virus

PepMV is mechanically transmitted and highly contagious to tomato plants. The virus spreads within crops when plants are pruned or suffer minor abrasions, as occurs when plants are handled and also when plants touch each other (Jones, Koenig & Lesemann 1980; Spence et al. 2006; Wright & Mumford 1999). It is transmitted when tools and worker's hands and clothes become contaminated (Hanssen & Thomma 2010; van der Vlugt 2009). Once the virus enters a tomato production facility it is difficult to contain and prevent its spread, and all the plants may become infected (Hanssen & Thomma 2010). PepMV particles are relatively stable at room temperature and can remain infectious for several weeks in plant debris, and on contaminated surfaces and in water (van der Vlugt 2009), adding to the problems of control within crops.

The virus can be transmitted by insects. PepMV is transmitted by the greenhouse whitefly (*Trialeurodes vaporariorum*) and bumble bees (*Bombus impatiens*), the former achieving transmission when feeding on the plants, the later when pollinating (Lacasa et al. 2003; Noël, Hance & Bragard 2014; Shipp et al. 2008). It has been suggested that infection often occurs when flowers are pollinated by bumble bees and then spreads to other parts of tomato plants.

PepMV is transmitted through water after being released from the roots of infected plants. The motile zoospores of the fungus *Oplidium virulentus* may be required for transmission through water or may just assist transmission (Alfaro-Fernández et al. 2010; Ling & Scott 2007; Mehle et al. 2014; Schwarz et al. 2010).

Transport with live plant material, including seeds and seedlings, is likely to be responsible for the introduction of the virus into crops (Córdoba-Sellés et al. 2007) and into geographic regions (Werkman & Sansford 2010). Long-distance transport of the virus within fruit is also considered possible (van der Vlugt 2009; Werkman & Sansford 2010), as fruit from infected plants contain high concentrations of the virus, and people can transmit the virus from plant to plant if it is on their hands or clothes (Hanssen et al. 2009; van der Vlugt 2009). Bumble bees will also transmit the virus between adjacent crops (Shipp et al. 2008).

4.4 Symptoms in tomato

PepMV disease reduces the quality of tomatoes and causes crop losses (Spence et al. 2006). The leaves of PepMV-infected plants typically develop distorted leaves that have a blistered appearance. The leaves may also develop chlorosis, yellow angular spots, severe leaf mosaics and necrosis. Brown streaks may appear on the stems and stems may become necrotic (Hanssen & Thomma 2010). Plants may develop the 'nettle-head' form, with the upper young leaves and shoots becoming stunted (Hanssen & Thomma 2010). PepMV-infected plants may be stunted or distorted or may wilt and collapse (EPPO 2014d).

Fruit from infected plants may be discoloured and have a marbled or mosaic appearance with patches of yellow and red or green and red (Hanssen & Thomma 2010). Fruit may split and become open so that the seed and flesh is exposed (Hanssen & Thomma 2010).

The degree of visible disease on the vegetative parts of PepMV-infected tomato plants varies widely, with some plants exhibiting severe symptoms, others expressing mild symptoms and some being symptomless. Importantly, symptomless infected plants and plants with mild

symptoms are difficult to recognise and may be missed when crops are inspected. The range of symptom expression may be due to environmental factors (Chitambar 2015). Low temperatures and low light conditions are reported to favour the appearance of more pronounced symptoms (van der Vlugt 2009). Infected tomato plants with no vegetative symptoms may still develop symptoms on their fruits. Co-infection of plants with both the EU and CH2 genotypes results in enhanced PepMV symptoms (Hanssen et al. 2008), but no other correlation has been confirmed between different PepMV genotypes and the severity of symptom expression (Blystad et al. 2015; Hanssen et al. 2008; Pagán et al. 2006).

4.5 Seed transmission and seed disinfestation

The transmission rate of a pathogen in seeds is defined as the proportion of seedlings that become infected when plants are grown from seeds from pathogen-infected parent plants. The rate is measured using only seeds from infected parent plants, not mixed with seed from healthy plants, and is most accurately measured when a great proportion of the seeds carry the pathogen.

It is now generally accepted that PepMV is transmitted from infected seeds to seedlings (Moreno-Pérez et al. 2014; Werkman & Sansford 2010), but this fact was not previously clear, as some early studies generated contradictory information. It is now known that the transmission rate of PepMV via seed is exceedingly low and it is reduced when the seed is cleaned. Importantly, chemical and heat treatments that could eliminate PepMV from tomato seeds have been compared (Ling 2010).

One of the first reported experiments on seed transmission of PepMV was done by germinating a small number of seeds ($n=50$), a method known as a 'grow-out test' or 'grow-out experiment' (Salomone & Roggero 2002). No infected plants were detected in the first reported grow-out experiment, suggesting the virus was not transmitted via seed. However, using a larger number of seeds ($n=168$), indicated a transmission rate of about 2 per cent (Córdoba-Sellés et al. 2007). A much greater number of seeds was used ($n=87,000$) in a third published grow-out experiment that confirmed transmission via seed (Hanssen et al. 2010). In this third experiment, the seed was cleaned using an acid and enzymatic treatment, following an industry standard method (Hanssen et al. 2010). The overall seed transmission rate was estimated to be 0.026 per cent, after cleaning, and seed transmission rates for different tomato lines were estimated to vary from 0.057 per cent to as low as 0.005 per cent (Hanssen et al. 2010).

Experiments where tomato seeds were baked (dry heat treated) indicated that 80°C for 72 hours was sufficient to eliminate the virus, while maintaining a high seed germination rate (Ling 2010). In comparison, experiments using chemical treatments did not eliminate the virus from seeds (Hanssen et al. 2010; Ling 2010).

PepMV has not been found in seed embryonic tissue and for this reason it is usually considered to be 'seed-borne' by virologists rather than 'seed-transmitted' (Ling 2008). The experiments on seed transmission indicate that PepMV virus particles are largely present on the outside of seeds, but also indicate that particles may be present in the seed coat (testa) (Ling 2008). Virus on the exterior of seeds can infect seedlings as they break through the seed coat.

4.6 Outbreaks in other countries and interceptions

Many outbreaks of PepMV in tomato crops have been reported, as have interceptions of PepMV-infected tomato fruit and seeds. The reports come from three sources: the scientific literature, a European PRA for PepMV (Werkman & Sansford 2010) and the European and Mediterranean Plant Protection Organization (EPPO). Tables 4.1 and 4.2 collate the reports from the EPPO and the scientific literature, but not the European PRA. Together the reports show there has been a widespread international outbreak of the virus across Africa, Asia, Europe and the Americas (Table 4.1) (CABI 2011; Clark & Crook 2012; Gómez, Sempere & Aranda 2012; Werkman & Sansford 2010). The reports suggest that control of the virus by current processes has not been fully effective, that the geographic distribution of the virus is uncertain, and that there are many unreported infections.

From 1999 to 2010, 102 detections of PepMV in tomato crops were reported by the EPPO and by others in the scientific literature (Table 4.1). The international outbreak probably began in 1999 and has continued since then (Table 4.1) (CABI 2011; Clark & Crook 2012; Gómez, Sempere & Aranda 2012). Before 1999 there were no reports of the virus in tomato crops, but in 1999 the virus was found in greenhouse tomato crops in Germany, the Netherlands and the United Kingdom (EPPO 2002c). In the year 2000, PepMV was detected in tomato fruit production at 15 locations in four countries in Europe (Table 4.1). In 2001, the virus was detected in tomato crops at 18 locations in ten countries in Europe and the Americas, and in 2002, it was detected at nine locations in seven countries in Europe and the Americas (Table 4.1).

The European PRA for PepMV provided a summary of surveys for PepMV by member states (MS) between 2000 and 2010 (Werkman & Sansford 2010). Fruit production sites and three categories of plant material were surveyed: tomato seed, plants for planting and fruit being marketed. Significantly, the European surveys provided evidence of many more outbreaks and detections than the 102 detections reported in other public sources (Werkman & Sansford 2010). The PRA indicated that 80 fruit lots were infected out of 461 lots on the European market that were surveyed in 2009 (Werkman & Sansford 2010). However, many reports summarised in the European PRA were made anonymously and the PRA noted:

In reviewing the surveys for this PRA it is important to note that not all MS appear to have reported to the EC or there are no data available, and, of those that have undertaken surveys, some have not reported on all 4 categories. The intensity of surveillance has also varied between years and MS.

The European PRA also stated there were 261 notifications of non-compliant PepMV-infected fruit from Europe and North Africa between 2000 and 2010 (Werkman & Sansford 2010).

It is suspected that the international outbreak was due in part to trade in infected seed (CABI 2011; Clark & Crook 2012). Many infected seed lots have been intercepted in Europe (section 4.7). The nearly simultaneous outbreaks in 1999 in three countries in Europe (EPPO 2002c), suggest that infected tomato seed was probably the source. Local spread by other transmission mechanisms between the three locations is unlikely to account for the correlated timing, as local spread takes longer to cover such distances. Tellingly, PepMV was detected in seed from the Netherlands in 2000 (EPPO 2000a) and in a seed crop in Chile in 2001 (Werkman & Sansford 2010).

Considering the detections of the virus in crops and in seed, the European PRA for PepMV concluded that ‘there is uncertainty regarding the exact distribution of PepMV’ (Werkman & Sansford 2010). New Zealand also published a risk analysis of PepMV in tomato seeds and considered that this virus was likely more widespread than currently officially recognised (CABI 2011; Clark & Crook 2012).

Outside Europe, PepMV has been found affecting tomato crops in Canada, China, Chile, Ecuador, Guatemala, Peru, Mexico, Morocco, South Africa, Thailand and in the USA (Werkman & Sansford 2010).

Table 4.1 Reports of PepMV outbreaks in tomato

Year	Facility	Country	Number of reports	Reference
1999	Tomato crops	UK	Unknown	(EPP0 2000a; Wright & Mumford 1999)
1999	Tomato crops	Netherlands	Unknown	(van der Vlugt et al. 2000)
1999	Fruit production	Germany	1	(EPP0 2002c)
2000	Fruit production	UK	3	(EPP0 2001a)
2000	Fruit production	Germany	1	(EPP0 2001a, 2002c; Lesemann et al. 2000)
2000	Fruit production	Netherlands	5	(EPP0 2001a)
2000	Fruit production	Spain	6	(EPP0 2003e)
2001	Glasshouse tomato plants	Italy	1	(EPP0 2001a; Roggero 2001)
2001	Fruit production (glasshouses)	Finland	6	(EPP0 2001a; KTTK 2002; Lemmetty et al. 2011)
2001	Glasshouse tomato plants	Germany	1	(EPP0 2001b, 2002c)
2001	Glasshouse tomato plants	Canada	1	(French et al. 2001)
2001	Fruit production	USA	4	(French et al. 2001)
2001	Glasshouse tomato plants	Sweden	1	(EPP0 2002c)
2001	Fruit production	Norway	1	(EPP0 2002c)
2001	Fruit production	Belgium	1	(EPP0 2003e)
2001	Fruit production (Nursery)	Denmark	1	(EPP0 2003e)
2001	Tomato crops for seed	Chile	1	(EPP0 2005b)
2002	Glasshouse tomato plants	Poland	1	(EPP0 2002c, 2003b)
2002	Fruit production (Nursery)	Denmark	1	(EPP0 2003e)
2002	Fruit production	Belgium	1	(EPP0 2003e)
2002	Fruit production (glasshouses)	France	1	(EPP0 2003e)
2002	Tomato crops	Peru	1	(EPP0 2002b)
2002	Fruit production	Ireland	1	(EPP0 2003e)
2002	Fruit production	UK	3	(EPP0 2003e)
2003	Fruit production (glasshouses)	Finland	1	(EPP0 2003a)
2003	Fruit production (glasshouses)	Germany	1	(EPP0 2003c)
2003	Tomato plants (non-commercial plastic house)	Slovakia	1	(EPP0 2004a)
2004	Fruit production (glasshouse)	Bulgaria	1	(EPP0 2004b)

2004	Fruit production	UK	5	(EPP0 2004e)
2004	Fruit production (glasshouses)	Hungary	1	(EPP0 2004c)
2004	Tomato crops	Switzerland	1	(EPP0 2006; Staubli 2005)
2002	Tomato crops for seed	Chile	1	(EPP0 2005b)
2005	Tomato crops	Chile	1	(EPP0 2005b)
2005	Glasshouse tomato plants	Italy	1	(Davino et al. 2006)
2005	Fruit production	Poland	Unknown	(Pospieszny & Borodynko 2006)
2006	Fruit production	Austria	3	(EPP0 2007)
2007	Glasshouse tomato plants	USA	Unknown	(EPP0 2008d)
2008	Fruit production (glasshouse)	Czech Republic	1	(EPP0 2008c)
2008	Tomato crops	Spain	1	(Alfaro-Fernández et al. 2008)
2008	Tomato crops (glasshouse)	Turkey	1	(Özdemir 2010)
2008	Fruit production	South Africa	1	(Carmichael et al. 2011)
2009	Tomato crops (glasshouse)	Turkey	1	(Özdemir 2010)
2009	Tomato crops (glasshouse)	Cyprus	1	(EPP0 2012d)
2010	Fruit production (glasshouse)	Italy	6	(EPP0 2011g)
2010	Fruit production (field)	Cyprus	3	(Papayiannis, Kokkinos & Alfaro-Fernández 2012)
2010	Fruit production (glasshouse)	Greece	2	(Efthimiou et al. 2011; EPP0 2012d)
2010	Fruit production (glasshouse)	Mexico	1	(EPP0 2012d; Ling & Zhang 2011)
2011	Fruit production (glasshouse)	Syria	1	(Fakhro et al. 2010)
2011	Glasshouse tomato plants	Italy	2	(EPP0 2011h)
2011	Fruit production	Finland	1	(EPP0 2011f)
2011	Fruit production	UK	3	(EPP0 2012e)
2011	Fruit production (glasshouse)	Croatia	3	(Novak, Milanovic & Kajic 2012)
2011	Tomato crops	Greece	Unknown	(Efthimiou et al. 2011)
2011	Tomato crops	Mexico	Unknown	(Ling & Zhang 2011)
2011	Tomato crops	South Africa	Unknown	(Carmichael et al. 2011)
2012	Fruit production	Switzerland	3	(EPP0 2012f)
2012	Fruit production (glasshouse)	Lithuania	2	(Šneideris et al. 2013)
2015	Fruit production	Spain	2	(EPP0 2016a)
2016	Fruit production (glasshouse)	Morocco	7	(Imane 2016; Souiri et al. 2017)

4.7 Interceptions of PepMV-infected tomato seed by other countries

The publicly available EPP0 records from 2000 to 2010 include reports of 23 interceptions of infected seed (Table 4.2). However, the European PRA for PepMV indicates there were 64 notifications of non-compliance on seed between 2000 and May 2010, and that 103 infected tomato seed lots were detected through surveys in 2009 (Werkman & Sansford 2010). These detections showed that the virus was present in several countries where it had not been previously reported. The virus was found in seed from countries in Africa, Asia, Europe, the Middle East and the Americas. Notably, many seed lots from Asia were infected. Together the detections indicate that tomato seed crops were often infected, but it is worth noting that

despite the great number of interceptions of infected tomato fruit and seed, only two infected seed crops were reported over the same time (EPPO 2005b).

Table 4.2 Tomato seed carrying PepMV intercepted by other countries

Year	Type of commodity	Country of origin	Country of destination	Number	Reference
2000	Seed	Netherlands	UK	1	(EPPO 2000a)
2004	Seed	Netherlands	Bulgaria	1	(EPPO 2004b)
2004	Seed	Netherlands	UK	1	(EPPO 2004e)
2008	Seed	Netherlands	Czech Republic	1	(EPPO 2008c)
2010	Seed	China	France	2	(EPPO 2010b)
2010	Seed	China	Poland	1	(EPPO 2010b)
2010	Seed	India	France	2	(EPPO 2010b)
2010	Seed	Israel	France	1	(EPPO 2010b)
2010	Seed	Senegal	France	1	(EPPO 2010b)
2010	Seed	Thailand	France	9	(EPPO 2010b)
2010	Seed	USA	France	1	(EPPO 2010b)
2010	Seed	China	France	2	(EPPO 2010a)
2011	Seed	Israel	Bulgaria	1	(EPPO 2011c)
2011	Seed	USA	France	1	(EPPO 2011c)
2011	Seed	China	Italy	1	(EPPO 2011b)
2011	Seed	China	France	1	(EPPO 2011d)
2012	Seed	China	Germany	1	(EPPO 2012c)
2012	Seed	Italy	Malta	1	(EPPO 2012b)
2012	Seed	Vietnam	France	1	(EPPO 2012b)
2013	Seed	Italy	Malta	1	(EPPO 2013a)
2014	Seed	Chile	France	1	(EPPO 2014b)

5 Pospiviroids

5.1 Pospiviroid biology

Viroids are the simplest and smallest plant pathogens, and are constituted from minimal molecular genetic components. They consist of circular duplexed ribonucleic acid (RNA) molecules, typically 239 to 401 nucleotides long (Gross et al. 1978; Steger & Riesner 2003), which is about ten times smaller than the smallest viral genome. Viroids have no genes, and are replicated by plant-encoded enzymes; the diseases caused by viroids probably result from interactions with the plant's RNA regulatory, defence and trafficking systems (Ding 2009; Flores et al. 2005; Machida et al. 2007).

Viroids cause significant disease in some crops and fruit trees. Related viroids have similar transmission properties and host ranges, and cause a similar range of symptoms (EFSA 2011; Singh, Ready & Nie 2003b), presumably because their interactions with hosts are nearly identical (Ding 2009). Viroids from the same genus have very similar RNA sequences, which may be significant as the interactions with the host are sequence dependent.

Pospiviroids are generally easily transmitted 'mechanically' when plants are intentionally cut or accidentally abraded during normal horticultural activities, including handling of plants. This type of transmission typically occurs through contact with contaminated pruning tools, farm equipment, clothing and people's hands (Owens & Verhoeven 2009; Sabaratnam 2012; Singh, Ready & Nie 2003b). Pospiviroids can also be transmitted through grafting and vegetative propagation, and they are naturally transmitted by contact between neighbouring plants, through pollen and seed and, in some specific circumstances, by aphids and bumble bees (Galindo 1988; Galindo, Lopez & Aguilar 1986; Owens & Verhoeven 2009; Salazar et al. 1983; Singh 1970; Singh, Boucher & Somerville 1992; Singh, Ready & Nie 2003b). Transmission from non-crop plants, such as ornamental plants, probably happens when farm vehicles, equipment or workers make contact with the plants, and would be likely if the plants are pruned and the pruning equipment is then used in a tomato crop.

5.2 Pospiviroid disease in tomatoes

Seven viroid species, all in the *Pospiviroid* genus, have been found infecting tomatoes, namely CEVd, CLVd, PCFVd, PSTVd, TASVd, TCDVd and TPMVd (Galindo, Smith & Diener 1982; Verhoeven et al. 2009; Verhoeven et al. 2004; Walter 1987). Mexican papita viroid, which has also been found in tomatoes, is now considered to be a variant of TPMVd (Martínez-Soriano et al. 1996; Verhoeven, Roenhorst & Owens 2011). *Chrysanthemum stunt viroid* (CSVd) and *Iresine viroid 1* are the only recognised pospiviroid species that have not been found naturally infecting tomato.

Infection levels in tomato crops vary widely, typically being below 10 per cent and sometimes below 1 per cent, but in some cases reaching a large proportion of the crop and in a few cases nearly 100 per cent (Antignus et al. 2002; EFSA 2011). Most spread within tomato crops is by mechanical transmission (Antignus et al. 2002; Owens & Verhoeven 2009; van Brunshot et al. 2014b; Verhoeven et al. 2004). Whereas there are many reports of pospiviroid infections in greenhouse tomato crops, few reports of infected tomato field crops were found (Barbetti et al. 2012; Ling et al. 2012; Mackie et al. 2016; Pur Rahim et al. 2009).

Infected tomato plants show a range of symptoms. In most reported infections, tomato plants are stunted and have chlorotic or bronzed leaves that are distorted or small. Some plants may lose leaves or have brittle leaves or patches of necrosis on the leaves, and in some cases on the stems (Behjatnia 1996; Galindo, Smith & Diener 1982; Martínez-Soriano et al. 1996; McClean 1948; Mishra et al. 1991; Owens 1990; Owens, Candresse & Diener 1990; Singh 1973; Singh, Nie & Singh 1999; Verhoeven et al. 2004; Walter 1987). Infected plants usually produce fewer and smaller fruit, and in some severe cases no fruit at all (Owens & Verhoeven 2009; Singh, Ready & Nie 2003b).

Diagnosis is difficult because the symptoms are not distinctive and not diagnostic. Different pospiviroids cause similar symptoms, which may be easily confused with symptoms caused by other pathogens, and possibly by herbicide damage (Blancard 2012; EFSA 2011).

The severity of symptoms varies considerably, with the most severely affected plants being stunted and expressing most of the typical symptoms, and the most mildly affected plants having few or no symptoms. Symptom severity is believed to depend upon the variant of the viroid, the cultivar of the tomato, and temperature and light levels (EFSA 2011; Singh, Ready & Nie 2003a). Symptom severity also varies as much within some pospiviroid species as between species (EFSA 2011; Runia & Peters 1980).

Some tomato cultivars do not produce symptoms (that is are asymptomatic) when infected by certain pospiviroid variants, or may only produce very mild disease (Barbetti et al. 2012; O'Brien 1972; Owens & Verhoeven 2009; Singh 1973; Stark-Lorenzen et al. 1997). Asymptomatic infections cannot be detected visually, but only by sampling and laboratory testing.

Seedlings infected with pospiviroid species may not produce symptoms for more than 6 weeks after germination, and some may not produce symptoms at all when grown from infected seeds (Kryczynski, Paduch-Cichal & Skreczkowski 1988; Singh et al. 2009). Viroid replication and symptom development is enhanced by high temperatures and light levels (Singh 1983; Singh, Ready & Nie 2003a), which might explain some reports of outbreaks in countries where temperature and light levels are higher (Table 5.2), if these conditions lead to worse disease and more frequent detection.

5.3 Viroid presence in seeds and floral parts

All pospiviroids that naturally infect tomato have been detected in samples of tomato seed (Table 5.1) (Antignus, Lachman & Pearlsman 2007; Chambers et al. 2013; FERA 2009b; Marach 2008; Singh & Dilworth 2009). However, whether a pospiviroid is present within the seed or is simply a contaminant on the seed surface has been questioned. This localisation may influence transmission through seed, may influence seed testing protocols, and may determine whether treating tomato seed with cleaning agents will eliminate the viroid.

The localisation of pospiviroid RNA in plant tissues in general has been investigated using PSTVd and TCDVd. PSTVd RNA has been detected within tomato seed and in all the floral parts of infected tomatoes that have been tested, including the ovaries, ovules and pollen (EUPHRESO 2010; Koenraadt et al. 2009; Singh & Dilworth 2009; Singh et al. 2006; Zhu et al. 2001). Similarly, experiments have shown the RNA of TASVd and TCDVd is present within the seed of infected tomatoes, and TASVd RNA is present in the petals, sepals, ovaries and stamens of infected tomatoes (Antignus, Lachman & Pearlsman 2007; Koenraadt et al. 2009; Singh &

Dilworth 2009; Singh, Nie & Singh 1999). These findings are consistent with earlier work that showed that PSTVd RNA is present in the pollen, sepals, fruit and true botanical seed of infected potato plants (Fernow, Peterson & Plaisted 1970; Salazar et al. 1983; Singh, Boucher & Somerville 1992; Singh, Boucher & Wang 1991), and are also consistent with later work showing that PSTVd RNA is present within petunia seeds, ovaries and embryos (EPPO 2016b; Matsushita & Tsuda 2014).

Pospiviroid RNA is also found in tomato fruit flesh (van Brunschot et al. 2014b) and hence will be present on seed surfaces. However, PSTVd, TASVd and TCDVd were not eliminated from seeds when the seeds were soaked in bleach (sodium hypochlorite) or alkali solutions to destroy RNA, confirming that the viroids are protected within seeds as well as being present on seed surfaces (Antignus, Lachman & Pearlsman 2007; Singh & Dilworth 2009).

The seed cleaning and localisation data suggest that attempts to eliminate pospiviroids by treating seeds will not be effective, given that the pospiviroid RNA is within the seeds; such processes are, however, likely to reduce the amount of pospiviroid RNA present in seed samples making it more difficult to detect if seed samples are tested.

5.4 Viroid transmission through tomato seeds to seedlings

Pospiviroids have been shown to be transmitted through tomato seeds in experiments where seedlings are grown from seeds collected from infected parent plants (Table 5.1). There is experimental evidence of transmission through tomato seeds for CEVd, CLVd, CSVd, PCFVd, PSTVd, TASVd, TCDVd and TPMVd (Antignus, Lachman & Pearlsman 2007; Benson & Singh 1964; Kryczynski, Paduch-Cichal & Skreczkowski 1988; Marach 2008; Owens & Verhoeven 2009; Semancik 1980; Singh 1970; Singh & Dilworth 2009; Yanagisawa & Matsushita 2017)). Based on the accumulated evidence, scientists from the Panel on Plant Health (PPH) instituted by the European Food Safety Authority (EFSA), generally agreed that all pospiviroids are likely to be transmitted through tomato seed (FERA 2011) (Table 5.1).

Seed-transmission of pospiviroids may be due to the presence of pospiviroid RNA in embryos, ovules and pollen. However some seedlings may be infected by pospiviroid RNA present on seed surfaces, given that pospiviroids are easily mechanically transmitted and because abrasion occurs when the seeds germinate and break through the seed coat.

Experiments indicate that the rate of transmission varies widely (Table 5.1). Experiments suggest the rate of transmission of PSTVd through tomato seeds is commonly close to 10 per cent, although it can approach 100 per cent in the true botanical seed of potato (Table 5.1). The rate of transmission varies with the host plant and probably also varies between pospiviroid species. The rate of transmission of both TASVd and TCDVd through tomato seed has been estimated to be 80 per cent (Antignus, Lachman & Pearlsman 2007; Singh & Dilworth 2009).

The proportion of chrysanthemum seeds that are infected with CSVd was found to be higher when the mother plant was infected, than when an uninfected mother plant was pollinated with pollen from an infected male parent plant (Chung & Pak 2008). By contrast, pollen transmission in petunia with PCFVd and TPMVd yielded a similar or greater proportion of infected seeds than direct infection of the seed from the mother plant (Yanagisawa & Matsushita 2017).

There may be several explanations for experiments where no transmission was detected through tomato seeds to seedlings, and for variations in the rate of transmission. It was

suggested that the temperature in which parent plants are grown and when seedlings are germinating may affect transmission (Chung & Pak 2008; Fox & Monger 2011). Pospiviroid seed transmission may also be reduced in some tomato cultivars (Singh, Boucher & Somerville 1992).

Sequence variation is known to influence viroid biology and might affect seed transmission (FERA 2011; Singh, Ready & Nie 2003a). In one example, a variant of PSTVd with mild effects was transmitted through small numbers of seeds, whereas transmission of a variant with severe effects was not detected (Khoury et al. 1988). In another example, transmission through tomato seed was detected using a variant of TCDVd that differed at a few nucleotide positions from a variant of TCDVd that apparently was not transmitted (Singh & Dilworth 2009; Singh, Nie & Singh 1999).

It is possible that variations in pospiviroid RNA levels in the seed also affect seed transmission. Tests of PSTVd in infected tomato seeds found that the level of viroid RNA was very variable, with some individual seeds being 'highly contaminated' whereas other seeds had 'low PSTVd concentrations' (EUPHRESCO 2010).

Table 5.1 Experiments on pospiviroid transmission through seed of Solanaceae to seedlings

Publication	Viroid species	Host plant	Transmission rate ^a
McClellan (1948)	Pospiviroid unknown species	<i>Nicotiana glutinosa</i>	0%
		<i>Physalis peruviana</i>	29.3%
		<i>Physalis viscosa</i>	0%
		<i>Solanum incanum</i>	53.9%
		<i>Solanum lycopersicum</i>	0%
Benson and Singh (1964)	PSTVd	<i>Solanum lycopersicum</i>	7.9–11.1%
Hunter et al. (1969)	PSTVd	<i>Solanum tuberosum</i>	87–100%
Fernow et al. (1970)	PSTVd	<i>Solanum tuberosum</i>	0–100%
Singh (1970)	PSTVd	<i>Solanum lycopersicum</i>	6–11%
		<i>Solanum tuberosum</i>	6–12%
Singh and Finnie (1973)	PSTVd	<i>Scopolia sinensis</i>	71%
Belalcazar and Galindo-Alonso (1974)	TPMVd	<i>Solanum lycopersicum</i>	No transmission observed
Semancik (1980)	CEVd	<i>Solanum lycopersicum</i>	Transmitted but transmission rate not reported
Grasmick and Slack (1987)	PSTVd	<i>Solanum tuberosum</i>	100%
Khoury et al. (1988)	PSTVd	<i>Solanum lycopersicum</i>	5%
Kryczynski et al. (1988)	PSTVd	<i>Solanum lycopersicum</i>	>10%
	Chrysanthemum stunt viroid	<i>Solanum lycopersicum</i>	>10%
Singh et al. (1999)	TCDVd	<i>Nicotiana physaloides</i>	No transmission observed
		<i>Nicotiana debneyi</i>	
		<i>Physalis angulata</i>	
		<i>Physalis oreadana</i>	
		<i>Solanum lycopersicum</i>	
		<i>Solanum tuberosum</i>	
Lebas et al. (2005)	PSTVd	<i>Capsicum annuum</i>	No transmission observed
		<i>Solanum lycopersicum</i>	

Publication	Viroid species	Host plant	Transmission rate ^a
Antignus et al. (2007)	TASVd	<i>Solanum lycopersicum</i>	80%
Marach (Marach 2008)	CLVd	<i>Solanum lycopersicum</i>	Transmitted but transmission rate not reported
Koenraad et al. (2009)	TCDVd	<i>Solanum lycopersicum</i>	No transmission observed
Singh and Dilworth (2009)	TCDVd	<i>Solanum lycopersicum</i>	80%
Verhoeven et al. (2009)	PCFVd	<i>Capsicum annuum</i>	19%
Candresse et al. (2010)	TCDVd	<i>Solanum lycopersicum</i>	<1% ^b
van Brunschot et al. (2014b)	PSTVd	<i>Solanum lycopersicum</i>	<1% ^b
Matsushita and Tsuda (2014)	PSTVd	<i>Petunia × hybrida</i>	51.7–78%
Simmons et al. (2015)	PSTVd	<i>Solanum lycopersicum</i>	50.9%
Yanagisawa & Matsushita (2017)	PCFVd	<i>Capsicum annum</i>	0%
		<i>Petunia × hybrida</i>	0–91.9%
		<i>Solanum lycopersicum</i>	0–1.4%
	PSTVd	<i>Petunia × hybrid</i>	20.8–97.7%
	TPMVd	<i>Capsicum annum</i>	0%
		<i>Petunia × hybrida</i>	17.5–96.4%
		<i>Solanum lycopersicum</i>	0–4.4%

a. Transmission rate estimates have usually been made by determining the number of infected seedlings grown from a known number of seeds from infected parent plants and has not involved any statistical method or tests of the proportion of seeds carrying the viroid. **b.** Candresse et al. (2010) and van Brunschot et al. (2014b) reported results of grow-out tests from commercially produced seed lots and hence the rates of transmission are probably affected by dilution of seeds from infected parent plants with seeds from healthy parent plants.

5.5 Pospiviroids outbreaks in tomato in other countries

Unexpected outbreaks of pospiviroids in tomato crops in disparate locations across the world are reported almost every year (Table 5.2). There has been at least one such unexpected outbreak of each of the tomato-infecting pospiviroid species (Antignus et al. 2002; Candresse et al. 2007; Ling et al. 2009; Mishra et al. 1991; Mumford et al. 2007; Punyapitak 2004; Reanwarakorn, Klinkong & Porsoongnurn 2011; Semancik 1980; Verhoeven, Jansen & Roenhorst 2006b; Verhoeven et al. 2009; Verhoeven et al. 2004; Walter 1987; Walter, Thouvenel & Fauquet 1980). These outbreaks and the paucity of prior records indicate the pospiviroids are emerging pathogens (Anderson et al. 2004).

The reports of outbreaks in disparate locations present a picture of global distribution that is best explained by international transport of the pathogens with seed, and their introduction to crops through seed, along with instances of local transmission.

Table 5.2 Reports of pospiviroid outbreaks in tomato outside Australia

Viroid species	Countries	Report
<i>Citrus exocortis viroid</i>	India	(Mishra et al. 1991)
	Spain	(Fadda et al. 2003)
	Thailand	(Sangdee, Thummabenjapone & Sirithorn 2004)
	The Netherlands	(Verhoeven et al. 2004; Werkman, Verhoeven & Roenhorst 2007)

Viroid species	Countries	Report
<i>Columnnea latent viroid</i>	Iceland	(Mumford et al. 2007)
	Belgium	(Verhoeven et al. 2004)
	The Netherlands	(Verhoeven et al. 2004)
	Thailand	(Tangkanchanapas et al. 2005)
	Portugal	(CSL 2007; Monger & Mumford 2006)
	France	(CSL 2007; Steyer et al. 2009)
	United Kingdom	(CSL 2007; Nixon et al. 2009; Sansford & Morris 2009)
	Italy	(Parrella, Crescenzi & Pacella 2011)
	Ghana	(Batuman et al. 2013)
	Mali	(Batuman & Gilbertson 2013)
<i>Pepper chat fruit viroid</i>	The Netherlands	(Verhoeven et al. 2009)
	Canada	(Verhoeven et al. 2011)
	Thailand	(Reanwarakorn, Klinkong & Porsoongnurn 2011)
<i>Potato spindle tuber viroid</i>	New Zealand	(Elliot et al. 2001; Lebas et al. 2005)
	The Netherlands	(EPPO 2003d; NPPO the Netherlands 2013; Verhoeven et al. 2004; Werkman, Verhoeven & Roenhorst 2007)
	Germany	(EPPO 2004d; Werkman, Verhoeven & Roenhorst 2007)
	United Kingdom	(CSL 2008; FERA 2011; Mumford, Jarvis & Skelton 2004)
	Belgium	(Verhoeven et al. 2007a; Werkman, Verhoeven & Roenhorst 2007)
	Canada	(Werkman, Verhoeven & Roenhorst 2007)
	Austria	(EPPO 2008b)
	Japan	(Matsuura et al. 2010)
	United States	(Li et al. 2012; Ling et al. 2012; Ling & Sfetcu 2010)
	Ghana	(Batuman et al. 2013)
	Dominican Republic	(Ling & Li 2014)
<i>Tomato apical stunt viroid</i>	Cote d'Ivoire	(Walter 1987; Walter, Thouvenel & Fauquet 1980)
	Indonesia	(Candresse, Smith & Diener 1987)
	Israel	(Antignus et al. 2000)
	Tunisia	(Verhoeven, Jansen & Roenhorst 2006b)
	Senegal	(Candresse et al. 2007)
	The Netherlands	(Verhoeven et al. 2012)
	France	(EPPO 2013b)
	Ghana	(Batuman et al. 2013)
<i>Tomato chlorotic dwarf viroid</i>	Canada	(Singh, Nie & Singh 1999)
	United States	(Ling et al. 2009; Verhoeven et al. 2004)
	United Kingdom	(EPPO 2008a)
	Netherlands	(EPPO 2008a)
	Japan	(Matsushita et al. 2008)
	Mexico	(Ling & Zhang 2009)
	France	(Candresse et al. 2010)

Viroid species	Countries	Report
<i>Tomato planta macho viroid</i>	Norway	(Fox et al. 2013)
	Canada	(Ling & Bledsoe 2009)
	Mexico	(Ling & Zhang 2009)

5.6 International interceptions and initiation of outbreaks from seed

Since 2002, countries in Europe and the Middle East have intercepted tomato seed carrying PSTVd (Table 5.3). These interceptions provide a likely explanation for the international outbreaks of PSTVd (Table 5.2), as they suggest the outbreaks were probably caused by the inadvertent distribution of PSTVd-infected tomato seeds through the international seed trade.

It is likely that outbreaks of other viroid species in tomato crops (Table 5.2) have the same cause, namely trade in infected tomato seed. Australia has intercepted tomato seed infected with several pospiviroid species, supporting this broader inference (Section 5.8). It seems likely that other countries have not detected the pospiviroid species because seed imports were not tested for the wider range of pospiviroid species.

The hypothesis that traded tomato seed is a source of outbreaks of pospiviroids is also supported by evidence that pospiviroids are transmitted through tomato seed to seedlings (Section 5.4), that crops are typically grown from traded seed (Chapter 3), and that outbreaks occur at disparate locations (Table 5.2).

Table 5.3 Tomato seed carrying PSTVd intercepted outside Australia

Year	Type of commodity	Country of origin	Country of destination	Number	Reference
2002	Seed	India	Austria	1	(CABI-EPP0 2002)
2002	Seed	Thailand	Austria	2	(EPPO 2002a)
2007	Seed	Israel	Not named	1	(European Commission 2008)
2008	Seed	Netherlands	Austria	1	(EPPO 2008b)
2008	Seed	Israel	Not named	1	(European Commission 2008)
2011	Seed	China	Not named	1	(EUROPHYT 2012b)
2011	Seed	China	Israel	1	(EPPO 2011a)
2011	Seed	Kenya	Israel	1	(EPPO 2011a)
2011	Seed	Netherlands	Israel	1	(EPPO 2011a)
2011	Seed	USA	Israel	1	(EPPO 2011a)
2011	Seed	China	Italy	1	(EPPO 2011b)
2012	Seed	China	Not named	1	(EUROPHYT 2012a)
2012	Seed	China	Austria	1	(EPPO 2012b)
2012	Seed	China	Not named	1	(EUROPHYT 2012c)
2012	Seed	China	Austria	1	(EPPO 2012a)
2014	Seed	China	Italy	1	(EPPO 2014a)
2014	Seed	China	Slovenia	1	(EPPO 2014c)
2015	Seed	China	Denmark	1	(EPPO 2015)
2017	Seed	China	Italy	1	(EPPO 2017)

Furthermore, there is also evidence that links outbreaks directly to infected tomato seed. In one instance, a trial line of tomato seed that was planted in the same greenhouse as a large crop was found to be the source of an outbreak of PSTVd in that crop (van Brunschot et al. 2014a). A sample of 370 seeds from the trial line was grown out and one seedling was found to be infected with PSTVd. The PSTVd isolate from the seedling had the same genetic sequence as the PSTVd identified in the outbreak, and it was concluded that the infected trial line was the source.

In another example, outbreaks of CLVd in four tomato crops of the same cultivar in the United Kingdom were linked to seed (Fox & Monger 2011; Nixon et al. 2009). An investigation of the outbreaks found that a seed lot used for the crops was carrying the same viroid at a low level (Fox & Monger 2011).

In a third example, an outbreak of TCDVd in tomato crops in France was linked to a seed lot that was found to be carrying the viroid (Candresse et al. 2010).

By contrast, Koenraad et al. (2009) did not find a single seedling infected among 4000 grown from seed from parent plants that were infected with TCDVd (EFSA 2011). It is possible that some environmental factor reduced or eliminated seed transmission in the experiment, or that the transmission rate may have been so small as to be undetectable when 4000 seeds were germinated.

Taken together, the evidence indicates some outbreaks are initiated by infected seeds in traded seed lots. The evidence also indicates outbreaks may be initiated when very few infected seeds are present in otherwise uninfected seed lots (Candresse et al. 2010; Fox & Monger 2011; van Brunschot et al. 2014a). When few seeds are infected and transmission rates are small, the very great numbers of seeds that are planted to establish tomato crops is an important factor. It is noted that outbreaks of other seed-transmitted pathogens can arise from very few infected seeds, and can arise even when the pathogen has a low seed transmission rate (Agarwal & Sinclair 1996).

5.7 Pospiviroid sources: reservoirs and alternative hosts

Scientists have proposed two other possible causes of outbreaks of pospiviroids in tomato crops, both being relevant to Australia. Firstly, it was suggested that the viroids are transmitted from ornamental plants, as the viroids have been detected many times in ornamental plants from the family Solanaceae (Verhoeven et al. 2004). Secondly, pospiviroids have been detected in wild plants and weeds in two countries, and it has been proposed that these plants may be sources of the viroids.

5.7.1 Ornamental plants as reservoirs

Some symptomless solanaceous ornamental plant species growing in nurseries and intercepted in trade have been found to be infected with pospiviroids. Five tomato-infecting pospiviroids, CEVd, CLVd, PSTVd, TASVd and TCDVd, have been detected in these plants (FERA 2011). Most detections in ornamental species occurred in Europe where surveys were done (EFSA 2011; Verhoeven et al. 2004) and there is little doubt that a few outbreaks in Europe in tomato crops can be attributed to these plants.

Verhoeven et al. (2010) proposed that infected ornamental plants are the main sources of PSTVd found in tomato crops in the Netherlands, but there is little published evidence of transmission

from ornamentals to tomato crops (FERA 2011), and no evidence linking trade of solanaceous ornamental plants to outbreaks in tomato crops in disparate locations.

Verhoeven et al. (2010) also suggested that outbreaks of PSTVd in Australia arose from *Physalis peruviana* (cape gooseberry), which is grown as an ornamental and is sometimes infected. However, the suggested link with these plants in Australia appears not to be supported, as the genetic sequences of the viroids detected in the two types of plants were not found to closely match.

It is worth noting that infection of tomato seed crops from ornamental plant reservoirs is just as likely as infection of fruit crops from such reservoirs. Furthermore, the evidence used by Verhoeven et al. (2010) is indirect and relies on interpretations of phylogenetic trees that were relatively poorly supported. Given the balance of evidence, the proposition that solanaceous ornamental plants are a major source of outbreaks in tomato is considered arguable at best.

5.7.2 Weeds as reservoirs

Relatively few wild plants have been tested for pospiviroids and the geographic origins and original hosts of pospiviroids are unknown. However, some pospiviroid populations are sustained in wild plants and it is likely that sometimes cultivated plants are infected from these wild sources. In Mexico, the Mexican pepita variant of TPMVd (TPMVd-MP) has been found in wild plants, suggesting that these plants are a natural reservoir in that country (Orozco Vargas & Galindo-Alonso 1986). Similarly, in parts of Iran and Australia, PSTVd has been detected in weeds within and near field crops, suggesting that the weeds in certain regions act as reservoirs. In Iran, PSTVd has been detected in *Physalis floridana*, *Datura* sp. and *Solanum nigrum* growing as weeds in potato and tomato fields suggesting these weed species were natural reservoirs of the viroid in that region (Pur Rahim et al. 2009).

In Australia, PSTVd was detected in the Carnarvon Shire in the Gascoyne region of WA in the weed species *Conyza bonariensis*, *Datura leichhardtii*, *Nicandra physalodes* and *Solanum nigrum*, and it was detected in *Physalis angulata* in the Ord River Irrigation Area in the Kimberley region in north-west WA (Barbetti et al. 2012; Mackie et al. 2016). In the Carnarvon Shire the infected weeds were found growing beside chilli and tomato crops and along roadsides. PSTVd was also detected in the Carnarvon Shire infecting the Australian native plant species *Atriplex semilunaris* and *Rhagodia eremaea* and a *Streptoglossa* species. The epidemiology of the viroid in these areas of Western Australia is not well known. The Gascoyne region of WA and the Ord River Irrigation Area are geographically remote, and isolated from the rest of Australia by expanses of uncultivated arid land. Given the sequence similarities, it is likely that PSTVd was introduced to the Gascoyne region in imported tomato or capsicum seed, seedlings or transplants (Mackie et al. 2016).

Although it is possible some weeds act as reservoirs at some locations, the unexpected outbreaks of pospiviroids in different countries (Table 5.2) cannot be explained simply in terms of natural spread from these weeds. No natural transmission pathway involving weeds has been identified or proposed that could explain the long distance movement between crops in different countries. Furthermore, no wild populations of infected host plants are known to connect the locations of the outbreaks.

5.8 Data from tomato seed testing under the emergency measures

5.8.1 Testing and data collation approach

Every year since 2009 Australian laboratories have detected tomato seed lots contaminated with pospiviroid-infected seeds through on-arrival testing under the emergency measures. Seed lots were tested following the Australian approved protocols (Appendix A). No seed lots were detected that were found to be carrying PepMV.

Data was compiled on seed lots imported and tested on-arrival during 2013. This seed test data from 2013 does not represent all tests of tomato seed lots sent to Australia in that year. Many seed lots were tested by laboratories in other countries (off-shore testing), and then certified as viroid-free before being exported to Australia. It is possible that tomato seed lots contaminated with pospiviroid-infected seeds (infected positives) were detected by the off-shore testing and that those seed lots were not sent to Australia. No suitable data on seed lots tested off-shore was available for this analysis, and it is not known how many seed lots were found to be contaminated by this off-shore testing.

Tomato seed lots were tested for the pospiviroids on-shore by extracting RNA from seed samples and by using RT-PCR assays (Appendix A). All pospiviroid detections were confirmed by nucleotide sequencing and by matching with viroid sequences in the GenBank international database.

The origins of seed lots were ascertained from Phytosanitary Certificates and other documents that accompanied the consignments. Many seed lots were sent directly to Australia from countries where the seed was produced, namely where the seed crop was grown. However, a proportion of the seed lots sent to Australia had been shipped to at least one other country before being sent to Australia (transhipped).

5.8.2 Results of testing tomato seed imports

During 2013, 507 tomato seed lots sent to Australia were tested by the Australian diagnostic laboratories and pospiviroids were detected in 52 of the lots, which is 10.3 per cent of the total number of lots. A slightly higher proportion of imported seed lots was found to be infected in 2012, but in later years the proportion of infected seed lots declined substantially (Constable et al. 2017).

Of the pospiviroid-infected seed lots detected in 2013, 19 lots were found to be carrying viroids that are quarantine pests for Australia. Among these seed lots, there was seed infected with CLVd, PCFVd, TASVd or TCDVd (Table 5.4). No seed lot has been found to be carrying TPMVd since testing for the viroid began in 2012, nor has a seed lot been found to be carrying PepMV since 2012.

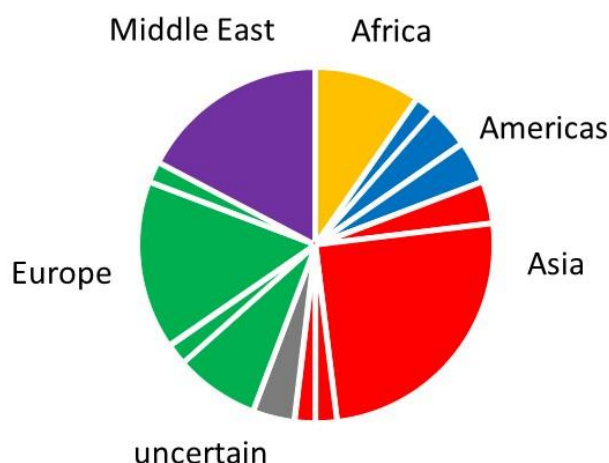
In 2013, 20 lots were found to be infected with PSTVd, 17 lots were found infected with CEVd and several lots were detected carrying more than one pospiviroid species (Table 5.4).

Table 5.4 Numbers of seed lots intercepted by Australia in 2013 carrying pospiviroid species

Viroid species detected	Number of lots intercepted in 2013
CEVd alone	13
CEVd & PSTVd	3
CEVd, CLVd & PCFVd	1
CLVd alone	11
CLVd & PCFVd	1
CLVd & TASVd	1
PCFVd alone	3
PCFVd & TASVd	1
PSTVd alone	17
TCDVd alone	1

Between 2012 and 2017, more seed lots infected with PSTVd, CEVd or CLVd were sent to Australia than seed lots carrying any other viroid species. After 2013, there were fewer detections of these three species, but the Australian diagnostic laboratories reported a greater number of seed lots carrying PCFVd.

Overall, 21 countries produced pospiviroid-infected seed that was sent to Australia between 2012 and 2017, including three countries in Africa, five in the Americas, eight in Asia, four in Europe and one in the Middle East. When viewed collectively, this data from seed testing shows the pathogens have a very wide geographic distribution, and that seed crops are infected in every major production region. In 2013, pospiviroid-infected seed lots were detected from countries representing the same geographic regions: Africa, the Americas, Asia, Europe and the Middle East (Figure 5.1). In that year three countries, one each in Asia, Europe and the Middle East, sent the great majority of infected seed lots to Australia (Figure 5.1).

Figure 5.1 Proportion of infected seed lots intercepted in 2013 attributed by region

a. Segments represent countries; the size of the segment is proportional to the number of contaminated seed lots sent from the country; the origins of two seed lots are uncertain as they were not recorded.

Under the current emergency measures, seed lots that weigh 300 g or less, comprising fewer than 100,000 seeds, are tested using a sample of 20 per cent of the lot (Section 1.2.1). During 2013, 22 small lots were found to be contaminated with pospiviroid-infected seeds. Among these contaminated seed lots, there were 14 lots that comprised 2500 or fewer seeds in total. Seed lots that are used for selections and trials are typically of this small size.

For some larger seed lots, the proportion of infected seeds in the lot could be estimated from the number of positive and negative subsamples. This was possible because when the sample comprised more than 400 seeds, the seed sample was divided into subsamples each of approximately 400 seeds or as close to 400 seeds as could be achieved, and each subsample was tested independently.

The proportion of infected seeds could be estimated most accurately when large seed lots were tested following the subsampling regime. Large seed lots were defined as those weighing more than 300 g and comprising 100,000 seeds or more. A 20,000 seed sample (the maximum size sample) was taken from these large lots and usually tested as 50 subsamples.

In 2013, 30 large seed lots were found to be contaminated with pospiviroid-infected seeds. Among these 30 large seed lots, there were several in which only one subsample returned a positive (infected) result. When only one subsample was positive and 49 were negative, the proportion of infected seeds was estimated to be 0.000062, within a 95 per cent confidence interval of 0.0000045 to 0.0002513. The best estimate of 0.000062 equates to approximately one infected seed in 16,000 uninfected seeds, with the confidence interval equating to a range of one seed in more than 222,000 to one seed in 3980.

Positive results indicating pospiviroid infection were returned from every subsample that was tested from one large seed lot that arrived in 2013, and for this seed lot the proportion of infected seeds was estimated to be greater than 0.0025, which equates to more than one infected seed in 400 uninfected seeds. Very few positive (infected) seed lots had levels of contamination with infected seeds that approached this high level; most were estimated to have much lower levels of contamination.

Several scenarios could explain how a large seed lot could become contaminated with very few infected seeds. A viroid infection of a seed crop may have only spread to a few plants. Supporting this possibility, a very small number of pospiviroid-infected plants were detected in a commercial crop in an incursion in Australia in 2013. It is not known if this is a common phenomenon, for although there are reports of widely and rapidly spreading outbreaks, no epidemiological studies were found during the preparation of this draft report that describe pospiviroid spread through crops or compare epidemiologies between outbreaks.

It is plausible that the proportion of infected plants would sometimes be very low because an outbreak was suppressed, perhaps by removing most, but not all, infected plants. Another likely possibility is that a pospiviroid outbreak might be initiated in a seed crop late in a season so that there is insufficient time for the viroid to spread, and so that very few plants are infected. A late infection might not be noticed as symptoms might not be well expressed.

Another plausible possibility is that an infected seed lot may have been intentionally or inadvertently blended with a healthy seed lot, affecting the proportion of infected seeds. Blending different lots of vegetable seeds is a normal commercial practice (Bello & Bradford 2016; ISF 2017). Cross contamination may also occur when equipment used to process seed is not fully cleaned and small quantities of seeds from one lot contaminate the equipment and are accidentally transferred to the next lot that is processed.

In conclusion, testing of tomato seed lots sent to Australia during the emergency measures has provided evidence that pospiviroid-infected seeds are present in some commercially traded tomato seed lots. Contaminated seed lots were produced in countries in every major production region and several pospiviroid species were detected. Some commercially traded seed lots were contaminated with very small numbers of pospiviroid-infected seeds. How seed lots become contaminated with very small numbers of infected seeds is not known but there are plausible explanations related to epidemiology and seed production. Small numbers of infected seeds are difficult to detect within seed lots, therefore, drawing sufficiently large samples will be necessary to effectively mitigate the risks.

6 Pest risk assessments for quarantine pests

Consistent with the IPPC and ISPM No. 1 (FAO 2016a), this pest risk assessment was initiated to fulfil Australia's obligations to review the emergency phytosanitary measures introduced in June 2008 and revised in February, May and December of 2012 and August of 2013 (Section 1.2). Australia introduced the emergency measures after incursions of PSTVd in tomato crops, consistent with published evidence that PSTVd is transmitted through tomato seed. The emergency measures were revised when other pospiviroid species were detected in tomato seed sent to Australia, consistent with published evidence that pospiviroid species were spreading to many countries and are seed-transmitted in tomato.

Table 6.1 lists the plant pathogens that are assessed in this draft report that are currently managed through the emergency measures on tomato seed and seed of wild tomato species. All but one of these plant pathogens are categorised as quarantine pests for Australia. The pathogens are categorised as quarantine pests because they are absent from Australia, have the potential to enter Australia, the potential to establish and spread in Australia in solanaceous crops, native plants and weeds, and because they have the potential to cause economic damage to tomato crops and crops of other solanaceous plant species.

This PRA assesses imports of tomato seed as a potential pathway by which the listed pathogens may enter Australia. CLVd, PCFVd, TASVd and TCDVd have been detected in tomato seed lots sent to Australia during the period of application of emergency measures, and PepMV has been detected in tomato seed lots by other countries. Confirming that this pathway could lead to outbreaks, there is evidence that all of the pathogens are transmitted from infected tomato seed to seedlings when the seed is germinated, and that traded seed is likely to be responsible for outbreaks (Section 4.4, 5.4 and 5.5; Table 6.1).

Pest risk assessments are presented in sections 6.2 to 6.7. The risks are estimated for imports as if they occurred without any testing for PepMV or pospiviroids (the unrestricted risks). The existing emergency measures on tomato seed for PepMV and pospiviroids, described in the previous sections and in Chapter 7 of this risk analysis, are not considered when the risks are estimated.

Additional information, provided in the previous chapters related to tomato seed production and trade, as well as the epidemiology of the pathogens and Australian testing for the pathogens, is summarised and considered in the risk assessments.

Imports of seed of wild tomato species (*Solanum chilense*, *S. chmielewskii*, *S. parviflorum*, *S. peruvianum* and *S. pimpinellifolium*) have not been assessed in the pest risk assessments as no reports were found showing the listed pathogens are transmitted through the seed of these species.

In summary, the risk assessments and the additional information indicate the following points.

- It is likely that the listed pathogens will be present in tomato seed lots sent to Australia for commercial tomato production, as international controls to prevent the production and trade of pathogen-infected tomato seeds do not adequately reduce these risks.

- Many tomato seed consignments sent to Australia have been shown to contain pospiviroid-infected seeds, and the rates of detection show that internationally traded tomato seed lots are often infected by pospiviroids.
- Pospiviroids and PepMV have been detected by other countries in tomato seed lots.
- Analysis of international outbreaks indicates the pathogens are transported with seed, and introduced to crops through planting infected seed.
- It is likely that the listed pathogens will establish and spread in Australia if no measures are taken to mitigate the risks of introduction.
 - PepMV and pospiviroids are transmitted through tomato seed to seedlings, as indicated by molecular testing of seed, seed transmission experiments and information from outbreaks.
 - Outbreaks of the pathogens in tomato crops often occur in other countries and some of these outbreaks have been linked to infected seed.
 - Several incursions of PSTVd have occurred in the past 16 years in Australian tomato crops.
- If the listed pathogens spread in Australia, substantial damage to crops and considerable control and eradication costs are likely.
 - Infections of PepMV and pospiviroids can cause substantial disease in tomato crops, and reduce tomato crop yields and the quality of fruit. Pospiviroids also present a risk to potato and capsicum crops and PepMV can affect pepino and basil crops.

Table 6.1 Summary of seed transmission and detection of listed plant pathogens

Pathogens subject to emergency measures	Acronym	Detected in commercial tomato seed lots	Transmitted through tomato seed	Quarantine pest
<i>Pepino mosaic virus</i>	PepMV	Yes ¹	Yes (Córdoba-Sellés et al. 2007; Hanssen et al. 2010; Hanssen & Thomma 2010; Ling 2008)	Yes
<i>Columnnea latent viroid</i>	CLVd	Yes ²	Yes (Marach 2008)	Yes
<i>Pepper chat fruit viroid</i>	PCFVd	Yes ²	Yes (Yanagisawa & Matsushita 2017)	Yes
<i>Potato spindle tuber viroid</i>	PSTVd	Yes ²	Yes (Antignus, Lachman & Pearlsman 2007; Antignus et al. 2006; Khoury et al. 1988)	No
<i>Tomato apical stunt viroid</i>	TASVd	Yes ²	Yes (Antignus, Lachman & Pearlsman 2007; Antignus et al. 2006)	Yes
<i>Tomato chlorotic dwarf viroid</i>	TCDVd	Yes ²	Yes (Singh & Dilworth 2009)	Yes
<i>Tomato planta macho viroid</i>	TPMVd	No	Yes (Yanagisawa & Matsushita 2017)	Yes

1. Detected in traded tomato seed by countries in Europe (see Table 4.2)

2. Detected in tomato seed lots sent to Australia between 2013 and 2017 by Australian testing (see Table 5.4). Seed lots that were found to be carrying the listed pathogens were re-exported or destroyed.

6.1 *Potato spindle tuber viroid*

Tomato seed sent to Australia is currently subject to emergency measures that require testing for PepMV and pospiviroids, including PSTVd. Australia was believed to be free of PSTVd, and the emergency measures were initiated after incursions of PSTVd in tomato crops. Several of the

incursions were eradicated, but despite these actions, the viroid became established, as reported to the IPPC in 2015 (AUS-66/1). The viroid is only present in some areas of Australia, but since the viroid is now present in the country, and because it is not under official control in any region, it is not categorised as a quarantine pest for Australia or any region in Australia.

Under the IPPC, a country may regulate a plant pest if it meets the definition of a quarantine pest or a regulated non-quarantine pest (RNQP). A quarantine pest is one of 'potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled' (ISPM 5), whereas an RNQP is one 'whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party' (ISPM 5). If phytosanitary measures are implemented at a national boundary based on a claim that a pest is an RNQP, the measures must be supported by a pest risk assessment that specifically addresses the requirements of the relevant ISPMs (ISPM 16 and 21), including the requirement for regulation of the pest in plants for planting within the territory.

The department is currently evaluating the risks presented by PSTVd and the possibility of regulating PSTVd as an RNQP. This draft report does not present a pest risk assessment for PSTVd. Assessments of potential quarantine pests differ from assessments of potential RNQPs; whereas the former evaluate 'the probability of the introduction and spread of a pest and the magnitude of the associated potential economic consequences,' the latter evaluate 'the probability that a pest in plants for planting affects the intended use of those plants with an economically unacceptable impact' (ISPM 5).

The presence of PSTVd in Australia and repeated introductions of the viroid have the potential to affect the Australian avocado, potato and tomato industries. While the department evaluates the status and impacts of PSTVd, the emergency measures for tomato seed importation requiring testing for PSTVd will be maintained to protect these Australian industries.

A preliminary evaluation indicates that PSTVd is seed transmitted in tomato and several other solanaceous crop species, and that trade in seed is a pathway for the introduction of the viroid. The preliminary evaluation also suggests that the viroid can become established in production systems from infected seeds and can cause economic damage, although it is probably excluded from certain Australian production systems by government and industry biosecurity activities. The department intends to develop and publish a pest risk assessment if it finds that the viroid meets the criteria for categorisation as an RNQP.

6.2 *Pepino mosaic virus*

Pepino mosaic virus (PepMV), a member of the *Potexvirus* genus, was first found in 1974 when it was isolated from diseased *Solanum muricatum* (pepino) in Peru (Jones, Koenig & Lesemann 1980). It was first reported in tomato in 1999 when it appeared in crops in Germany, the Netherlands and the United Kingdom (van der Vlugt et al. 2002). An intercontinental outbreak ensued with the virus infecting tomato crops in Asia, Africa, the Americas and in many countries in Europe.

The virus spreads readily in tomato crops, being mechanically transmitted by horticultural workers when infected plants are handled and workers become contaminated (Hanssen & Thomma 2010; Jones, Koenig & Lesemann 1980; Spence et al. 2006; Wright & Mumford 1999). The virus is also transmitted by plant to plant contact, by the greenhouse whitefly (*Trialeurodes*

vaporariorum) and bumble bees (*Bombus impatiens*), and it is transmitted through water in hydroponic crops (Lacasa et al. 2003; Noël, Hance & Bragard 2014; Shipp et al. 2008).

PepMV is seed-transmitted and has been detected many times in consignments of traded tomato seed since 2001 (Clark & Crook 2012). Although the transmission rate from contaminated seeds can be very small, between 2 per cent and 0.005 per cent, it is believed that transmission via contaminated seeds is responsible for initiating outbreaks and for transport of the virus to regions where it was previously not known (Moreno-Pérez et al. 2014; Werkman & Sansford 2010).

Long-distance transmission of the virus with trade of infected tomato fruit is also possible (van der Vlugt 2009) as the virus can contaminate people who consume fruit and contaminate fruit waste (Werkman & Sansford 2010).

Since the virus is readily transmitted, entire crops can become infected (Wright & Mumford 1999). The virus can kill tomato plants or cause wilting or stunting. However, it commonly causes a range of less severe symptoms and in many instances does not cause any symptoms on the vegetative parts of plants (EPPO 2014d; Hanssen & Thomma 2010; Spence et al. 2006). Fruit from infected plants may be unmarketable or may be downgraded, and fruit can be affected on plants that are otherwise symptomless (Hanssen & Thomma 2010).

The virus also infects basil, potato and a wide range of wild and weedy plants, including species from the Amaranthaceae, Asteraceae, Boraginaceae, Brassicaceae, Chenopodiaceae, Convolvulaceae, Malvaceae, Plantaginaceae, Polygonaceae and Solanaceae (Córdoba, Martínez-Priego & Jordá 2004; Papayiannis, Kokkinos & Alfaro-Fernández 2012; Soler et al. 2002). Infected wild plants and weeds may act as reservoirs of the virus, increasing the likelihood of establishment and spread (Córdoba, Martínez-Priego & Jordá 2004; Papayiannis, Kokkinos & Alfaro-Fernández 2012; Soler et al. 2002). The virus also infects close relatives of tomato (*Solanum lycopersicum*), some of which are cultivated and are commonly called wild tomato, namely: *Solanum chilense*, *S. chmielewskii*, *S. parviflorum*, *S. peruvianum* and *S. pimpinellifolium*.

In this pest risk assessment, a risk scenario is considered whereby PepMV enters Australia in tomato seed, the seed is planted, and the virus is transmitted within a tomato crop and to other hosts.

6.2.1 Likelihood of entry

Likelihood of importation

The likelihood of entry is considered in two parts, relating to importation and distribution. One part concerns the arrival of the pathogen in Australia in tomato seeds that are imported for sowing. The second part concerns the distribution of the infected tomato seed in Australia and whether the seed and the pathogen will remain viable and survive before germination.

The likelihood that PepMV will be present in tomato seed imported into Australia is assessed as Moderate. This assessment is made because PepMV has been detected by other countries in many traded tomato seed lots, and the virus has been detected in crops in many countries, including seed-producing countries. Additionally, large volumes of tomato seed are imported into Australia each year and planted. Australian laboratories have not detected the virus in

imported tomato seed lots during the period of application of emergency measures, which is considered to be an ameliorating factor in the assessment.

- PepMV is present in countries in Asia, Africa, the Americas and Europe, but there is uncertainty about the details of its geographic distribution (CABI 2017; EPPO 2000b, 2001b; Roggero 2001; Werkman & Sansford 2010), partly because outbreaks can go unreported and the pathogen is moved with seed trade (Section 4.5).
- Seed producers may be unaware that fruit selected for seed extraction is infected with PepMV. PepMV infected plants may be symptomless or exhibit symptoms similar to those caused by other pathogens (Clark & Crook 2012).
- PepMV is easily spread by standard crop handling procedures. It is spread when tools, hands and clothing become contaminated and it is spread by direct plant to plant contact (Hanssen & Thomma 2010; Spence et al. 2006; Wright & Mumford 1999). The virus may be spread from weeds to seed crops and from fruit production crops to seed crops.
- PepMV contaminates the exterior of seeds extracted from infected plants and may be located in the seed coat, but is probably not in the embryo of tomato seeds (Ling 2008).
- The European Plant Protection Organisation has reported PepMV contaminated tomato seed lots in consignments from Chile, China, India, Israel, Italy, the Netherlands, Senegal, Thailand, USA and Vietnam (Clark & Crook 2012).
- PepMV has been reported in tomato crops grown for seed production in Chile (Carreno 2005; Munoz et al. 2002).
- Tomato seeds are cleaned using standard processes during or after extraction. This cleaning process is thought to substantially reduce the quantity of virus inoculum (Córdoba-Sellés et al. 2007; EPPO 2005b; Hanssen et al. 2010). Current evidence suggests cleaning does not eliminate the virus from large batches of PepMV-contaminated seeds (Córdoba-Sellés et al. 2007; Hanssen et al. 2010), and no study was found during the preparation of this draft report showing the effect on transmission when commercial quantities of seed were cleaned.
- PepMV-contaminated seed has not been detected in tomato seed imports tested during the period of application of emergency measures by Australian laboratories. However, the seed tests done by Australian laboratories have relied upon small samples consisting of at most 3,000 seeds, which are too small to reliably detect virus-contaminated seeds if only very small numbers are present in a large seed lot.
- Large quantities of tomato seed are imported into Australia annually from suppliers in many countries, including from countries that are known to have PepMV in tomatoes. The department estimates that on average 760 kilograms of tomato seed are imported into Australia annually.
- PepMV-infected seeds contaminating a seed consignment cannot be detected by visual inspection.

Likelihood of distribution

To have an impact a pathogen must be transported within Australia and must be capable of infecting a suitable host plant. The chance of this occurring depends upon the intended use of the imported commodity and the dispersal mechanisms of the pathogen. The likelihood that PepMV will be distributed in Australia in tomato seed and be present in the seed in an infectious state when it is planted is assessed as High. This assessment is made because imported tomato seed is distributed for planting throughout Australia, and if PepMV-infected seeds are present in

an imported seed lot, it is likely the seeds and virus will remain viable, and the seeds will be sown.

- Tomato seed is imported for planting for greenhouse and field production of tomato fruit. Seed will be distributed for planting in domestic gardens and in nurseries, greenhouses and farms in production areas throughout Australia.
- If present, PepMV is very likely to be present in an infectious state in the seed when it is planted.
- PepMV is likely to survive in seed for long periods of time as the virus particles are very stable. PepMV can survive more than 90 days in dried plant material (Blancard 2012).

Overall likelihood of entry (importation × distribution)

The likelihoods of importation and distribution of identified quarantine pests are combined to give an overall likelihood of entry using the matrix shown in Table 2.2.

The overall likelihood that PepMV will enter Australia and be transferred to a suitable host via tomato seed for sowing is assessed as Moderate.

6.2.2 Likelihood of establishment

The likelihood of establishment of PepMV within Australia will depend upon the availability of host plants and the reproductive and survival strategies of the virus. Based on an evaluation of these factors, the likelihood of establishment is assessed as High. This assessment is made primarily because very large numbers of imported tomato seeds are sown in Australia, and PepMV is transmitted from infected seeds to seedlings and because there is evidence of the virus establishing in tomato crops and weeds in other countries.

- Since 1999 the virus has become established in many countries in Africa, Asia, Europe and the Americas (CABI 2016).
- PepMV is transmitted from tomato seeds to seedlings (Carreno 2005).
- The rate of PepMV transmission via seeds depends on the time of seed harvest, the tomato variety, and seed cleaning and disinfection methods (Córdoba-Sellés et al. 2007; Hanssen & Thomma 2010; Ling 2008). The rate may be up to 2 per cent but can be as small as 0.005 per cent after seed cleaning (Hanssen et al. 2010).
- Millions of tomato plants are grown each year in Australia from imported seed. Although the rate of transmission of PepMV from cleaned contaminated seeds is probably very small (Córdoba-Sellés et al. 2007; Hanssen & Thomma 2010; Ling 2008), given the numbers of tomato seeds that are planted, it is probable that infected seedlings will emerge.
- An expert opinion indicated that 'one seed giving rise to an infected seedling is very likely to spread PepMV to other plants and finally infect the whole crop' (Werkman & Sansford 2010).
- PepMV infections may go unnoticed as sometimes infected plants are symptomless.
- PepMV infections in tomato may not be recognised because they are difficult to distinguish visually from infections of other disease agents (Blancard 2012; EPPO 2011e).
- Tomatoes are grown commercially in greenhouses and in the field in all states of Australia, including regions with temperate and tropical climates. They are grown during spring and summer in the field in the cooler regions of southern Australia, and all year round in other regions of Australia and in greenhouses.

- The climates of regions in Australia where tomatoes are grown are generally similar to the climates of the areas where PepMV has established in other countries. The environmental conditions in commercial greenhouses in Australia are very similar to those in greenhouses in countries where outbreaks of PepMV have occurred.

6.2.3 Likelihood of spread

When a pathogen has entered Australia and become established, the nature of an outbreak will depend upon whether it spreads to new areas from the point where it first became established. Based on a comparison of factors relevant to the potential expansion of the geographic range of the pathogen in the source and destination areas, the likelihood that PepMV will spread is assessed as High. This assessment is made primarily because there is evidence the virus has spread widely and quite rapidly in other countries. Additionally, the virus infects weed species and it is spread by normal horticultural activities. Moreover it may be transported inadvertently by contaminated agricultural equipment, with infected crop residues and with fruit, and is also likely to be spread by infected pollen, seed and seedlings, and by insects.

- PepMV has spread in many countries in Africa, Asia, Europe and the Americas (CABI 2016). Surveys indicated that the virus became very widespread in Europe in the 2000s (Werkman & Sansford 2010)
- Tomato is widely grown in home gardens, greenhouses and the field in all states and territories of Australia.
- PepMV is easily spread by standard crop handling procedures. It is spread when tools, hands and clothing become contaminated and it is spread by direct plant-to-plant contact (Hanssen & Thomma 2010; Spence et al. 2006; Wright & Mumford 1999).
- PepMV could be spread to tomato crops in new areas if contaminated machinery or tools are moved between areas, and could be moved on worker's hands if people work in more than one area within a short period of time (Hanssen & Thomma 2010; Spence et al. 2006; Wright & Mumford 1999).
- PepMV also infects basil, pepino and potato (CSL 2005; Davino et al. 2009a; Papayiannis, Kokkinos & Alfaro-Fernández 2012).
- The virus may spread from tomato to other host plant species including weed species. PepMV was recorded in field and greenhouse tomatoes in Cyprus as well as in 20 weed species in the field of that country in 2009 (Papayiannis, Kokkinos & Alfaro-Fernández 2012). The weeds infected in Cyprus included the species *Malva parviflora*, *Sonchus oleraceus*, *Solanum nigrum*, *Convolvulus arvensis*, *Chrysanthemum segetum* and *Calendula arvensis*. These weed species occur in Australia and some are widespread and abundant.
- PepMV infects plant species in the families Amaranthaceae, Asteraceae, Boraginaceae, Brassicaceae, Chenopodiaceae, Convolvulaceae, Malvaceae, Plantaginaceae, Polygonaceae and Solanaceae (Córdoba, Martínez-Priego & Jordá 2004; Papayiannis, Kokkinos & Alfaro-Fernández 2012; Soler et al. 2002). Since many wild and weedy species in these plant families exist in Australia, PepMV in tomato plants may be transmitted to weeds in a crop or weeds or wild plants growing near a crop. The virus may be sustained in the alternative hosts.
- Weeds probably act as reservoirs of the virus (Córdoba, Martínez-Priego & Jordá 2004; Werkman & Sansford 2010). Infection of weeds may accelerate or consolidate the spread of the virus, as more inoculum may be present at a location when weeds are infected, and opportunities for spread may occur more frequently.

- PepMV can be transmitted by bumble bees which contribute to the spread of the virus between tomato plants (Lacasa et al. 2003; Shipp et al. 2008). Bumble bees can also transmit PepMV between different species, for example, from tomato plants to the weed species *Solanum ptycanthum*, *S. sarrachoides* and *Datura stramonium*, and from these species back to tomato (Stobbs et al. 2009). *Bombus terrestris* is present in Tasmania but bumblebees are not present on the mainland of Australia.
- PepMV could be transmitted through soil by the chytrid fungus *Olpidium virulentus* (Alfaro-Fernández et al. 2010). *Olpidium virulentus* is present in Australia (Maccarone et al. 2010). PepMV may be transmitted by the fungus between field-grown tomatoes, and may be transmitted by the fungus in hydroponic systems via the irrigation water.
- PepMV infections may go unrecognised. The symptoms are similar to those caused by other viruses and viroids. An unrecognised infection may not be controlled or eradicated, and thus, may spread.

6.2.4 Overall likelihood of entry, establishment and spread

The overall likelihood of entry, establishment and spread is determined by combining the individual likelihoods of entry, of establishment and of spread using the matrix shown in Table 2.2.

The overall likelihood that PepMV will be imported in tomato seed for sowing, be distributed in the seed, establish in an area and subsequently spread within Australia is assessed as Moderate.

6.2.5 Consequences

The potential consequences (direct and indirect) of PepMV are assessed as Moderate. This assessment is made because the virus may cause substantial losses in tomato crops, and these losses would be amplified by spread of the virus. If there is an incursion, it is likely that eradication and control would be attempted by state and territory governments, but given the characteristics of the virus, these activities would prove costly and difficult. Moreover, if there is an incursion, it is possible domestic trade in tomato fruit would be disrupted as state and territory governments might restrict cross-border trade in an attempt to prevent transport of the virus between jurisdictions.

Criterion	Estimate and rationale
Direct	
Plant life or health	<p>E – significant at the regional level</p> <p>PepMV causes disease in tomato and basil and may cause disease in potato. Tomato and basil are grown commercially throughout Australia and potato is grown commercially in the southern states of the country. Australian potato and tomato production in the Australian financial year 2015-2016 were estimated to have gross values of \$659.7 million and \$541.6 million respectively (Australian Bureau of Statistics 2012; HIA 2017). Australian basil production is estimated to have a gross value of several tens of millions of dollars per annum.</p> <p>PepMV infection directly affects tomato fruit production by reducing yields and affecting the quality of fruit. In the United Kingdom, business losses from PepMV infections in tomato crops were estimated to range from £3.8 million per season to £37.5 million per season over a period of three years (Alleweldt 2011). The Australian tomato industry is several times larger than that in the United Kingdom and hence, there is the potential for greater losses in Australia.</p> <p>The incidence of infected plants varies in tomato crops, with one estimate indicating a range of incidence from 10 to 90 per cent (Soler-Aleixandre et al. 2005).</p> <p>Trials have shown the effect on tomato yields and quality vary depending on virus variant (Alleweldt 2011; Peters et al. 2011; Peters et al. 2010). One variant of the virus</p>

	<p>was found to have little effect on tomato yields and quality. However other variants were found to cause a reduction of yield of about 5 to 15 per cent (Peters et al. 2011). Trials have also shown yields of class 1 fruits to be reduced by certain variants by about 14 per cent to more than 38 per cent (Spence et al. 2006). Additional grading of tomato fruit due to PepMV infection would add to production costs.</p> <p>Fruit from infected tomato plants may be discoloured and have a marbled or mosaic appearance, or may split and become open so that the seed and flesh is exposed (Hanssen & Thomma 2010). The degree of symptoms in the fruit depends on the virus variant and probably on the cultivar of tomato (Fakhro et al. 2011; Peters et al. 2011; Peters et al. 2010).</p> <p>In some cases, PepMV-infected tomato plants only express mild symptoms on the vegetative parts or may be symptomless (Peters et al. 2011; Peters et al. 2010). However, in other cases, tomato plants have developed more serious symptoms including necrosis, deformed growth, wilting, plant collapse and plant death (Hanssen & Thomma 2010; Polston 2008; Soler-Aleixandre et al. 2005).</p> <p>Symptoms and adverse effects may be worse when the virus infects tomato plants together with other pathogens. One report of plants infected with a mixed infection of PepMV and the chytrid fungus <i>Olpidium brassicae</i> indicated that wilting and collapse were common symptoms (Soler-Aleixandre et al. 2005). A mixed infection with tomato chlorosis virus (ToCV) also produced heightened symptoms and greater losses than were expected to be induced by either pathogen alone (Davino et al. 2008). Increased effects were also noted when PepMV and <i>Verticillium</i> sp. co-infected plants (Mumford & Jones 2005).</p> <p>How PepMV infection might effect potato production is uncertain. Although experimental infections induced systemic necrosis, naturally infected potatoes have been reported to have milder vegetative symptoms (Jones, Koenig & Lesemann 1980; Mumford & Jones 2005). No report of an infected potato crop outside of Peru was found during the preparation of this draft report, nor was a report found that described effects on tubers. It is possible that infection and disease in potato depends on the cultivar of potato and variant of PepMV, and only occurs under certain environmental conditions (Blystad et al. 2015).</p> <p>One report indicates PepMV-infected basil crops in Sicily showing chlorosis on young leaves (Davino et al. 2009b). Chlorosis is likely to reduce the value of basil production.</p>
Other aspects of the environment	<p>D – minor significance at the regional level</p> <p>PepMV also infects species from the Amaranthaceae, Asteraceae, Brassicaceae, Boraginaceae, Chenopodiaceae, Convolvulaceae, Malvaceae, Plantaginaceae, Polygonaceae and Solanaceae (Córdoba, Martinez-Priego & Jordá 2004; Papayiannis, Kokkinos & Alfaro-Fernández 2012). There are Australian native species of plants in all these families, and many other species in these families are naturalised weeds in Australia. Some of these wild and naturalised plant species may be infected by PepMV and their abundance or health might be affected.</p>
Indirect	
Eradication, control	<p>E – major significance at the district level</p> <p>If an incursion of PepMV was to occur in an Australian tomato crop it is likely that eradication would be attempted. In the past decade several incursions of pospiviroids in tomato crops have been eradicated.</p> <p>The extent of an outbreak will depend on many factors including the transmission characteristics of the virus variant, environmental conditions, the transport of plants and machinery between properties and the movement workers and the activity of insect vectors. A PepMV outbreak in a tomato crop might be detected if the plants express significant symptoms, but if the plants are symptomless or the symptoms are indistinct the infection may go unnoticed. If an outbreak is initially unrecognised or unnoticed the virus may spread, and when control or eradication is attempted, a greater area will need to be managed and the costs will be greater.</p> <p>Spread in field crops is likely to be difficult to control, and lead to greater costs because the virus may also infect wild and weedy plants. This appears to have happened in Europe where endemic weed species have been reported as natural alternative hosts, (Córdoba, Martinez-Priego & Jordá 2004; OEPP-EPPO 2009), and these plants may have become reservoirs that perpetuate PepMV infection. If weeds are infected, eradication and control of PepMV may be more costly and less likely to succeed.</p>

	<p>During an outbreak, the infected property and adjoining and nearby properties will be surveyed. Samples from surveys will be tested in state government laboratories. Enzyme-linked immunosorbent assays (ELISA) will be used to test most samples, and RT-PCR will probably be used to confirm detections. Surveillance activities and laboratory tests are costly.</p> <p>PepMV is not eliminated when infected plants are killed with herbicide, so to control the virus and prevent spread, infected plant material would need be buried or burnt. One strategy may be to identify infected plants in a crop by surveying and testing, but if the aim is eradication and the virus spreads quickly, the entire crop may need to be destroyed. If the virus is contained in a greenhouse, the greenhouse would need to be cleaned before it could be used again. Equipment would need to be sterilized using bleach. Typically, when eradication is attempted, plants, propagating material, machinery and implements may not be moved from properties where an outbreak has been detected.</p>
Domestic trade	<p>E – significant at the regional level</p> <p>If PepMV became established in an Australian state, restrictions might be introduced on the interstate trade of affected produce, including tomato fruit, plants and seed. These restrictions could lead to loss of domestic markets.</p> <p>Long-distance transport of the virus within fruit is believed to be possible (van der Vlugt 2009; Werkman & Sansford 2010) as fruit from infected plants contain high concentrations of the virus and people can transmit the virus to plants if it is on their hands or clothes (Hanssen & Thomma 2010; van der Vlugt 2009). Infected fruit can be symptomless.</p> <p>The virus might be spread to crops if horticultural workers purchase and handle infected fruit, and then inadvertently introduce the virus to crops. It might also be spread to plants in domestic gardens by members of the public. PepMV has been detected in tomato fruit many times in Europe (Table 4.1).</p> <p>Tomato fruit, seed, seedlings and transplants are traded across Australia. This trade might be interrupted if an outbreak of PepMV occurred. It is suspected that the international outbreak of PepMV and its spread across Europe was due in part to trade in infected seed (Córdoba-Sellés et al. 2007).</p>
International trade	<p>D – significant at the district level</p> <p>Part of the Australian fresh tomato fruit crop is exported. These exports might be affected if PepMV became established in Australia as long distance transmission of the virus within fruit is possible (Table 4.1), and exports might be temporarily restricted.</p> <p>In 2015, Australia exported about 748 tonnes of tomatoes worth about \$2.4 million (HIA 2017). Australia has markets for fresh tomatoes to New Zealand, Singapore, Hong Kong, Brunei-Darussalam, Malaysia, New Caledonia, Indonesia, French Polynesia, Fiji, and USA (DAFF 2008; HAL 2012). Australia has a market for tomato seed to Thailand.</p>
Non-commercial and environmental	<p>A –indiscernible at the local level</p> <p>No evidence was found indicating environmental and non-commercial indirect effects.</p>

6.2.6 Unrestricted risk estimate

Unrestricted risk is the result of combining the likelihood of entry, establishment and spread with the assessment of overall consequences. Likelihoods and consequences are combined using the risk estimation matrix shown in Table 2.5.

Unrestricted risk estimate for PepMV	
Overall likelihood of entry, establishment and spread	Moderate
Consequences	Moderate
Unrestricted risk	Moderate

The unrestricted risk estimate for PepMV of Moderate does not achieve Australia's ALOP. Therefore, specific risk management measures are required for this virus.

6.3 *Columnnea latent viroid*

Columnnea latent viroid (CLVd) was found in several cultivars of tomato in the Netherlands in 1989, 1993 and 1995, and in tomato samples from Belgium in 1996 (EPPO 2005a; Verhoeven et al. 2004). It was identified in tomato plants from Portugal in 2006, and was confirmed in nurseries producing tomato plants in England in 2007 (Monger & Mumford 2006; Nixon et al. 2009). CLVd was also reported in France in 2007 (CSL 2007). Tomato plants infected with CLVd develop disease symptoms including stunting, chlorosis, bronzing and leaf distortion (NAPPO 2007).

CLVd is transmitted through tomato seed to seedlings (FERA 2009b; Marach 2008). The viroid is probably introduced into greenhouses with infected seed (FERA 2009b; Marach 2008). The viroid is mechanically transmitted, and it has been reported to spread rapidly through greenhouse tomato crops, likely by plant to plant contact and through normal horticultural activities when agricultural equipment becomes contaminated (FERA 2009b).

In this pest risk assessment, a risk scenario is considered whereby CLVd enters Australia in tomato seed, the seed is planted, and the viroid is transmitted within a tomato crop and to other hosts.

6.3.1 Likelihood of entry

Likelihood of importation

The likelihood of entry is considered in two parts, relating to importation and distribution. One part concerns the arrival of the pathogen in Australia in tomato seeds that are imported for sowing. The second part concerns the distribution of the infected tomato seed in Australia, and whether the seed and the pathogen will remain viable and survive to the point of germination.

The likelihood that CLVd will be present in tomato seed imported into Australia is assessed as High. This assessment is made because CLVd has been detected in tomato seed lots sent to Australia during the period of the application of emergency measures, and the viroid has been reported in crops in many countries, including tomato seed producing countries. Additionally, large volumes of tomato seed are imported into Australia each year.

- CLVd has been found in tomato crops in Asia (Thailand), Europe (United Kingdom, Belgium, France, the Netherlands, Germany and Portugal) and North America (Canada and USA) (CSL 2008; Hadidi et al. 2003; Mumford et al. 2006; NCBI 2007; Steyer et al. 2009; Verhoeven et al. 2004; Werkman, Verhoeven & Roenhorst 2007).
- Pospiviroids infect tomato plants systemically. Pospiviroid RNA has been found in the sap, leaves, roots, sepals, petals, ovaries, stamens and seed of tomato (Antignus, Lachman & Pearlsman 2007; EUPHRESO 2010; Koenraadt et al. 2009; Singh & Dilworth 2009; Singh et al. 2006; Singh, Nie & Singh 1999; Zhu et al. 2001). Pospiviroid RNA has been detected in the embryonic tissues of the seed of some hosts (Antignus, Lachman & Pearlsman 2007; EPPO 2016b; Matsushita & Tsuda 2014).
- Seed extracted from tomato fruit naturally infected with CLVd has been found to carry CLVd RNA (FERA 2009b).
- CLVd was detected in 13 lots of tomato seed sent to Australia from the Middle East and Asia between February 2012 and October 2013.

- Large quantities of tomato seed are imported into Australia annually from suppliers in many countries for the production of tomato crops. The department estimates that on average 760 kilograms of tomato seed are imported into Australia annually.

Likelihood of distribution

To have an impact a pathogen must be transported within Australia and must be capable of infecting a suitable host plant. The chance of this occurring depends upon the intended use of the imported commodity and the dispersal mechanisms of the pathogen. The likelihood that CLVd will be distributed in Australia in tomato seed and be present in the seed in an infectious state when it is planted is assessed as High. This assessment is made because imported tomato seed is distributed for planting throughout Australia, and if CLVd-infected seeds are present in an imported seed lot, it is likely the seeds and viroid will remain viable, and that the seeds will be sown.

- Tomato seed is imported for planting for greenhouse and field production of tomato fruit. Seed will be distributed for planting to greenhouses and farms in production areas throughout Australia, and for planting in domestic gardens.
- It is very likely CLVd will survive in tomato seed for long periods, as PSTVd has been found to endure for many years in seed when stored at room temperature (Singh, Boucher & Wang 1991).
- CLVd will be distributed with the seed to production areas throughout Australia, and if present, is very likely to be present in an infectious state in the seed when it is planted.

Likelihood of entry (importation × distribution)

The overall likelihood of entry is determined by combining the likelihood of importation with the likelihood of distribution using the matrix shown in Table 2.2.

The likelihood that CLVd will enter Australia in imported tomato seed and be in an infectious state in the seed when it is planted is assessed as High.

6.3.2 Likelihood of establishment

The likelihood that CLVd will establish in Australia will depend upon the availability of host plants and the reproductive and survival strategies of the viroid. Based on an evaluation of these factors, the likelihood of establishment is assessed as High. This assessment is made primarily because very large numbers of imported tomato seeds are sown in Australia, CLVd is transmitted from infected seed to seedlings and there is evidence of the viroid establishing in tomato crops in other countries.

- CLVd has been found in Thailand and in several countries in Europe and North America (CSL 2008; Hadidi et al. 2003; Mumford et al. 2006; NCBI 2007; Steyer et al. 2009; Verhoeven et al. 2004; Werkman, Verhoeven & Roenhorst 2007). CLVd has been found in tomato plants for seed production in an open field in Thailand (Tangkanchanapas et al. 2005).
- CLVd is transmitted through tomato seed to seedlings (Marach 2008); some seedlings grown from CLVd-infected tomato seed are thus likely to be infected by the viroid. The germination rates of tomato seed infected with CLVd may be reduced, as has been reported for tomato seed infected with other pospiviroids (Benson & Singh 1964).
- Millions of tomato plants are grown each year in Australia from imported seed.

- Outbreaks of CLVd in tomato crops in the United Kingdom in 2007 are believed to have been caused by infected seed, based on an assessment that seed was the common factor linking the outbreaks (FERA 2009b).
- CLVd is mechanically transmitted from plant to plant by contaminated cutting tools and machinery, on worker's hands and by plant to plant contact (FERA 2009b).
- CLVd may spread in greenhouse crops before disease symptoms become apparent or are recognised. Infected tomato plants in a field crop are less likely to be detected because of the lower intensity of horticultural activity in these crops.
- CLVd may survive between tomato crops in contaminated greenhouses and in infected crop residues and infect plants subsequently grown in the vicinity in later seasons. The related viroid PSTVd is stable under most environmental conditions, and can remain infective in hydrated plant material for several months and in dry material for over a year, and is expected to survive composting processes (Mikkelsen, Elphinstone & Jensen 2005).
- Tomatoes are grown commercially in greenhouses and fields in all states of Australia, including regions with tropical and Mediterranean climates. They are grown during the spring and summer in the field in the cooler regions of southern Australia, and all year round in other regions of Australia and in greenhouses.
- The climates of these regions in Australia where tomatoes are grown are generally similar to the climates of the areas where CLVd has been found. The environmental conditions in commercial greenhouses in Australia are very similar to those in greenhouses in countries where outbreaks of CLVd have occurred.
- It is possible that CLVd may infect solanaceous weeds in the vicinity of infected greenhouses and field crops, and become established in an area.
- CLVd outbreaks in tomato crops have been reported from the Netherlands and Belgium (EPPO 2005a; Verhoeven et al. 2004), Portugal (Monger & Mumford 2006), England (Nixon et al. 2009) and France (CSL 2007).
- Incursions of PCFVd and PSTVd, two pospiviroids, have been recorded in Australia. PCFVd was eradicated and almost all incursions of PSTVd were eradicated.
- The related viroids CEVd, CSVd and PSTVd have established in Australia (Gillings, Broadbent & Gollnow 1991; Hill & Moran 1996; Mackie et al. 2016; van Brunschot et al. 2014b).

6.3.3 Likelihood of spread

When a pathogen has entered Australia and become established, the nature of an outbreak will depend upon whether it spreads to new areas from the point where it first became established. Based on a comparison of factors relevant to the potential expansion of the geographic range of the pathogen in the source and destination areas, the likelihood that CLVd will spread is assessed as Moderate. This assessment is made because the viroid is spread by normal horticultural activities, and it may also be dispersed with trade in tomato seed, seedlings and fruit, and with the disposal of crop residues. The viroid might also be transmitted by bumble bees and certain aphids.

- Tomato is widely grown in home gardens, greenhouses and the field in all states and territories of Australia.
- Pospiviroids can spread rapidly through greenhouse crops because of the density of planting and the intensity of horticultural activity (Diener 1971; Hammond & Owens 2006).

- CLVd could be spread to tomato crops in new areas if contaminated machinery or tools are moved between areas, and could be moved on worker's hands if people work in more than one area within a short period of time (FERA 2009a).
- Transport of crop residues infected with CLVd to new areas and their disposal near tomato, weed or ornamental hosts could result in the mechanical transfer of the viroid to these hosts and result in its spread.
- CLVd could be introduced into new areas in tomato seed, as it can be present in seed and seed to seedling transmission has been demonstrated.
- Seedlings are moved over long distances for planting and infected seedlings could introduce CLVd into new areas. An incursion of the related viroid PSTVd was initiated at Mansfield, Victoria in 2012 after seedlings were transported from Perth, Western Australia, to the property in Victoria.
- Distribution and sale of tomato fruit infected with CLVd might transport the viroid over long distances to new areas, as seed in tomato fruit is usually viable and is sometimes planted by members of the public (van Brunschot et al. 2014b).
- CLVd could also be transmitted by plant-feeding insects or in pollen, as other pospiviroids are sometimes transmitted by these mechanisms (Antignus, Lachman & Pearlsman 2007; EPPD 1997; Fernow, Peterson & Plaisted 1970; Kryczynski, Paduch-Cichal & Skreczkowski 1988; Querci et al. 1997; Singh, Boucher & Somerville 1992; Syller, Marczewski & Pawlowicz 1997).
- Potato is experimentally susceptible to CLVd (Verhoeven et al. 2004) and potato seed and ware crops may be at risk of infection by this viroid. If potato crops were to become infected, the viroid could be spread in infected potato tubers as the related viroid PSTVd is spread by this means (Diener 1971; Hammond & Owens 2006). Some other species from the Asteraceae, Cucurbitaceae and Solanaceae also became infected when inoculated experimentally with CLVd (Singh, Ready & Nie 2003b).

6.3.4 Overall likelihood of entry, establishment and spread

The overall likelihood of entry, establishment and spread is determined by combining the likelihoods of entry, of establishment and of spread using the matrix shown in Table 2.2.

The overall likelihood that CLVd will be imported in tomato seed for sowing, be distributed in the seed, establish in an area and subsequently spread within Australia is assessed as Moderate.

6.3.5 Consequences

The potential consequences (direct and indirect) of CLVd are assessed as Moderate. This assessment is made because the viroid may cause substantial losses in tomato crops, and these losses would be amplified by spread of the viroid. The viroid might also damage other solanaceous crops including potato. If there is an incursion, it is likely that eradication and control would be attempted by state and territory governments, but given the characteristics of the viroid, these activities could prove costly and difficult.

Criterion	Estimate and rationale
Direct	
Plant life or health	E – significant at the regional level CLVd causes disease in tomato and may cause disease in potato if it is transmitted to that crop. Australian potato and tomato production in the Australian financial year 2015-2016 were estimated to have gross values of \$659.7 million and \$541.6 million respectively (Australian Bureau of Statistics 2012; HIA 2017). Tomatoes are grown

	<p>commercially throughout Australia and potatoes are grown commercially in all Australian states, but not in the Northern Territory.</p> <p>Tomato plants infected with CLVd are stunted and their leaves become distorted, yellow and bronzed (Antignus et al. 2002; Candresse et al. 2007; Spieker, Marinkovic & Sanger 1996; Verhoeven, Jansen & Roenhorst 2006b). Fruit from infected plants may be small and discoloured and ripening may be delayed as is found in plants infected with other pospiviroids (Antignus et al. 2002; Candresse et al. 2007). Yield losses of tomato fruit of 26 to 100 per cent have been measured in experiments (Marach 2008).</p> <p>During an outbreak of CLVd in the United Kingdom, 50-60 percent of a tomato crop became infected, symptoms were severe and losses were heavy (Constable & Moran 1996; FERA 2009b; Nixon et al. 2009; Sansford & Morris 2009).</p> <p>Potato plants experimentally infected with CLVd exhibit symptoms similar to potato plants infected with PSTVd (Diener 1987; Verhoeven, Jansen & Roenhorst 2006a). Potato crops might suffer substantial yield losses if infected by CLVd and the losses might be similar to those caused by PSTVd, but control measures may limit or eliminate the losses.</p> <p>Additional grading of tomato fruit or potato tubers due to CLVd infection would add to production costs. A delay in detection may result in extensive spread of the pathogen in greenhouses and crops. Control of CLVd may add substantially to potato production costs in Australia since currently seed potatoes are divided by cutting before planting and this practice may have to cease if CLVd were present. Greater numbers of seed tubers would be required if seed tubers were not cut before planting.</p>
Other aspects of the environment	<p>C – minor significance at the district level</p> <p>CLVd may infect other plant species of the Solanaceae in addition to tomato. Native and naturalised Solanaceae are components of Australian ecosystems that might be infected by CLVd and their abundance or health might be affected.</p>
Indirect	
Eradication, control	<p>D – significant at the district level</p> <p>Incursions of other pospiviroids in Australian tomato crops have been eradicated. If an incursion of CLVd were to occur in a tomato crop, and it was detected, it would probably be controlled by eradication. The extent of an outbreak will depend on many factors including the transmission characteristics of the viroid variant, environmental conditions, the transport of plants and machinery between properties, the movement of workers and the activity of insect vectors, if present.</p> <p>CLVd infections may go unrecognised because a number of other viroid species produce symptoms identical or very similar to those induced by CLVd (Hammond, Smith & Diener 1989; Verhoeven et al. 2004). An outbreak of CLVd may not be recognised or reported until it has spread to several crops, properties and species.</p> <p>During an outbreak, the infected property and adjoining and nearby properties will be surveyed. Samples from surveys will be tested in state government laboratories. RT-PCR will be used to test the samples and detect the viroid. Surveillance and laboratory tests are costly.</p> <p>Viroid incursions in tomato greenhouse crops have been eradicated by destroying the infected plants and by cleaning the greenhouse at the end of the season. Infected plants are identified by testing and removed. Viroids are not eliminated when infected plants are killed so infected plant material is buried. Equipment is sterilized using bleach. Typically plants, propagating material, machinery and implements may not be moved from properties where outbreaks have been detected.</p> <p>Plants grown in potato seed certification schemes in Australia are inspected for symptoms of viroid infection (DAFWA 2009a, b; ViCSPA 2009).</p>
Domestic trade	<p>C – minor significance at the district level</p> <p>If CLVd became established in an Australian state, restrictions might be introduced on the interstate trade of affected seed and seedlings, and if this occurred it could lead to the loss of markets.</p> <p>No movement of machinery from the affected properties is permitted during an eradication campaign.</p>
International trade	<p>D – significant at the district level</p> <p>Part of the Australian fresh tomato fruit crop is exported, as are parts of the Australian ware potato crop and seed potato crop. These exports might be affected if CLVd became</p>

	established in Australia. Australia has markets for fresh tomatoes to New Zealand, Singapore, Hong Kong, Brunei-Darussalam, Malaysia, New Caledonia, Indonesia, French Polynesia, Fiji, and USA (DAFF 2008; HAL 2012). Australia has markets for ware potatoes to the Republic of Korea, Malaysia, Mauritius, Singapore, Hong Kong, Indonesia, Philippines, the United Arab Emirates, Thailand, Taiwan and Brunei-Darussalam. Australia has markets for tomato seed and seed potatoes to Thailand.
Non-commercial and environmental	A –indiscernible at the local level No evidence was found indicating environmental and non-commercial indirect effects.

6.3.6 Unrestricted risk estimate

Unrestricted risk is the result of combining the likelihood of entry, establishment and spread with the assessment of overall consequences. Likelihoods and consequences are combined using the risk estimation matrix shown in Table 2.5.

Unrestricted risk estimate for CLVd	
Overall likelihood of entry, establishment and spread	Moderate
Consequences	Moderate
Unrestricted risk	Moderate

The unrestricted risk estimate for CLVd of Moderate does not achieve Australia's ALOP. Therefore, specific risk management measures are required for this viroid.

6.4 *Pepper chat fruit viroid*

Pepper chat fruit viroid (PCFVd) is a newly identified pospiviroid that causes disease in tomato and capsicum (*Capsicum annuum* L.). It was first reported in greenhouse capsicum crops in the Netherlands in 2006 and reoccurred in 2007 in the same species and location (Verhoeven et al. 2009). In 2009, it was isolated from tomato plants in Thailand, and in 2010 in capsicum plants in Canada (Punyapitak 2004; Reanwarakorn, Klinkong & Porsoongnurn 2011; Verhoeven et al. 2011). It was suspected that the viroid had been present in tomato in Thailand for several years. Infected tomato plants are stunted, the leaves becoming necrotic, distorted and discoloured, and the fruits are small.

PCFVd is transmitted through tomato seed to seedlings at rates of up to 1.4 per cent percent (Singh & Dilworth 2009). PCFVd is also seed transmitted at a relatively high rate in capsicum (Verhoeven et al. 2010). Sequenced isolates published in GeneBank from Thailand are 95 percent to 99 percent similar to sequences from outbreaks in Canada and the Netherlands. The geographic distribution of the viroid suggests it was probably transported to Canada and the Netherlands in infected seed.

In this pest risk assessment, a risk scenario is considered whereby PCFVd enters Australia in tomato seed, the seed is planted, and the viroid is transmitted within a tomato crop and to other hosts.

6.4.1 Likelihood of entry

Likelihood of importation

The likelihood of entry is considered in two parts, relating to importation and distribution. One part concerns the arrival of the pathogen in Australia in tomato seeds that are imported for

sowing. The second part concerns the distribution of the infected tomato seed in Australia, and whether the seed and the pathogen will remain viable and survive to the point of germination.

The likelihood that PCFVd will be present in tomato seed imported into Australia is assessed as High. This assessment is made because PCFVd has been detected in tomato seed lots sent to Australia during the period of application of the emergency measures, and the viroid has been reported in several countries, including tomato seed producing countries. Additionally, large volumes of tomato seed are imported into Australia each year.

- In the past 6 years, PCFVd has been found in tomato in Thailand and in capsicum from the Netherlands and Canada (Punyapitak 2004; Reanwarakorn, Klinkong & Porsoongnurn 2011; Verhoeven et al. 2011; Verhoeven et al. 2009). It has been found both in greenhouse and field-grown plants.
- Pospiviroids infect tomato plants systemically. Pospiviroid RNA has been found in the sap, leaves, roots, sepals, petals, ovaries, stamens and seed of tomato (Antignus, Lachman & Pearlsman 2007; EUPHRESCO 2010; Koenraad et al. 2009; Singh & Dilworth 2009; Singh et al. 2006; Singh, Nie & Singh 1999; Zhu et al. 2001). Pospiviroid RNA has been detected in the embryonic tissues of the seed of some hosts (Antignus, Lachman & Pearlsman 2007; EPPO 2016b; Matsushita & Tsuda 2014).
- PCFVd was detected in eleven lots of tomato seed sent to Australia and tested on arrival between February 2012 and October 2013, with infected seed produced in two countries in Europe, one in the Middle East and two countries in Asia.
- Large quantities of tomato seed are imported into Australia annually from suppliers in many countries for the production of tomato crops. The department estimates that on average 760 kilograms of tomato seed are imported into Australia annually.

Likelihood of distribution

To have an impact a pathogen must be transported within Australia and must be capable of infecting a suitable host plant. The chance of this occurring depends upon the intended use of the imported commodity and the dispersal mechanisms of the pathogen. The likelihood that PCFVd will be distributed in Australia in tomato seed and be present in the seed in an infectious state when it is planted is assessed as High. This assessment is made because imported tomato seed is distributed for planting throughout Australia, and if PCFVd-infected seeds are present in an imported seed lot, it is likely the infected seed and the viroid will remain viable, and that the seed will be planted.

- Tomato seed is imported for planting for greenhouse and field production of tomato fruit. Seed will be distributed for planting to greenhouses and farms in production areas throughout Australia, and for planting in domestic gardens.
- It is very likely PCFVd will survive in tomato seed for long periods. The related viroid PSTVd has been found to endure for at least 21 years in botanical seed of potato when stored at room temperature (Singh, Boucher & Wang 1991).
- PCFVd will be distributed with the seed to production areas throughout Australia, and if present, is very likely to be present in an infectious state in the seed when it is planted.

Likelihood of entry (importation × distribution)

The overall likelihood of entry is determined by combining the likelihood of importation with the likelihood of distribution using the matrix shown in Table 2.2.

The likelihood that PCFVd will enter Australia in imported tomato seed and be in an infectious state in the seed when it is planted is assessed as High.

6.4.2 Likelihood of establishment

The likelihood that PCFVd will establish in Australia will depend upon the availability of host plants and the reproductive and survival strategies of the viroid. Based on an evaluation of these factors, the likelihood of establishment is assessed as High. This assessment is made primarily because very large numbers of imported tomato seeds are sown in Australia, PCFVd is transmitted from infected seed to seedlings, and there is evidence of the viroid establishing in tomato crops in other countries.

- PCFVd has been found in Thailand, the Netherlands and Canada (Punyapitak 2004; Reanwarakorn, Klinkong & Porsoongnurn 2011; Verhoeven et al. 2011; Verhoeven et al. 2009).
- Some seedlings grown from PCFVd-infected tomato seed are very likely to be infected by the viroid, as seed to seedling transmission has been demonstrated for PCFVd (Yanagisawa & Matsushita 2017). The viroid is also seed transmitted in capsicum (Verhoeven et al. 2009).
- The germination rates of tomato seed infected with PCFVd may be reduced, as has been reported for tomato seed infected with other pospiviroids (Benson & Singh 1964).
- Millions of tomato plants are grown each year in Australia from imported seed.
- PCFVd is mechanically transmitted in tomato (Reanwarakorn, Klinkong & Porsoongnurn 2011; Verhoeven et al. 2009). Pospiviroids are spread mechanically from infected plants by contaminated agricultural equipment and on worker's hands during horticultural activities and by plant to plant contact (Conde, Connelly & Pitkethley 1996; Diener 1971; Hammond & Owens 2006; Horticulture New Zealand 2008; Manzer & Merriam 1961; Singh & Dhar 1998).
- PCFVd may spread in greenhouse crops before disease symptoms become apparent or are recognised. Infected tomato plants in a field crop are less likely to be detected because of the lower intensity of horticultural activity in these crops.
- PCFVd may survive between tomato crops in contaminated greenhouses and in infected crop residues, and infect plants subsequently grown in the vicinity in later seasons. The related viroid PSTVd is stable under most environmental conditions, and can remain infective in hydrated plant material for several months and in dry material for over a year, and is expected to survive composting processes (Mikkelsen, Elphinstone & Jensen 2005).
- Tomatoes are grown commercially in greenhouses and in the field in all states of Australia, including regions with tropical and Mediterranean climates. They are grown during the spring and summer in the field in the cooler regions of southern Australia, and all year round in other regions of Australia and in greenhouses.
- The climates of these regions in Australia where tomatoes are grown are generally similar to the climates of the areas where PCFVd has been found. The environmental conditions in commercial greenhouses in Australia are very similar to those in greenhouses in countries where outbreaks of PCFVd have occurred.
- It is possible that PCFVd may infect solanaceous weeds in the vicinity of infected greenhouses and field crops, and become established in an area.
- An incursion of PCFVd in a greenhouse tomato crop in South Australia was eradicated. The incursion was the first record of PCFVd in Australia, suggesting it was introduced in tomato seed, and that seed to seedling transmission occurred.

- Incursions of PSTVd, another pospiviroid, have also been recorded in Australia.
- The related viroids CEVd, CSVd and PSTVd have established in Australia (Gillings, Broadbent & Gollnow 1991; Hill & Moran 1996; Mackie et al. 2016; van Brunschot et al. 2014b).

6.4.3 Likelihood of spread

When a pathogen has entered Australia and become established, the nature of an outbreak will depend upon whether it spreads to new areas from the point where it first became established. Based on a comparison of factors relevant to the potential expansion of the geographic range of the pathogen in the source and destination areas, the likelihood that PCFVd will spread is assessed as Moderate. This assessment is made because the viroid is spread by normal horticultural activities, and it may also be dispersed with trade in tomato seed, seedlings and fruit, and with the disposal of crop residues. The viroid might also be transmitted by bumble bees and certain aphids. Tomato is widely grown in Australia in home gardens, greenhouses and the field in all states and territories of Australia.

- Pospiviroids can spread rapidly through greenhouse crops because of the density of planting and the intensity of horticultural activity (Diener 1971; Hammond & Owens 2006).
- PCFVd could be spread to tomato crops in new areas if contaminated machinery or tools are moved between areas, and could be moved on worker's hands if people work in more than one area within a short period of time (Reanwarakorn, Klinkong & Porsoongnurn 2011; Verhoeven et al. 2009).
- Transport of crop residues infected with PCFVd to new areas and their disposal near tomato, weed or ornamental hosts could result in the mechanical transfer of the viroid to these hosts and result in its spread.
- PCFVd could be introduced into new areas in tomato seed, as it can be present in seed and seed to seedling transmission has been demonstrated (Singh & Dilworth 2009).
- Seedlings are moved over long distances for planting and infected seedlings could introduce PCFVd into new areas. An incursion of the related viroid PSTVd was initiated at Mansfield, Victoria in 2012 after seedlings were transported from Perth, Western Australia, to the property in Victoria.
- Distribution and sale of tomato fruit infected with PCFVd might transport the viroid over long distances to new areas, as seed in tomato fruit is usually viable and is sometimes planted by members of the public (van Brunschot et al. 2014b).
- PCFVd could also be transmitted by plant feeding insects or in pollen, as other pospiviroids are sometimes transmitted by these mechanisms (Antignus, Lachman & Pearlsman 2007; EPPO 1997; Fernow, Peterson & Plaisted 1970; Kryczynski, Paduch-Cichal & Skreczkowski 1988; Querci et al. 1997; Singh, Boucher & Somerville 1992; Syller, Marczewski & Pawlowicz 1997).
- Potato is experimentally susceptible to PCFVd (Verhoeven et al. 2004) and potato seed and ware crops may be at risk of infection by this viroid. If potato crops were to become infected, the viroid could be spread in infected potato tubers as the related viroid PSTVd is spread by this means (Diener 1971; Hammond & Owens 2006).

6.4.4 Overall likelihood of entry, establishment and spread

The overall likelihood of entry, establishment and spread is determined by combining the likelihoods of entry, of establishment and of spread using the matrix shown in Table 2.2.

The overall likelihood that PCFVd will be imported in tomato seed for sowing, be distributed in the seed, establish in an area and subsequently spread within Australia is assessed as Moderate.

6.4.5 Consequences

The potential consequences (direct and indirect) of PCFVd are assessed as Moderate. This assessment is made because the viroid may cause substantial losses in tomato and capsicum crops, and these losses would be amplified by spread of the viroid. The viroid might also damage other solanaceous crops including potato. If there is an incursion, it is likely that eradication and control would be attempted by state and territory governments, but given the characteristics of the viroid, these activities could prove costly and difficult.

Criterion	Estimate and rationale
Direct	
Plant life or health	<p>E – significant at the regional level</p> <p>PCFVd causes disease in capsicum and tomato and may cause disease in potato if it is transmitted to that crop. Australian capsicum and chilli production in the Australian financial year 2015-2016 was estimated to have a gross value of \$138.9 million. Australian potato and tomato production in the Australian financial year 2015-2016 were estimated to have gross values of \$659.7 million and \$541.6 million respectively (Australian Bureau of Statistics 2012; HIA 2017). Tomatoes and capsicum are grown commercially throughout Australia and potatoes are grown commercially in all Australian states, but not the Northern Territory.</p> <p>Fruit from infected capsicum plants are small, being reduced in size by up to 50 percent, and probably unmarketable (Punyapitak 2004; Reanwarakorn, Klinkong & Porsoongnurn 2011; Verhoeven et al. 2011; Verhoeven et al. 2009). Capsicum plants may be mildly stunted.</p> <p>Tomato plants infected with PCFVd may be stunted and their leaves may become necrotic, distorted and yellow (Verhoeven et al. 2011; Verhoeven et al. 2009). PCFVd may reduce tomato yields, as the symptoms in tomato plants are similar to those caused by PSTVd, and PSTVd infection sometimes reduces tomato yields substantially (Kryczynski, Paduch-Cichal & Skreczkowski 1988; NSW DPI 2012).</p> <p>Potato plants experimentally infected with PCFVd produce small, elongated, misshapen tubers and otherwise exhibit symptoms similar to potato infected with PSTVd (Punyapitak 2004; Reanwarakorn, Klinkong & Porsoongnurn 2011; Verhoeven et al. 2011; Verhoeven et al. 2009). Potato crops might suffer substantial yield losses if infected by PCFVd and the losses might be similar to those caused by PSTVd, but control measures may limit or eliminate the losses.</p> <p>Additional grading of capsicum and tomato fruit or potato tubers due to PCFVd infection would add to production costs. A delay in detection may result in extensive spread of the pathogen in greenhouses and crops. Control of PCFVd may add substantially to potato production costs in Australia since currently seed potatoes are divided by cutting before planting and this practice may have to cease if PCFVd were present. Greater numbers of seed tubers would be required if seed tubers were not cut before planting.</p>
Other aspects of the environment	<p>C – minor significance at the district level</p> <p>PCFVd may infect other plant species of the Solanaceae, in addition to capsicum and tomato. Native and naturalised Solanaceae are components of Australian ecosystems that might be infected by PCFVd and their number or health might be affected. These plants provide food for native animals. <i>Solanum centrale</i> (bush tomato) is widespread in arid regions of central Australia.</p>
Indirect	
Eradication, control	<p>D – significant at the district level</p> <p>An incursion of PCFVd was eradicated from Australia in 2012. If further incursions occur in tomato crops action to eradicate the viroid may be taken.</p> <p>The extent of an outbreak will depend on many factors including environmental conditions, the transport of plants and machinery between properties, the movement of workers and the activity of insect vectors, if present.</p>

	<p>PCFVd infections may go unrecognised because a number of other viroid species produce symptoms identical or very similar to those induced by PCFVd (Hammond, Smith & Diener 1989; Verhoeven et al. 2004). An outbreak of PCFVd may not be detected until it has spread to several crops, properties and species.</p> <p>During an outbreak, the infected property and adjoining and nearby properties will be surveyed. Samples from surveys will be tested in state government laboratories. RT-PCR will be used to test the samples and detect the viroid. Surveillance and laboratory tests are costly.</p> <p>Viroid incursions in tomato greenhouse crops have been eradicated by destroying the infected plants and by cleaning the greenhouse at the end of the season. Infected plants are identified by testing and removed. Viroids are not eliminated when infected plants are killed so infected plant material is buried. Equipment is sterilized using bleach. Typically plants, propagating material, machinery and implements may not be moved from properties where outbreaks have been detected.</p> <p>Plants grown in potato seed certification schemes in Australia are inspected for symptoms of viroid infection (DAFWA 2009a, b; ViCSPA 2009).</p>
Domestic trade	<p>C – minor significance at the district level</p> <p>If PCFVd became established in an Australian state, restrictions might be introduced on the interstate trade of affected seed and seedlings, and if this occurred it could lead to the loss of markets.</p> <p>No movement of machinery from the affected properties is permitted during an eradication campaign.</p>
International trade	<p>D – significant at the district level</p> <p>Part of the Australian fresh tomato fruit crop is exported, as are parts of the Australian ware potato crop and seed potato crop. These exports might be affected if PCFVd became established in Australia. Australia has markets for fresh tomatoes to New Zealand, Singapore, Hong Kong, Brunei-Darussalam, Malaysia, New Caledonia, Indonesia, French Polynesia, Fiji, and USA (DAFF 2008; HAL 2012). Australia has markets for ware potatoes to the Republic of Korea, Malaysia, Mauritius, Singapore, Hong Kong, Indonesia, Philippines, the United Arab Emirates, Thailand, Taiwan and Brunei-Darussalam. Australia has markets for tomato seed and seed potatoes to Thailand.</p>
Non-commercial and environmental	<p>A – indiscernible at the local level</p> <p>No evidence was found indicating environmental and non-commercial indirect effects.</p>

6.4.6 Unrestricted risk estimate

Unrestricted risk is the result of combining the likelihood of entry, establishment and spread with the assessment of overall consequences. Likelihoods and consequences are combined using the risk estimation matrix shown in Table 2.5.

Unrestricted risk estimate for PCFVd	
Overall likelihood of entry, establishment and spread	Moderate
Consequences	Moderate
Unrestricted risk	Moderate

The unrestricted risk estimate for PCFVd of Moderate does not achieve Australia's ALOP. Therefore, specific risk management measures are required for this viroid.

6.5 *Tomato apical stunt viroid*

Tomato apical stunt viroid (TASVd) has infected tomato crops in several countries since the first known outbreak in Côte d'Ivoire in 1981, and has caused significant disease and yield loss in outbreaks in Israel and Tunisia (Antignus et al. 2002; Verhoeven, Jansen & Roenhorst 2006b; Walter, Thouvenel & Fauquet 1980). In Côte d'Ivoire, TASVd was found in tomato plants grown in the open field, whereas it was found in greenhouse crops in Israel and Tunisia (Antignus, Lachman & Pearlsman 2007; Verhoeven, Jansen & Roenhorst 2006b; Walter, Thouvenel &

Fauquet 1980). TASVd has also been found from tomato crops in Indonesia, Niger and Senegal (Candresse et al. 2007; Candresse, Smith & Diener 1987; Walter 1987). The viroid has been reported in *Cestrum* sp. in the Netherlands recently (Verhoeven, Jansen & Roenhorst 2008).

TASVd is transmitted through tomato seed and may be introduced into greenhouses via infected seed (Antignus, Lachman & Pearlsman 2007; Antignus et al. 2002). TASVd has a moderately wide host range, and some infected hosts are symptomless (Singh, Ready & Nie 2003b). It is mechanically transmitted and is reported to spread rapidly through greenhouse tomato crops, probably on contaminated agricultural equipment and by plant to plant contact (Antignus et al. 2002). It is also transmitted by bumble bees (Antignus, Lachman & Pearlsman 2007).

In this pest risk assessment, a risk scenario is considered whereby TASVd enters Australia in tomato seed, the seed is planted, and the viroid is transmitted within a tomato crop and to other hosts.

6.5.1 Likelihood of entry

Likelihood of importation

The likelihood of entry is considered in two parts, relating to importation and distribution. One part concerns the arrival of the pathogen in Australia in tomato seeds that are imported for sowing. The second part concerns the distribution of the infected tomato seed in Australia and whether the seed and the pathogen will remain viable and survive to the point of germination.

The likelihood that TASVd will be present in tomato seed imported into Australia is assessed as High. This assessment is made because TASVd has been detected in tomato seed lots sent to Australia during the period of application of emergency measures, and the viroid has been reported in several countries, including tomato seed producing countries. Additionally, large volumes of tomato seed are imported into Australia each year.

- TASVd has been found in tomato crops in Côte d'Ivoire (Walter, Thouvenel & Fauquet 1980), Indonesia (Candresse, Smith & Diener 1987), Israel (Antignus, Lachman & Pearlsman 2007), Niger (Walter 1987), Senegal (Candresse et al. 2007) and Tunisia (Verhoeven, Jansen & Roenhorst 2006b).
- TASVd was found consistently in greenhouse tomato crops in Israel over several years from 1999 (Antignus, Lachman & Pearlsman 2007; Antignus et al. 2002; Candresse et al. 2007).
- TASVd infects tomato plants systemically. TASVd RNA has been found in the sap, leaves, roots, sepals, petals, ovaries, stamens and seed of tomatoes (Antignus, Lachman & Pearlsman 2007). It is probably present in the embryonic tissues of tomato seed as well as on the seed surface (Antignus, Lachman & Pearlsman 2007).
- TASVd was detected in three tomato seed lots sent to Australia and tested on arrival between February 2012 and October 2013, with the infected seed produced in a country in Asia.
- Large quantities of tomato seed are imported into Australia annually from suppliers in many countries for the production of tomato crops. The department estimates that on average 760 kilograms of tomato seed are imported into Australia annually.

Likelihood of distribution

To have an impact a pathogen must be transported within Australia and must be capable of infecting a suitable host plant. The chance of this occurring depends upon the intended use of the imported commodity and the dispersal mechanisms of the pathogen. The likelihood that TASVd will be distributed in Australia in tomato seed and be present in the seed in an infectious state when it is planted is assessed as High. This assessment is made because imported tomato seed is distributed for planting throughout Australia, and if TASVd-infected seeds are present in an imported seed lot, it is likely the seeds and viroid will remain viable, and that the seeds will be sown.

- Tomato seed is imported for planting for greenhouse and field production of tomato fruit. Seeds will be distributed for planting to greenhouses and farms in production areas throughout Australia, and for planting in domestic gardens.
- It is very likely TASVd will survive in tomato seed for long periods, as PSTVd has been found to endure for many years in seed when stored at room temperature (Singh, Boucher & Wang 1991).
- TASVd in seed will be distributed with the seed to production areas throughout Australia, and if present, is very likely to be present in an infectious state in the seed when it is planted.

Overall likelihood of entry (importation × distribution)

The overall likelihood of entry is determined by combining the likelihood of importation with the likelihood of distribution using the matrix shown in Table 2.2.

The likelihood that TASVd will enter Australia in imported tomato seed and be in an infectious state in the seed when it is planted is assessed as High.

6.5.2 Likelihood of establishment

The likelihood that TASVd will establish in Australia will depend upon the availability of host plants and the reproductive and survival strategies of the viroid. Based on an evaluation of these factors, the likelihood of establishment is assessed as High. This assessment is made primarily because very large numbers of imported tomato seeds are sown in Australia, TASVd is transmitted from infected seed to seedlings, and there is evidence of the viroid establishing in tomato crops in other countries.

- TASVd has been found in Israel and Indonesia, and in several countries in Africa (Antignus, Lachman & Pearlsman 2007; Candresse et al. 2007; Candresse, Smith & Diener 1987; Verhoeven, Jansen & Roenhorst 2006b; Walter, Thouvenel & Fauquet 1980).
- Some seedlings grown from TASVd-infected tomato seeds are very likely to be infected by the viroid. The viroid is transmitted through tomato seed to seedlings and a transmission rate of about 80 percent has been reported (Antignus, Lachman & Pearlsman 2007).
- The germination rates of TASVd infected tomato seeds may be reduced, as has been reported for tomato seeds infected with other pospiviroids (Benson & Singh 1964).
- Millions of tomato plants are grown each year in Australia from imported seeds.
- Outbreaks of TASVd in greenhouses in Israel probably arose from infections of single isolated plants (Antignus et al. 2002).

- TASVd is mechanically transmitted (Antignus, Lachman & Pearlsman 2007; Singh & Dhar 1998; Singh, Ready & Nie 2003b) and is spread on contaminated machinery, tools and worker's hands during horticultural activities, including grafting, pruning and cutting.
- TASVd may spread in greenhouse crops before disease symptoms become apparent or are recognised. Infected tomato plants are less likely to be detected in field crops because of the lower intensity of horticultural activity in these crops.
- TASVd may survive between tomato crops in contaminated greenhouses and in infected crop residues, and infect plants subsequently grown in the vicinity in later seasons. The related viroid PSTVd is stable under most environmental conditions, and can remain infective in hydrated plant material for several months and in dry material for over a year, and is expected to survive composting processes (Mikkelsen, Elphinstone & Jensen 2005).
- Tomatoes are grown commercially in greenhouses and in the field in all states of Australia, including in regions with tropical and Mediterranean climates. They are grown during the spring and summer in the field in the cooler regions of southern Australia, and all year round in other regions of Australia and in greenhouses.
- The climates of these regions in Australia where tomatoes are grown are generally similar to the climates of the areas where TASVd has been found. The environmental conditions in commercial greenhouses in Australia are very similar to those in greenhouses in countries where outbreaks of TASVd have occurred.
- It is possible that TASVd may infect solanaceous weeds in the vicinity of infected greenhouses and field crops, and become established in a new area; *Solanum nigrum*, *Datura innoxia*, *D. metel* and other solanaceous species can be infected when inoculated with the viroid (Singh, Ready & Nie 2003b).
- In addition, species in the Asteraceae, Chenopodiaceae and Scrophulariaceae can be infected when inoculated with TASVd (Singh, Ready & Nie 2003b).
- Incursions of PCFVd and PSTVd, two pospiviroids, have been recorded in Australia. PCFVd was eradicated, and almost all incursions of PSTVd were eradicated.
- The related viroids CEVd, CSVd and PSTVd have established in Australia (Gillings, Broadbent & Gollnow 1991; Hill & Moran 1996; Mackie et al. 2016; van Brunschot et al. 2014b).

6.5.3 Likelihood of spread

When a pathogen has entered Australia and become established, the nature of an outbreak will depend upon whether it spreads to new areas from the point where it first became established. Based on a comparison of factors relevant to the potential expansion of the geographic range of the pathogen in the source and destination areas, the likelihood that TASVd will spread is assessed as Moderate. This assessment is made because the viroid is spread by normal horticultural activities and it may also be dispersed with trade in tomato seed, seedlings and fruit, and with the disposal of crop residues. The viroid might also be transmitted by bumble bees and certain aphids.

- Hosts of TASVd are widespread in Australia, as tomatoes are grown commercially in all states and territories of Australia. The solanaceous ornamental hosts *Cestrum* sp., *Solanum laxum* and *Solanum pseudocapsicum* are widely grown.
- TASVd is mechanically transmitted (Singh, Ready & Nie 2003b) and could be spread to new areas on contaminated machinery, tools and worker's hands during horticultural activities, including grafting, pruning and cutting.

- In greenhouse crops in Israel, TASVd spread quickly, almost always along plant rows, with entire crops becoming infected (Antignus et al. 2002).
- An entire greenhouse crop in Tunisia was quickly infected by TASVd (Verhoeven, Jansen & Roenhorst 2006b).
- Bumble bees can transmit TASVd during pollination (Antignus, Lachman & Pearlsman 2007). Bumble bees are not present on mainland Australia, but *Bombus terrestris* is present in Tasmania.
- Transport of crop residues infected with TASVd to new areas and their disposal near tomato, weed or ornamental hosts could result in the mechanical transfer of TASVd to these hosts and result in its spread.
- TASVd could be introduced into new areas via tomato seed, as it can be present in seed and seed to seedling transmission has been demonstrated for this viroid in tomato (Antignus, Lachman & Pearlsman 2007).
- Seedlings are moved over long distances for planting and infected seedlings could introduce TASVd into new areas. An incursion of the related viroid PSTVd was initiated at Mansfield, Victoria in 2012 after seedlings were transported from Perth, Western Australia, to the property in Victoria.
- Movement and sale of tomato fruit infected with TASVd might transport the viroid over long distances to new areas, as seed in tomato fruit is usually viable and is sometimes planted by members of the public (van Brunshot et al. 2014b)
- TASVd could also be transmitted by plant feeding insects or in pollen, as other pospiviroids are sometimes transmitted by these mechanisms (EPPO 1997; Fernow, Peterson & Plaisted 1970; Kryczynski, Paduch-Cichal & Skreczkowski 1988; Querci et al. 1997; Singh et al. 2006; Syller, Marczewski & Pawlowicz 1997).
- The cowpea aphid (*Aphis craccivora*) might transmit the viroid, albeit inefficiently (Walter 1987).

6.5.4 Overall likelihood of entry, establishment and spread

The overall likelihood of entry, establishment and spread is determined by combining the likelihoods of entry, of establishment and of spread using the matrix shown in Table 2.2.

The overall likelihood that TASVd will be imported in tomato seed for sowing, be distributed in the seed, establish in an area and subsequently spread within Australia is assessed as Moderate.

6.5.5 Consequences

The potential consequences (direct and indirect) of TASVd are assessed as Moderate. This assessment is made because the viroid may cause substantial losses in tomato crops and these losses would be amplified by spread of the viroid. The viroid might also damage other solanaceous crops including potato. If there is an incursion, it is likely that eradication and control would be attempted by state and territory governments, but given the characteristics of the viroid, these activities could prove costly and difficult.

Criterion	Estimate and rationale
Direct	
Plant life or health	E – significant at the regional level TASVd causes disease in tomato and may cause disease in potato if it is transmitted to that crop. Australian potato and tomato production in the Australian financial year 2015-2016 were estimated to have gross values of \$659.7 million and \$541.6 million

	<p>respectively (Australian Bureau of Statistics 2012; HIA 2017). Tomatoes are grown commercially throughout Australia and potatoes are grown commercially in all Australian states, but not the Northern Territory.</p> <p>TASVd-infected tomato plants are stunted and their leaves become deformed, yellow and brittle (Antignus et al. 2002; Candresse et al. 2007; Spieker, Marinkovic & Sanger 1996; Verhoeven, Jansen & Roenhorst 2006b). Fruit from infected plants is small and discoloured, ripening is delayed, and storage life is greatly reduced (Antignus et al. 2002; Candresse et al. 2007). In greenhouse tomato crops TASVd can spread quickly and infect entire crops (Antignus et al. 2002; Verhoeven, Jansen & Roenhorst 2006b).</p> <p>Tomato crops would be expected to suffer yield losses if infected by TASVd, and the losses would probably be similar to those caused by PSTVd. PSTVd infection can reduce tomato yields by up to 60 percent (Kryczynski, Paduch-Cichal & Skreczkowski 1988; NSW DPI 2012).</p> <p>If TASVd were present in weeds in potato growing areas, transmission could occur to potato as TASVd is mechanically transmitted and potato is susceptible. The tubers of potato plants experimentally infected with TASVd become severely cracked (Singh, Nie & Singh 1999). Potato crops might suffer substantial yield losses if infected by TASVd, and the losses might be similar to those caused by PSTVd, but control measures may limit or eliminate the losses.</p> <p>Additional grading of tomato fruit or potato tubers due to TASVd infection would add to production costs. A delay in detection may result in extensive spread of the pathogen in greenhouses and crops. Control of TASVd may add substantially to potato production costs in Australia since currently seed potatoes are divided by cutting before planting and this practice may have to cease if TASVd were present. Greater numbers of seed tubers would be required if seed tubers were not cut before planting.</p>
Other aspects of the environment	<p>C – minor significance at the district level</p> <p>Species from the Asteraceae, Chenopodiaceae, Scrophulariaceae and Solanaceae were infected when inoculated with TASVd (Singh, Ready & Nie 2003b). Native and naturalised species of these plant families may be infected by this viroid and their number or health may be affected. Native and naturalised species of Chenopodiaceae, Asteraceae, Scrophulariaceae and Solanaceae are components of Australian ecosystems and provide food for native animals. <i>Solanum centrale</i> (bush tomato) is widespread in arid regions of central Australia.</p>
Indirect	
Eradication, control	<p>D – significant at the district level</p> <p>Incursions of other pospiviroid species in Australian tomato crops have been eradicated. If an incursion of TASVd were to occur in a tomato crop, and it was detected, it would probably be controlled by eradication. The extent of an outbreak will depend many factors including environmental conditions, the transport of plants and machinery between properties, the movement of workers and the movement of insect vectors, if present.</p> <p>TASVd infections may go unrecognised because a number of other viroid species produce symptoms identical or very similar to those induced by TASVd (Singh, Ready & Nie 2003b). An outbreak of TASVd may not be detected until it has spread to several crops, properties and species.</p> <p>During an outbreak, the infected property and adjoining and nearby properties will be surveyed. Samples from surveys will be tested in state government laboratories. RT-PCR will be used to test the samples and detect the viroid. Surveillance and laboratory tests are costly.</p> <p>Viroid incursions in tomato greenhouse crops have been eradicated by destroying the infected plants and by cleaning the greenhouse at the end of the season. Infected plants are identified by testing and removed. Viroids are not eliminated when infected plants are killed so infected plant material is buried. Equipment is sterilized using bleach. Typically plants, propagating material, machinery and implements may not be moved from properties where outbreaks have been detected.</p> <p>Plants grown in potato seed certification schemes in Australia are inspected for symptoms of viroid infection (DAFWA 2009a, b; ViCSPA 2009).</p>
Domestic trade	<p>C – minor significance at the district level</p> <p>TASVd is listed in the National Vegetable Industry Biosecurity Plan released by Plant Health Australia (Plant Health Australia 2007). If TASVd became established in an</p>

	<p>Australian state, restrictions might be introduced on the interstate trade of affected seed and seedlings, and if this occurred it could lead to the loss of markets.</p> <p>No movement of machinery from the affected properties is permitted during an eradication campaign.</p>
International trade	<p>D – significant at the district level</p> <p>Part of the Australian fresh tomato fruit crop is exported, as are parts of the Australian ware potato crop and seed potato crop. These exports might be affected if TASVd became established in Australia. Australia sells tomatoes to New Zealand, Singapore, Hong Kong, Brunei-Darussalam, Malaysia, New Caledonia, Indonesia, French Polynesia, Fiji, and USA (DAFF 2008; HAL 2012). Australia sells ware potatoes to the Republic of Korea, Malaysia, Mauritius, Singapore, Hong Kong, Indonesia, Philippines, the United Arab Emirates, Thailand, Taiwan and Brunei-Darussalam. Australia sells tomato seed and seed potatoes to Thailand.</p>
Non-commercial and environmental	<p>A –indiscernible at the local level</p> <p>No evidence was found indicating environmental and non-commercial indirect effects.</p>

6.5.6 Unrestricted risk estimate

Unrestricted risk is the result of combining the likelihood of entry, establishment and spread with the assessment of overall consequences. Likelihoods and consequences are combined using the risk estimation matrix shown in Table 2.5.

Unrestricted risk estimate for TASVd	
Overall likelihood of entry, establishment and spread	Moderate
Consequences	Moderate
Unrestricted risk	Moderate

The unrestricted risk estimate for TASVd of Moderate does not achieve Australia's ALOP. Therefore, specific risk management measures are required for this viroid.

6.6 *Tomato chlorotic dwarf viroid*

Tomato chlorotic dwarf viroid (TCDVd) was first described from a greenhouse tomato crop in Canada produced from seed imported through the Netherlands in 1996 (Sabaratnam 2012; Singh, Nie & Singh 1999). The viroid causes significant disease and economic losses in greenhouse tomato crops (Matsushita et al. 2008; Singh, Nie & Singh 1999) and has now been reported from countries in Asia, Europe, the Middle East and North America (Table 5.2).

TCDVd is transmitted through tomato seed to seedlings at rates of up to 94 percent (Singh & Dilworth 2009). Its spread through greenhouse tomato crops in Japan was consistent with mechanical contact (Matsushita, Usugi & Tsuda 2009). Bumblebees have also been shown to transmit TCDVd during pollination (Matsushita, Usugi & Tsuda 2009).

In this pest risk assessment, a risk scenario is considered whereby TCDVd enters Australia in tomato seed, the seed is planted and the viroid is transmitted within a tomato crop and to other hosts.

6.6.1 Likelihood of entry

Likelihood of importation

The likelihood of entry is considered in two parts, relating to importation and distribution. One part concerns the arrival of the pathogen in Australia in tomato seeds that are imported for

sowing. The second part concerns the distribution of the infected tomato seed in Australia and whether the seed and the pathogen will remain viable and survive to the point of germination.

The likelihood that TCDVd will be present in tomato seed imported into Australia is assessed as High. This assessment is made because TCDVd has been detected in tomato seed lots sent to Australia during the period of application of emergency measures, and the viroid has been reported in several countries, including tomato seed producing countries. Additionally, large volumes of tomato seed are imported into Australia each year.

- In the past 14 years, TCDVd has been found in greenhouse tomato crops in Canada (Singh, Nie & Singh 1999), USA (Ling et al. 2009), Japan (Matsushita et al. 2008), France (Candresse et al. 2010) and Mexico (Ling & Zhang 2009).
- Pospiviroids infect tomato plants systemically. Pospiviroid RNA has been found in the sap, leaves, roots, sepals, petals, ovaries, stamens and seed of tomato (Antignus, Lachman & Pearlsman 2007; EUPHRESO 2010; Koenraad et al. 2009; Singh & Dilworth 2009; Singh et al. 2006; Singh, Nie & Singh 1999; Zhu et al. 2001). Pospiviroid RNA has been detected in the embryonic tissues of the seed of some hosts (Antignus, Lachman & Pearlsman 2007; EPPO 2016b; Matsushita & Tsuda 2014).
- A high percentage of tomato seed from TCDVd-infected plants can contain the viroid (Singh & Dilworth 2009).
- TCDVd was detected in seven lots of tomato seed sent to Australia and tested on arrival between February 2012 and October 2013. The seed was produced in two countries in Europe, one in the Americas, one in Africa and one in the Middle East.
- Large quantities of tomato seed are imported into Australia annually from suppliers in many countries for the production of tomato crops. The department estimates that on average 760 kilograms of tomato seed are imported into Australia annually.

Likelihood of distribution

To have an impact a pathogen must be transported within Australia and must be capable of infecting a suitable host plant. The chance of this occurring depends upon the intended use of the imported commodity and the dispersal mechanisms of the pathogen. The likelihood that TCDVd will be distributed in Australia in tomato seed and be present in the seed in an infectious state when it is planted is assessed as High. This assessment is made because imported tomato seed is distributed for planting throughout Australia, and if TCDVd-infected seeds are present in an imported seed lot, it is likely the seeds and viroid will remain viable, and that the seeds will be sown.

- Tomato seeds are imported for planting for greenhouse and field production of tomato fruit. Seeds will be distributed for planting to greenhouses and farms in production areas throughout Australia, and for planting in domestic gardens.
- It is very likely TCDVd will survive in tomato seed for long periods. The related pospiviroid PSTVd has been found to endure for at least 21 years in true potato seed when stored at room temperature (Singh, Boucher & Wang 1991).
- TCDVd present in seed will be distributed with the seed to production areas throughout Australia, and if present, is very likely to be present in an infectious state in the seed when it is planted.

Overall likelihood of entry (importation × distribution)

The overall likelihood of entry is determined by combining the likelihood of importation with the likelihood of distribution using the matrix shown in Table 2.2.

The likelihood that TCDVd will enter Australia in imported tomato seed and be in an infectious state in the seed when it is planted is assessed as High.

6.6.2 Likelihood of establishment

The likelihood that TCDVd will establish in Australia will depend upon the availability of host plants and the reproductive and survival strategies of the viroid. Based on an evaluation of these factors, the likelihood of establishment is assessed as High. This assessment is made primarily because very large numbers of imported tomato seeds are sown in Australia, TCDVd is transmitted from infected seed to seedlings, and there is evidence of the viroid establishing in tomato crops in other countries.

- Some seedlings grown from TCDVd infected tomato seed are very likely to be infected by the viroid. TCDVd is transmitted through tomato seed to seedlings and a transmission rate of up to 94 percent has been reported (Singh & Dilworth 2009).
- Seed to seedling transmission has been demonstrated for commercial seed, as two of 250 pools of 10 plants tested positive for TCDVd infection in a grow-out test of seed from which an outbreak developed in France (Candresse et al. 2010).
- The germination rates of TCDVd infected tomato seeds may be reduced, as has been reported for tomato seeds infected with other pospiviroids (Benson & Singh 1964).
- Millions of tomato plants are grown each year in Australia from imported seeds.
- TCDVd is mechanically transmitted (Matsushita, Usugi & Tsuda 2009; Singh & Dilworth 2009) and its spread along rows of tomato plants in Japan suggests that transmission resulted from mechanical contact (Matsushita et al. 2008). Pospiviroids are spread mechanically from infected plants by contaminated agricultural equipment and on worker's hands during horticultural activities and by plant to plant contact (Conde, Connelly & Pitkethley 1996; Diener 1971; Hammond & Owens 2006; Horticulture New Zealand 2008; Manzer & Merriam 1961; Singh & Dhar 1998).
- TCDVd may spread in greenhouse crops before disease symptoms become apparent or are recognised. Infected tomato plants in a field crop are less likely to be detected because of the lower intensity of horticultural activity in these crops.
- TCDVd may survive between tomato crops in contaminated greenhouses and in infected crop residues, and infect plants subsequently grown in the vicinity in later seasons. The related viroid PSTVd is stable under most environmental conditions, and can remain infective in hydrated plant material for several months and in dry material for over a year, and is expected to survive composting processes (Mikkelsen, Elphinstone & Jensen 2005).
- Tomato crops are grown commercially in greenhouses and in the field in all states of Australia. TCDVd has been found overseas infecting greenhouse crops in temperate areas.
- The environmental conditions in commercial greenhouses in Australia are very similar to those in greenhouses in countries where outbreaks of TCDVd have occurred.
- Beside tomato, TCDVd has been found infecting the ornamentals *Brugmansia sanguine* (Verhoeven et al. 2010), *Petunia x hybrida* (James et al. 2008; Verhoeven et al. 2007b) and *Vinca minor* (Singh & Dilworth 2009). These ornamentals could act as reservoirs of TCDVd if they were infected.

- Outbreaks of TCDVd have occurred in Canada, USA, Japan, and Mexico (Candresse et al. 2010; Ling & Zhang 2009; Matsushita et al. 2008; Singh, Nie & Singh 1999).
- Incursions of PCFVd and PSTVd, two pospiviroids, have been recorded in Australia. PCFVd was eradicated and almost all incursions of PSTVd were eradicated.
- The related viroids CEVd, CSVd and PSTVd have established in Australia (Gillings, Broadbent & Gollnow 1991; Hill & Moran 1996; Mackie et al. 2016; van Brunschot et al. 2014b).

6.6.3 Likelihood of spread

When a pathogen has entered Australia and become established, the nature of an outbreak will depend upon whether it spreads to new areas from the point where it first became established. Based on a comparison of factors relevant to the potential expansion of the geographic range of the pathogen in the source and destination areas, the likelihood that TCDVd will spread is assessed as Moderate. This assessment is made because the viroid is spread by normal horticultural activities and it may also be dispersed with trade in tomato seed, seedlings, and fruit and with the disposal of crop residues. The viroid is transmitted by bumble bees and might be transmitted by aphids.

- Hosts of TCDVd are widespread in Australia, as tomatoes are grown commercially in all states and territories of Australia and the solanaceous ornamental hosts *Brugmansia sanguinea*, *Petunia x hybrida*, *Verbena x hybrida* and *Vinca minor* are widely grown.
- Pospiviroids can spread rapidly through greenhouse crops because of the density of planting and the intensity of horticultural activity (Diener 1971; Hammond & Owens 2006).
- Species from the Asteraceae and Solanaceae were infected with TCDVd when experimentally inoculated (Matsushita, Usugi & Tsuda 2009; Singh, Nie & Singh 1999) suggesting that other species could be infected.
- TCDVd could be spread to tomato crops in new areas if contaminated machinery or tools were moved between areas and could be moved on worker's hands if people work in more than one area within a short period of time (Sabaratnam 2012).
- Movement of TCDVd-infected crop residues to new areas, and their disposal near tomato, weed or other hosts could result in the mechanical transfer of the viroid to those hosts and result in its spread.
- TCDVd could be introduced into new areas by tomato seed, as it can be present in seed, and seed to seedling transmission has been demonstrated for this viroid in tomato (Singh & Dilworth 2009).
- Seedlings are moved over long distances for planting and infected seedlings could introduce TASVd into new areas. An incursion of the related viroid PSTVd was initiated at Mansfield, Victoria in 2012 after seedlings were transported from Perth, Western Australia, to the property in Victoria.
- Seedlings infected with PSTVd produced in the Netherlands caused an outbreak of this viroid in Belgium (Verhoeven et al. 2007a).
- The movement of nursery stock of the solanaceous ornamental hosts of TCDVd, *Brugmansia sanguinea*, *Petunia x hybrida*, *Verbena x hybrida* and *Vinca minor*, could spread the viroid to new areas.
- Movement and sale of tomato fruit infected with TCDVd could spread the viroid over long distances to new areas, as transmission studies have shown that the related viroid PSTVd can be mechanically transmitted from crushed fruit (van Brunschot et al. 2014b).

- TCDVd can be spread by bumble bees (Matsushita, Usugi & Tsuda 2009). Bumble bees are not present on mainland Australia, but *Bombus terrestris* is present in Tasmania.
- It is possible that TCDVd could be spread by other vectors, as the closely related viroid PSTVd can be transmitted by *Myzus persicae* (green peach aphid) in association with *Potato leafroll virus* (EPPO 1997; Querci et al. 1997; Syller, Marczewski & Pawlowicz 1997). In addition, PSTVd is probably transmitted by other plant-feeding insects (Schumann, Tingey & Thurston 1980) and nematodes (Jatala & Garzón 1990).
- Potato (*Solanum tuberosum*) is experimentally susceptible to TCDVd (Singh, Nie & Singh 1999), therefore potato seed and ware crops may be at risk of infection by this viroid. If potato crops were to become infected, the viroid could be spread in infected potato tubers as the related viroid PSTVd is spread by this means (Diener 1971; Hammond & Owens 2006).

6.6.4 Overall likelihood of entry, establishment and spread

The overall likelihood of entry, establishment and spread is determined by combining the likelihoods of entry, of establishment and of spread using the matrix shown in Table 2.2.

The overall likelihood that TCDVd will be imported in tomato seed for sowing, be distributed in the seed, establish in an area and subsequently spread within Australia is assessed as Moderate.

6.6.5 Consequences

The potential consequences (direct and indirect) of TCDVd are assessed as Moderate. This assessment is made because the viroid may cause substantial losses in tomato crops and these losses would be amplified by spread of the viroid. The viroid might also damage other solanaceous crops including potato. If there is an incursion, it is likely that eradication and control would be attempted by state and territory governments, but given the characteristics of the viroid, these activities could prove costly and difficult.

Criterion	Estimate and rationale
Direct	
Plant life or health	<p>E – significant at the regional level</p> <p>TCDVd causes disease in tomato and may cause disease in potato if it is transmitted to that crop. Australian potato and tomato production in the Australian financial year 2015-2016 were estimated to have gross values of \$659.7 million and \$541.6 million respectively (Australian Bureau of Statistics 2012; HIA 2017). Tomatoes are grown commercially throughout Australia and potatoes are grown commercially in all Australian states, but not the Northern Territory.</p> <p>Tomato plants infected by TCDVd show top bunching, leaf curling symptoms (Candresse et al. 2010). Commonly observed symptoms are stunting, bunchiness, reduced leaves and fruit, leaf chlorosis, leaf and petiole necrosis, downward bending of leaves and fruit distortion (Sabaratnam 2012). TCDVd has been found to spread in greenhouse tomato crops in Canada (Singh, Nie & Singh 1999), Japan (Matsushita et al. 2008) and France (Candresse et al. 2010). TCDVd may reduce tomato yields, as the symptoms in tomato plants are similar to those caused by PSTVd, and PSTVd infection sometimes reduces tomato yields substantially (Kryczynski, Paduch-Cichal & Skreczkowski 1988; NSW DPI 2012).</p> <p>If TCDVd were present in weeds in potato-growing areas, transmission could occur to potato as TCDVd is mechanically transmitted, and potato is susceptible. The tubers of potato plants experimentally infected with TCDVd become severely cracked (Singh, Nie & Singh 1999). Potato crops might suffer substantial yield losses if infected by TCDVd and the losses might be similar to those caused by PSTVd, but control measures may limit or eliminate the losses.</p> <p>Additional grading of tomato fruit or potato tubers due to TCDVd infection would add to production costs. A delay in detection may result in extensive spread of the pathogen in</p>

	greenhouses and crops. Control of TCDVd may add substantially to potato production costs in Australia since currently seed potatoes are divided by cutting before planting and this practice may have to cease if TCDVd were present. Greater numbers of seed tubers would be required if seed tubers were not cut before planting.
Other aspects of the environment	<p>C – minor significance at the district level</p> <p>Native and naturalised species of Solanaceae may be infected by TCDVd and their number or health may be affected. Native and naturalised Solanaceae are components of Australian ecosystems and provide food for native animals. <i>Solanum centrale</i> (bush tomato) is widespread in arid regions of central Australia.</p>
Indirect	
Eradication, control	<p>D – significant at the district level</p> <p>Incursions of other pospiviroid species, PSTVd and PCFVd, in Australian tomato crops have been eradicated. If an incursion of TCDVd were to occur in a tomato crop, and it was detected, it would probably be controlled by eradication. The extent of an outbreak will depend on many factors including the environmental conditions, the transport of plants and machinery between properties, the movement of workers and the activity of insect vectors, if present.</p> <p>PCFVd infections may go unrecognised because a number of other viroid species produce symptoms identical or very similar to those induced by TCDVd (Singh, Ready & Nie 2003b). An outbreak of TCDVd may not be detected until it has spread to several crops, properties and species.</p> <p>During an outbreak, the infected property and adjoining and nearby properties will be surveyed. Samples from surveys will be tested in state government laboratories. RT-PCR will be used to test the samples and detect the viroid. Surveillance and laboratory tests are costly.</p> <p>Viroid incursions in tomato greenhouse crops have been eradicated by destroying the infected plants and by cleaning the greenhouse at the end of the season. Infected plants are identified by testing and removed. Viroids are not eliminated when infected plants are killed so infected plant material is buried. Equipment is sterilized using bleach. Typically plants, propagating material, machinery and implements may not be moved from properties where outbreaks have been detected.</p> <p>Plants grown in potato seed certification schemes in Australia are inspected for symptoms of viroid infection (DAFWA 2009a, b; ViCSPA 2009).</p>
Domestic trade	<p>C – minor significance at the district level</p> <p>If TCDVd became established in an Australian state, restrictions might be introduced on the interstate trade of affected seed and seedlings, and if this occurred it could lead to the loss of markets.</p> <p>No movement of machinery from the affected properties is permitted during an eradication campaign.</p>
International trade	<p>D – significant at the district level</p> <p>Part of the Australian fresh tomato fruit crop is exported, as are parts of the Australian ware potato crop and seed potato crop. These exports might be affected if TCDVd became established in Australia. Australia sells tomatoes to New Zealand, Singapore, Hong Kong, Brunei-Darussalam, Malaysia, New Caledonia, Indonesia, French Polynesia, Fiji, and USA. Australia sells ware potatoes to the Republic of Korea, Malaysia, Mauritius, Singapore, Hong Kong, Indonesia, Philippines, the United Arab Emirates, Thailand, Taiwan and Brunei-Darussalam. Australia sells tomato seed and seed potatoes to Thailand (DAFF 2008; HAL 2012).</p>
Non-commercial and environmental	<p>A – indiscernible at the local level</p> <p>No evidence was found indicating environmental and non-commercial indirect effects.</p>

6.6.6 Unrestricted risk estimate

Unrestricted risk is the result of combining the likelihood of entry, establishment and spread with the assessment of overall consequences. Likelihoods and consequences are combined using the risk estimation matrix shown in Table 2.5.

Unrestricted risk estimate for TCDVd	
Overall likelihood of entry, establishment and spread	Moderate
Consequences	Moderate
Unrestricted risk	Moderate

The unrestricted risk estimate for TCDVd of Moderate does not achieve Australia's ALOP. Therefore, specific risk management measures are required for this viroid.

6.7 *Tomato planta macho viroid*

Tomato planta macho viroid (TPMVd) has infected tomato crops in Mexico since the disease was first observed in 1969, and has caused significant disease and yield loss (Diener 1987; Orozco Vargas & Galindo-Alonso 1986). Infected plants only produce small fruit that have no commercial value (Galindo, Smith & Diener 1982).

Mexican papita viroid is considered to be a variant of TPMVd (TPMVd-MP), as nucleotide sequences of the two viroids are more than 90 percent identical (Verhoeven, Roenhorst & Owens 2011). The TPMVd-MP was reported from *Solanum cardiophyllum* (papita) and in greenhouse tomato crops in Mexico in 1996, and Canada in 2008 (Ling & Bledsoe 2009; Ling & Zhang 2009; Martínez-Soriano et al. 1996). This variant of TPMVd viroid produces general stunting, leaf chlorosis and reduced-sized fruits in tomato (Ling & Zhang 2009).

TPMVd is transmitted through tomato seed to seedlings at rates of up to 4.4 per cent (Yanagisawa & Matsushita 2017). The viroid is also transmitted at low rates through seed of some wild natural solanaceous hosts (Orozco Vargas & Galindo-Alonso 1986). One report indicated that tomato plants infected with TPMVd produced fruit with few to no seeds (Orozco Vargas & Galindo-Alonso 1986), but another report indicating that hundreds of seeds had been gathered from infected plants (Yanagisawa & Matsushita 2017) suggests reasonable numbers of viable seeds are produced under some circumstances.

In this pest risk assessment, a risk scenario is considered whereby TPMVd enters Australia in tomato seed, the seed is planted, and the viroid is transmitted within a tomato crop and to other hosts.

6.7.1 Likelihood of entry

Likelihood of importation

The likelihood of entry is considered in two parts, relating to importation and distribution. One part concerns the arrival of the pathogen in Australia in tomato seeds that are imported for sowing. The second part concerns the distribution of the infected tomato seed in Australia, and whether the seed and the pathogen will remain viable and survive to the point of germination.

The likelihood that TPMVd will be present in tomato seed imported into Australia is assessed as Moderate. This assessment is made because TPMVd is present in a seed-producing country and appears to have moved historically with traded tomato seed, and like other pospiviroids, has the potential to enter the seed production system. Additionally, large volumes of tomato seed are imported into Australia each year. The lack of detections of the viroid in imported tomato seed lots during the period of application of emergency measures is considered to be an ameliorating factor in the assessment.

- TPMVd occurs in Mexico and has been reported in a tomato crop in Canada (Ling & Zhang 2009; Orozco & Galindo 1986; Orozco Vargas & Galindo-Alonso 1986).
- The outbreak in Canada was probably a result of transport of seed or seedlings (Ling & Bledsoe 2009; Yanagisawa & Matsushita 2017). The viroid was likely introduced in imported seed or seedlings, given the large geographical distance between Mexico and Canada and the absence of any other reports of this viroid in Canada, nor any reports of it in the United States. The viroid was probably transported in tomato seed or seedlings from Mexico or another seed producing country
- Pospiviroids infect tomato plants systemically. Pospiviroid RNA has been found in the sap, leaves, roots, sepals, petals, ovaries, stamens and seed of tomato (Antignus, Lachman & Pearlsman 2007; EUPHRESO 2010; Koenraad et al. 2009; Singh & Dilworth 2009; Singh et al. 2006; Singh, Nie & Singh 1999; Zhu et al. 2001). Pospiviroid RNA has been detected in the embryonic tissues of the seed of some hosts (Antignus, Lachman & Pearlsman 2007; EPPO 2016b; Matsushita & Tsuda 2014).
- TPMVd is present in seed of infected tomato plants (Yanagisawa & Matsushita 2017).
- TPMVd has not been detected in tomato seed sent to Australia during the period of application of emergency measures.
- One report indicates tomato plants infected with the viroid produce few or no seeds (Orozco Vargas & Galindo-Alonso 1986), possibly reducing the chance that the variant will be present in commercial tomato seed. However, other reports indicate infected fruits do contain seeds, although infection by the viroid reduces fruit size (Ling & Zhang 2009; Yanagisawa & Matsushita 2017).
- Large quantities of tomato seed are imported into Australia annually from suppliers in many countries for the production of tomato crops. The department estimates that on average 760 kilograms of tomato seed are imported into Australia annually.

Likelihood of distribution

To have an impact a pathogen must be transported within Australia and must be capable of infecting a suitable host plant. The chance of this occurring depends upon the intended use of the imported commodity and the dispersal mechanisms of the pathogen. The likelihood that TPMVd will be distributed in Australia in tomato seed and be present in the seed in an infectious state when it is planted is assessed as High. This assessment is made because imported tomato seed is distributed for planting throughout Australia, and if TPMVd-infected seeds are present in an imported seed lot, it is likely the seeds and viroid will remain viable, and that the seeds will be sown.

- Tomato seed is imported for planting for greenhouse and field production of tomato fruit. Seed will be distributed for planting to greenhouses and farms in production areas throughout Australia, and for planting in domestic gardens.
- It is very likely TPMVd will survive in tomato seed for long periods. The related pospiviroid PSTVd has been found to endure for at least 21 years in true potato seed when stored at room temperature (Singh, Boucher & Wang 1991).
- TPMVd present in seed will be distributed with the seed to production areas throughout Australia, and if present, is very likely to be present in an infectious state in the seed when it is planted.

Overall likelihood of entry (importation × distribution)

The overall likelihood of entry is determined by combining the likelihood of importation with the likelihood of distribution using the matrix shown in Table 2.2.

The likelihood that TPMVd will enter Australia in imported tomato seed and be in an infectious state in the seed when it is planted is assessed as Moderate.

6.7.2 Likelihood of establishment

The likelihood that TPMVd will establish in Australia will depend upon the availability of host plants and the reproductive and survival strategies of the viroid. Based on an evaluation of these factors, the likelihood of establishment is assessed as High. This assessment is made primarily because very large numbers of imported tomato seeds are sown in Australia, TPMVd is transmitted from infected seed to seedlings, and there is evidence of the viroid establishing in tomato crops in other countries.

- Some seedlings grown from TPMVd infected tomato seeds are very likely to be infected by the viroid.
- TPMVd is transmitted through tomato seed to seedlings at rates of up to 4.4 per cent (Singh & Dilworth 2009). TPMVd is also transmitted at low rates through the seed of some of its wild solanaceous hosts (Orozco Vargas & Galindo-Alonso 1986).
- The germination rates of tomato seed infected with TPMVd may be reduced, as has been reported for tomato seed infected with other pospiviroids (Benson & Singh 1964).
- TPMVd is mechanically transmitted (Galindo, Smith & Diener 1982) and the TPMVd-MP variant has spread through tomato crops in greenhouses in Mexico (Ling & Zhang 2009).
- If TPMVd was introduced into a greenhouse tomato crop in Australia, it could spread through the crop before disease symptoms become apparent or were recognised. Infected tomato plants in a field crop are less likely to be detected because of the lower intensity of horticultural activity in these crops.
- TPMVd may survive between tomato crops in contaminated greenhouses and in infected crop residues, and infect plants subsequently grown in the vicinity in later seasons. The related viroid PSTVd is stable under most environmental conditions, and can remain infective in hydrated plant material for several months and in dry material for over a year, and is expected to survive composting processes (Mikkelsen, Elphinstone & Jensen 2005).
- Tomatoes are grown commercially in greenhouses and in the field in all states of Australia, including in regions with tropical and Mediterranean climates. They are grown during the spring and summer in the field in the cooler regions of southern Australia, and all year round in other regions of Australia and in greenhouses.
- The climates of these regions in Australia where tomatoes are grown are generally similar to the climates of the areas where TPMVd-MP has been found.
- TPMVd-MP variant has been found to infect greenhouse tomato crops in Mexico and Canada. The environmental conditions of commercial greenhouses in Australia are very similar to those greenhouses in Mexico and Canada where outbreaks of TPMVd-MP variant have occurred.
- Incursions of PCFVd and PSTVd, two pospiviroids, have been recorded in Australia. PCFVd was eradicated and almost all incursions of PSTVd were eradicated.
- The related viroids CEVd, CSVd and PSTVd have established in Australia (Gillings, Broadbent & Gollnow 1991; Hill & Moran 1996; Mackie et al. 2016; van Brunschot et al. 2014b).

6.7.3 Likelihood of spread

When a pathogen has entered Australia and become established, the nature of an outbreak will depend upon whether it spreads to new areas from the point where it first became established. Based on a comparison of factors relevant to the potential expansion of the geographic range of the pathogen in the source and destination areas, the likelihood that TPMVd will spread is assessed as Moderate. This assessment is made because the viroid is spread by normal horticultural activities and it may also be dispersed with trade in tomato seed, seedlings and fruit, and with the disposal of crop residues. The viroid is transmitted by aphids and might be transmitted by bumble bees.

- Tomato is widely grown in home gardens, greenhouses and the field in all states and territories of Australia.
- TPMVd could be spread to tomato crops in new areas if contaminated machinery or tools are moved between areas, and could be moved on worker's hands if people work in more than one area within a short period of time (Singh, Ready & Nie 2003b).
- Movement of TPMVd-infected crop residues to new areas and their disposal near tomato or other hosts could allow the mechanical transfer of the viroid and result in its spread.
- TPMVd could be introduced into new areas via infected tomato seed.
- Seedlings are moved over long distances for planting and infected seedlings could spread TPMVd to new areas. An incursion of the related viroid PSTVd was initiated at Mansfield, Victoria in 2012 after seedlings were transported from Perth, Western Australia, to the property in Victoria, and seedlings infected with PSTVd produced in the Netherlands caused an outbreak of this viroid in Belgium (Verhoeven et al. 2007a).
- Movement and sale of tomato fruit infected with TPMVd might transport the viroid over long distances to new areas, as seed in tomato fruit is usually viable and is sometimes planted by members of the public (van Brunschot et al. 2014b)
- The aphid *Myzus persicae* has been found to acquire and transmit TPMVd (Galindo, López & Aguilar 1989), and could spread the viroid within and between tomato crops and to other host plants in new areas.

6.7.4 Overall likelihood of entry, establishment and spread

The overall likelihood of entry, establishment and spread is determined by combining the likelihoods of entry, of establishment and of spread using the matrix shown in Table 2.2.

The overall likelihood that TPMVd will be imported in tomato seed for sowing, be distributed in the seed, establish in an area and subsequently spread within Australia is assessed as Low.

6.7.5 Consequences

The potential consequences (direct and indirect) of TPMVd are assessed as Moderate. This assessment is made because the viroid may cause very substantial losses in tomato crops and these losses would be amplified by spread of the viroid. The viroid might also damage other solanaceous crops including potato. If there is an incursion, it is likely that eradication and control would be attempted by state and territory governments, and given the characteristics of the viroid, these activities could prove costly and difficult.

Criterion	Estimate and rationale
Direct	
Plant life or health	<p>E – significant at the regional level</p> <p>TPMVd causes severe disease in tomato and may cause disease in potato if it is transmitted to that crop. Australian potato and tomato production in the Australian financial year 2015-2016 were estimated to have gross values of \$659.7 million and \$541.6 million respectively (Australian Bureau of Statistics 2012; HIA 2017). Tomatoes are grown commercially throughout Australia, and potatoes are grown commercially in all Australian states, but not the Northern Territory.</p> <p>Tomato plants infected with TPMVd are severely stunted and exhibit strong curling of the leaves. Leaflets become crinkled and brittle, veins and stems may become necrotic, and old leaves will yellow and dry out (Galindo, Smith & Diener 1982). Fruit remains small and has no commercial value (Galindo, Smith & Diener 1982). In greenhouse tomato crops, TPMVd-MP variant can spread quickly and infect entire crops (Ling & Zhang 2009).</p> <p>TPMVd has been reported to cause severe damage in tomato crops and total crop loss has been recorded (Belalcazar & Galindo-Alonso 1974; Galindo, Smith & Diener 1982). Severe symptoms and heavy yield losses in greenhouse tomato crops have been reported in crops infected with the TPMVd-MP variant (Ling & Bledsoe 2009; Ling & Zhang 2009). Symptom development and heavy yield losses in tomato crops occur at temperatures above 22 degrees Celsius (Galindo 1987).</p> <p>If TPMVd were present in weeds in potato-growing areas then transmission could occur to potato, as TPMVd is mechanically transmitted and potato is susceptible. Potato plants experimentally infected with TPMVd produce small distorted tubers (Yanagisawa & Matsushita 2017). Potato crops might suffer substantial yield losses if infected by TPMVd and the losses might be similar to those caused by PSTVd, but control measures may limit or eliminate the losses.</p> <p>Additional grading of tomato fruit or potato tubers due to TPMVd infection would add to production costs. A delay in detection may result in extensive spread of the pathogen in greenhouses and crops. Control of TPMVd may add substantially to potato production costs in Australia since currently seed potatoes are divided by cutting before planting and this practice may have to cease if TPMVd were present. Greater numbers of seed tubers would be required if seed tubers were not cut before planting.</p>
Other aspects of the environment	<p>C – minor significance at the district level</p> <p>TPMVd has been found from wild solanaceous plants in Mexico (Orozco Vargas & Galindo-Alonso 1986). Naturally infected wild plants generally have no symptoms or mild symptoms.</p> <p>Native and naturalised Solanaceae are components of Australian ecosystems and provide food for native animals.</p>
Indirect	
Eradication, control	<p>D – significant at the district level</p> <p>Incursions of other pospiviroid species, PCFVd and PSTVd, in Australian tomato crops have been eradicated. If an incursion of TPMVd were to occur in a tomato crop, and it was detected, it would probably be controlled by eradication. The extent of an outbreak will depend many factors including environmental conditions, the transport of plants and machinery between properties, the movement of workers and the activity of insect vectors.</p> <p>TPMVd infections may go unrecognised because a number of other viroid species produce symptoms similar to those induced by TPMVd (Singh, Ready & Nie 2003b). An outbreak of TPMVd may not be detected until it has spread to several crops, properties and species.</p> <p>During an outbreak, the infected property and adjoining and nearby properties will be surveyed. Samples from surveys will be tested in state government laboratories. RT-PCR will be used to test the samples and detect the viroid. Surveillance and laboratory tests are costly.</p> <p>Viroid incursions in tomato greenhouse crops have been eradicated by destroying the infected plants and by cleaning the greenhouse at the end of the season. Infected plants are identified by testing and removed. Viroids are not eliminated when infected plants are killed so infected plant material is buried. Equipment is sterilized using bleach.</p>

	Typically plants, propagating material, machinery and implements may not be moved from properties where outbreaks have been detected. Plants grown in potato seed certification schemes in Australia are inspected for symptoms of viroid infection (DAFWA 2009a, b; ViCSPA 2009).
Domestic trade	C – minor significance at the district level TPMVd is listed in the National Vegetable Industry Biosecurity Plan released by Plant Health Australia (Plant Health Australia 2007). If TPMVd became established in an Australian state, restrictions might be introduced on the interstate trade of affected seed and seedlings, and if this occurred it could lead to the loss of markets. No movement of machinery from the affected properties is permitted during an eradication campaign.
International trade	D – significant at the district level Part of the Australian fresh tomato fruit crop is exported, as are parts of the Australian ware potato crop and seed potato crop. These exports might be affected if TPMVd became established in Australia. Australia has markets for fresh tomatoes to New Zealand, Singapore, Hong Kong, Brunei-Darussalam, Malaysia, New Caledonia, Indonesia, French Polynesia, Fiji, and USA (DAFF 2008; HAL 2012). Australia has markets for ware potatoes to the Republic of Korea, Malaysia, Mauritius, Singapore, Hong Kong, Indonesia, Philippines, the United Arab Emirates, Thailand, Taiwan and Brunei-Darussalam. Australia has markets for tomato seed and seed potatoes to Thailand.
Non-commercial and environmental	A – indiscernible at the local level No evidence was found indicating environmental and non-commercial indirect effects.

6.7.6 Unrestricted risk estimate

Unrestricted risk is the result of combining the likelihood of entry, establishment and spread with the assessment of overall consequences. Likelihoods and consequences are combined using the risk estimation matrix shown in Table 2.5.

Unrestricted risk estimate for TPMVd	
Overall likelihood of entry, establishment and spread	Low
Consequences	Moderate
Unrestricted risk	Low

The unrestricted risk estimate for TPMVd of Low does not achieve Australia's ALOP. Therefore, specific risk management measures are required for this viroid.

6.8 Pest risk assessment conclusion

The unrestricted risk posed by a pest is estimated by combining the assessments of the likelihoods of entry, establishment and spread with the overall assessment of consequences. Likelihoods and consequences are combined using the risk estimation matrix shown in Table 2.5.

Table 6.2 summarises the department's estimates of unrestricted risks for PepMV and five pospiviroids, CLVd, PCFVd, TASVd, TCDVd and TPMVd, when they are carried by traded tomato seed. It is the department's assessment that these pathogens in tomato seed pose unrestricted risks that do not achieve Australia's ALOP (Section 2.2.5).

The assessment of these pathogens as having unrestricted risks in tomato seed that do not achieve Australia's ALOP provides technical justification for the imposition of the emergency measures for tomato seed introduced by Australia in June 2008, and revised in 2012 and 2013.

Table 6.2 Unrestricted risk estimates (URE) for PepMV and pospiviroids borne by tomato seed

Pest name	Entry	Establishment	Spread	Consequences	URE
<i>Pepino mosaic virus</i>	Moderate	High	High	Moderate	Moderate
<i>Columnnea latent viroid</i>	High	High	Moderate	Moderate	Moderate
<i>Pepper chat fruit viroid</i>	High	High	Moderate	Moderate	Moderate
<i>Tomato apical stunt viroid</i>	High	High	Moderate	Moderate	Moderate
<i>Tomato chlorotic dwarf viroid</i>	High	High	Moderate	Moderate	Moderate
<i>Tomato planta macho viroid</i>	Moderate	High	Moderate	Moderate	Low

7 Pest risk management

It is the assessment of the Department of Agriculture and Water Resources that the listed pathogens, associated with tomato seed, present unrestricted risks that exceed Australia's ALOP. Risk management measures are therefore proposed in order to reduce those risks to achieve Australia's ALOP. The proposed measures are expected to reduce, to a very low level, the risks presented by tomato seed imports that might carry one or more of the listed pathogens, namely *Pepino mosaic virus*, *Columnea latent viroid*, *Pepper chat fruit viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid* and *Tomato planta macho viroid*.

Potato spindle tuber viroid is no longer considered to be a quarantine pest for Australia because it has become established in Queensland and in Western Australia (IPPC 2015). However, the emergency measures on tomato seed for *Potato spindle tuber viroid* will continue while the department evaluates the risks and the possibility of regulating *Potato spindle tuber viroid* as a regulated non-quarantine pest consistent with ISPMs 16 and 21.

Under the IPPC and World Trade Organisation (WTO) SPS Agreement, phytosanitary measures to mitigate the risk of introducing new pests, including emergency measures, must be technically justified. In this section of the draft report, the department evaluates the existing measures and emergency measures to determine whether they are appropriate, and if so whether they should be maintained or amended. Alternative and additional measures that might manage the risks are also considered.

7.1 Standard import conditions and their evaluation

Under Australia's existing policies, seeds of tomato and wild tomato species are subject to the department's standard import conditions for seeds, namely:

- each shipment must be packed in clean, new packaging and be clearly labelled with the full botanical name of the species.
- where the seed lot weight is greater than 10 kilograms and the seed size is less than eight millimetres in diameter, mandatory International Seed Testing Association (ISTA) sampling of each consignment must be used to establish freedom from weed seed contamination. This testing may be performed at department-approved ISTA laboratories overseas, or on arrival at Australian-accredited facilities.
- where seed lots are less than or equal to 10 kilograms in weight, or contain seeds greater than eight millimetres in diameter, a biosecurity officer must conduct a visual inspection of each consignment on arrival in Australia for freedom from live insects, soil, disease symptoms, contaminant seed, other plant material (for example, leaf and stem material, fruit pulp, and pod material), animal material (for example, animal faeces and feathers) and any other extraneous contamination of biosecurity concern.

All consignments imported into Australia regardless of end use (including seed for sowing) must meet departmental standards for seed contamination and tolerance. In addition, the department requires that tomato seeds for sowing imported into Australia be commercially produced and subject to quality control measures by industry.

It is the importer's responsibility to ensure compliance with the requirements of all other regulatory and advisory bodies associated with importing commodities into Australia. These include the Australian Government Department of Home Affairs, the Therapeutic Goods Administration, the Australian Pesticides and Veterinary Medicines Authority, the Australian

Government Department of the Environment and Energy, the Office of the Gene Technology Regulator, and state and territory departments of agriculture.

7.1.1 Mandatory visual inspection to verify freedom from live insects

Limitations: Samples of seed lots are inspected rather than the entire seed lot.

Recommendation: The department proposes that this requirement remains in place.

Reasoning: Mandatory inspection on arrival to verify freedom from live insects is an appropriate measure to detect insect pests contaminating tomato seeds and wild tomato seeds.

7.1.2 Mandatory visual inspection to verify freedom from disease symptoms

Limitations: Visual inspection is only effective if the pathogens of interest produce visible symptoms on the seed. Samples of seed lots are inspected rather than entire seed lots.

Recommendation: The department proposes that this requirement remains in place.

Reasoning: Some pathogens cause visible symptoms on seeds and therefore may be detected by visual inspection. However, *Pepino mosaic virus*, *Columnea latent viroid*, *Pepper chat fruit viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid* and *Tomato planta macho viroid* do not cause visible symptoms on seeds, and therefore visual inspection of seeds is inadequate for detecting these pathogens.

7.1.3 Mandatory visual inspection to verify freedom from soil and extraneous material

Limitations: Samples of seed are inspected rather than entire seed lots.

Recommendation: The department proposes that this requirement remains in place, as pests that are soil-borne or trash-borne can be spread by movement of contaminated soil and plant debris (trash).

Reasoning: Tomato seeds and wild tomato seeds that are contaminated with even small quantities of soil or plant debris may provide a pathway for pests that can remain viable in soil or plant debris for several years.

7.2 Mandatory testing of seeds under the emergency measures

Under the current emergency measures, tomato seeds and wild tomato seeds entering Australia must be tested for and found free of regulated seed-transmitted pathogens, namely, *Pepino mosaic virus*, *Columnea latent viroid*, *Pepper chat fruit viroid*, *Potato spindle tuber viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid* and *Tomato planta macho viroid*.

Limitations: Samples of seed are tested rather than entire seed lots. Tests for the viroids are done on samples of 20,000 seeds, when the lot weighs more than 300 g. Tests for *Pepino mosaic virus* are done on samples of 3,000 seeds, when the lot weighs 300 g or more. Other seed-transmitted pathogens may not be detected. The testing is destructive.

Recommendation: Tomato seed lots to be imported into Australia are required to be free from the listed pathogens. The current emergency measures will remain in place until the report is finalised and continuing phytosanitary measures are implemented.

Reasoning: Australia introduced the emergency measures after incursions of pospiviroids in tomato crops, and after published reports indicated that *Pepino mosaic virus* and the listed pospiviroid species are spreading to many countries, and are seed-transmitted in tomato. The requirements of the emergency measures have protected Australia as shown by a reduction in the number of incursions.

7.3 Proposed risk management measures

The objective of the proposed measures is to maintain Australian freedom from *Pepino mosaic virus*, *Cumbea latent viroid*, *Pepper chat fruit viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid* and *Tomato planta macho viroid*.

Tomato seed: The department proposes that tomato seed, namely seeds of *Solanum lycopersicum* (syn. *Lycopersicon solanum*) and hybrids of this species, imported for sowing should be subject to one of the following regulatory options:

- the department's standard import conditions for seeds for sowing; AND
- additional mandatory testing for *Pepino mosaic virus*, *Cumbea latent viroid*, *Pepper chat fruit viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid* and *Tomato planta macho viroid*.

OR

- the department's standard import conditions for seeds for sowing; AND
- additional mandatory laboratory testing for *Cumbea latent viroid*, *Pepper chat fruit viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid* and *Tomato planta macho viroid*; AND
- dry heat treatment at 80°C for 72 hours.

The laboratory testing requirements established under the emergency measures will remain in place, except for some amendments (see Section 7.3.1). Samples of 20,000 seeds will be tested from lots that weigh more than 300 g, and samples of one fifth (20 per cent) of the lot will be tested from lots that weigh 300 g or less.

Testing for *Pepino mosaic virus* and the pospiviroids may be done using the same RNA preparation extracted from the seed sample.

Reasoning: Assessment indicates that tomato seed imports pose a risk of introducing the listed pathogens that exceeds Australia's ALOP (Chapters 4, 5 and 6). The unrestricted risk posed by the pathogens associated with tomato seed is assessed to be Low or Moderate, based on assessments of the likelihood of entry, establishment and spread and the potential for consequences. Continued testing of tomato seed lots sent to Australia will be required to ensure freedom from the listed pathogens; more effective risk management will also be achieved by amending some of the requirements (Section 7.3.1).

Testing is justified given the evidence from interceptions, the probability of detecting infected seeds, the cryptic nature of the pathogens, seed production practices, recorded outbreaks of the pathogens overseas and incursions of similar pathogens in Australia.

Australian interceptions have provided evidence that seed lots can become contaminated with pospiviroid infected seeds (section 5.8). Some seed lots consisting almost entirely of healthy seeds were found to be contaminated with small numbers of infected seeds. It was estimated that some intercepted seed lots included only one infected seed in 16,000 or more healthy seeds (Section 5.8). Testing samples of less than 20,000 seeds would not reliably detect the presence of the pathogens, when the level of contamination is so low. Continued testing of small seed lots (those less than 300 g) is justified on the basis that many small seed lots have been found to be infected when tested on-arrival in Australia.

Testing by European countries has shown that some tomato seed lots are contaminated with *Pepino mosaic virus*. The prevalence of *Pepino mosaic virus* in these seed lots intercepted in Europe has not been reported. An investigation by the IIGB suggested that tomato seed lots contaminated by the virus continue to be produced, which is consistent with evidence from European testing and reporting (IIGB 2016; Section 3.5; Tables 4.1 and 4.2).

Seed cleaning does not always eliminate the virus, and the virus may be difficult to detect after seed cleaning (Section 4.5), but the seed industry is cleaning seed to reduce the chance of transmission of *Pepino mosaic virus* (Chapter 3). It is considered likely that seed lots are produced that are contaminated with small numbers of seeds carrying very small quantities of *Pepino mosaic virus*.

In proposing phytosanitary measures, the statistics of sampling were considered and the probability of detecting infected seeds in samples was estimated. By testing samples of 20,000 seeds from lots that weigh more than 300 g, the proposed measures will provide an appropriate level of confidence (approaching 99%) that the target pathogens are not present in more than 0.02% of seeds in a lot that tests negative. This level of risk is consistent with Australia's ALOP.

While washing and chemical treatments do not eliminate PepMV from tomato seeds, the virus is eliminated from tomato seeds by dry heat treatment at 80°C for 72 hours (Ling 2010)(Chapter 3). This dry heat treatment is considered to be equivalent to testing extracts from seeds for the virus.

7.3.1 Proposed amendments to mandatory testing requirements

The department proposes the following changes to the mandatory testing requirements for tomato seed and wild tomato seed established under the emergency measures:

- Tomato seed lots that weigh more than 300 g will be tested for *Pepino mosaic virus* using a sample of 20,000 seeds, as seeds infected with the virus are likely to be present in some seed lots in very small numbers, just as seeds infected with pospiviroids are present in very small numbers.
- Seed lots that weigh less than 300 g will be tested for *Pepino mosaic virus* using a sample of at least 20 per cent of the lot, as small seed lots may be contaminated with seeds carrying the virus.
- Dry heat treatment at 80°C for 72 hours is offered as an option in place of testing for *Pepino mosaic virus*, as this treatment has been shown to eliminate the virus.
- Testing of seeds from wild tomato species for *Pepino mosaic virus*, *Columnea latent viroid*, *Pepper chat fruit viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid* and *Tomato planta macho viroid* will cease, as no report was found indicating that these pathogens are transmitted through the seed of wild tomato species.

- On-shore and off-shore laboratories will be required to obtain departmental approval for the protocols used for testing, as this will provide confidence in testing arrangements.
- Laboratory tests of tomato seed will be required to include an appropriate department-approved RNA extraction and RT-PCR test so that every listed pospiviroid can be detected and *Pepino mosaic virus* can be detected, unless the seed has been treated to eliminate the virus.
- Laboratories will not be permitted to pool (batch) samples for testing from different seed lots or seed batches.

7.3.2 Certification

The department proposes that tomato seed lots that weigh more than 300 g and that are tested or treated off-shore for *Pepino mosaic virus* and the regulated pospiviroids must be accompanied by a laboratory report indicating the test results and an official government Phytosanitary Certificate endorsed with one of the following additional declarations:

- The consignment of tomato seed comprises [insert number of seed lots] seed lot(s) grown in [insert name of country], and each lot [insert lot numbers] was tested and found to be free of [insert name of virus/viroids] using department-approved PCR tests on a sample of 20,000 seeds drawn from the lot and divided and tested as subsamples of no more than 400 seeds.

OR

- The consignment of tomato seed comprises [insert number of seed lots] seed lot(s) grown in [insert name of country], and each lot [insert lot numbers] was dry heat treated at 80°C for 72 hours and was tested and found to be free of [insert name viroids] using department-approved PCR tests on a sample of 20,000 seeds drawn from the lot and divided and tested as subsamples of no more than 400 seeds.

The department proposes that tomato seed lots that weigh 300g or less than 300 g and that are tested or treated off-shore for the regulated pathogens must be accompanied by an official government Phytosanitary Certificate endorsed with one of the following additional declarations:

- The consignment of tomato seed comprises [insert number of seed lots] seed lot(s) grown in [insert name of country], and each lot [insert lot numbers] was tested and found to be free of [insert name of virus/viroids] using department-approved PCR tests on a sample comprising 20 per cent of the lot that was divided and tested as subsamples of no more than 400 seeds.

OR

- The consignment of tomato seed comprises [insert number of seed lots] seed lot(s) grown in [insert name of country], and each lot [insert lot numbers] was dry heat treated at 80°C for 72 hours and was tested and found to be free of [insert name of viroids] using department-approved PCR tests on a sample comprising 20 per cent of the lot that was divided and tested as subsamples of no more than 400 seeds.

The department also proposes the following procedures when considering Phytosanitary Certificates:

- Seed lots that do not retain the same lot number on the Phytosanitary Certificate, the laboratory report and the seed packaging will not be permitted to enter Australia. A matching identifier (lot number) is required to officially identify the lot that has been tested and certified.
- Batch numbers may be used in place of lot numbers, but when this is done seed batches that do not retain the same batch number on the Phytosanitary Certificate, the laboratory report and the seed packaging will not be permitted to enter Australia. A matching identifier (batch number) is required to officially identify the batch that has been tested and certified.

7.3.3 Proposed procedures for seed testing

The department proposes the following procedures for certification and testing:

- off-shore testing must use a department-approved laboratory protocol that has been agreed between the testing laboratory and the department prior to testing. The reliability and sensitivity of testing protocols varies, and to ensure an effective test is done an approved testing protocol must be used.
- on-shore testing must use a laboratory protocol that has been agreed between the testing laboratory and the department prior to testing (departmental approval).
- seed lots from different production sites must not be blended prior to testing. Blending of the seed lots from different locations affects the risk profile and potentially can produce seed batches that include very few infected seeds that are difficult to detect.
- samples must be drawn for testing prior to fungicide treatment or coating of the seeds, as fungicide treatment and seed coating affect seed testing.

7.3.4 Inspection on arrival

All seed lots must be inspected on arrival to verify freedom from live insects, soil, disease symptoms, prohibited seeds, other plant material (for example, leaf, stem material, fruit pulp and pod material), animal material (for example, animal faeces and feathers) and any other extraneous contamination of quarantine concern.

7.4 Evaluation of proposed risk management measures

The proposed risk management measures are designed to reduce the pest risk for each identified quarantine pest to a very low level, which would achieve the ALOP for Australia.

Table 7.1 Risk management measures proposed for quarantine pathogens associated with tomato seed

Pest	Effect of the measure	Probability of quarantine pest entry post treatment
<i>Pepino mosaic virus</i>	The risk of the virus entering Australia will be reduced if tomato seed imports are subject to laboratory PCR testing or dry heat treatment.	Very low
<i>Columnea latent viroid</i> <i>Pepper chat fruit viroid</i> <i>Tomato apical dwarf viroid</i> <i>Tomato chlorotic dwarf viroid</i> <i>Tomato planta macho viroid</i>	Laboratory PCR testing will reduce the risk of these viroids entering Australia in tomato seed.	Very low

7.5 Consideration of alternative measures

Consistent with the principle of equivalence detailed in ISPM 1 (FAO 2016a) and ISPM 11 (FAO 2016e), the department will consider any alternative measure proposed by an NPPO, to assess whether it will achieve the ALOP for Australia. Evaluation of any such measure or treatment will require a technical submission from the NPPO that details the proposed treatment, including evidence in the form of data from suitable treatment trials to demonstrate efficacy.

There are a number of risk management measures that could potentially be adopted to protect and minimise the risk posed to Australia by exotic seed-borne pests under the IPPC standards.

7.5.1 Sourcing seeds from pest-free areas

The establishment and use of a pest-free area (PFA) by an NPPO provides assurance that specific pests are not present in a delimited geographic area. The delimitation of a PFA should be relevant to the biology of the pest concerned.

The requirements for establishing PFAs are set out in ISPM 4 (FAO 2016c). This ISPM defines a PFA as ‘an area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained’. A PFA may concern all or part of several countries and is managed by the NPPO of the exporting country. The establishment and use of a PFA by an NPPO allows an exporting country to export plants and other regulated articles to an importing country without having to apply additional phytosanitary measures providing certain requirements are met.

Requirements for an NPPO to establish and maintain a PFA include:

- systems to establish freedom (general surveillance and specific surveys)
- phytosanitary measures to maintain freedom (regulatory actions, routine monitoring, and extension advice to producers)
- checks to verify freedom has been maintained.

NPPOs that propose to use area freedom as a measure for managing the risk posed by the quarantine pests identified in this review must provide the Department of Agriculture and Water Resources with an appropriate submission demonstrating area freedom, for its consideration.

If an application is made for certification based on ISPM 4, the department will consider the evidence that is provided. The department has previously accepted phytosanitary certification of tomato seed lots based on claims by countries of area freedom, but testing of seed sent to Australia indicated that some certified seed lots were contaminated with infected seeds (Section 1.2.1). The department considers that tomato seed production crops in most countries are established from imported seed stocks (see Chapter 3). Area freedom, therefore, is difficult to establish without testing seed imports and substantive and specific surveys.

7.5.2 Sourcing seeds from pest-free places of production

Requirements for establishing pest-free places of production are set out in ISPM 10 (FAO 2016d). The concept of 'pest freedom' allows exporting countries to provide assurance to importing countries that plants, plant products and other regulated articles are free from a specific pest or pests and meet the phytosanitary requirements of the importing country. Where a defined portion of a place of production is managed as a separate unit and can be maintained pest-free, it may be regarded as a pest-free production site.

Requirements for an NPPO to establish and maintain a pest-free place of production or a pest-free production site as a phytosanitary measure include:

- systems to establish pest freedom
- systems to maintain pest freedom
- verification that pest freedom has been attained or maintained
- product identity, consignment integrity and phytosanitary security.

Where necessary, a pest-free place of production or a pest-free production site must also establish and maintain an appropriate buffer zone.

Administrative activities required to support a pest-free place of production or pest-free production site include documentation of the system and maintenance of adequate records about the measures taken. Review and audit procedures undertaken by an NPPO are essential to support assurance of pest freedom and for system appraisal. Bilateral agreements or arrangements may also be needed.

NPPOs that propose to use pest-free places of production as a measure for managing the risk posed by the quarantine pests identified in this review must provide the department with an appropriate submission demonstrating pest-free place of production status, for its consideration.

If an application is made for certification based on ISPM 10, the department will consider the systems used to establish and maintain freedom from the listed pathogens. Rigorous systems are required to be certain that *Pepino mosaic virus* and pospiviroids have been excluded from a place of production. These pathogens do not affect the appearance of tomato seeds, they are transmitted by certain insects and inadvertently by horticultural activities, and infected plants can be asymptomatic. To exclude the pathogens, a place of production will need to establish stringent biosecurity arrangements, and testing of seeds or parent plants will be necessary.

The department has previously accepted phytosanitary certification of tomato seed lots based on parent plant surveys, but testing of seed sent to Australia indicated that some certified seed lots were contaminated with infected seeds (section 1.2.1). The department concluded at that time that the measure was insufficient for the listed pathogens and the option was removed.

7.5.3 Sourcing seeds produced under a systems approach

ISPM 14 (FAO 2016f) provides guidelines on the use of systems approaches to manage pest risk. According to ISPM 14 (FAO 2016f), 'a systems approach requires the integration of different measures, at least two of which act independently, with a cumulative effect' to achieve the appropriate level of protection.

Systems approaches could provide an alternative to relying on a single measure to achieve the ALOP of an importing country, or could be used where no single measure is available. Systems approaches are often tailored to specific commodity–pest–origin combinations and may be developed and implemented collaboratively by exporting and importing countries. The importing country specifies the appropriate approach after considering technical requirements, minimisation of impact, transparency, non-discrimination, equivalence and operational feasibility.

NPPOs that propose to use a systems approach as a measure for managing risks posed by the quarantine pests identified in this review must provide the Department of Agriculture and Water Resources with an appropriate submission describing their preferred systems approach and rationale, for its consideration.

If an application is made for certification based under a systems approach, the department will consider the system used, the containment capabilities of the system and the testing that is proposed. Rigorous systems are required to be certain that *Pepino mosaic virus* and pospiviroids have been excluded from production. These pathogens do not affect the appearance of tomato seeds, they are transmitted by certain insects and inadvertently by horticultural activities, and infected plants can be asymptomatic. To exclude the pathogens, a production system will need to establish stringent biosecurity arrangements, and testing of seeds or parent plants will be necessary.

7.5.4 Seed disinfestation by chemical and heat treatments

The department has considered evidence that *Pepino mosaic virus* and pospiviroids may be eliminated from seed by using chemical or heat treatments (Sections 4.3, 4.4, 5.3). Whereas evidence was found that supported the use of a dry heat treatment to eliminate *Pepino mosaic virus*, no equivalent evidence was found in the preparation of this report that showed pospiviroids could be eliminated from large numbers of seeds by a chemical or heat treatment. If an application is received to use seed disinfestation as an alternative to testing for the pospiviroids, the department will require efficacy data that includes sufficient replicates and testing of sufficient numbers of seeds to show that the method will reliably eliminate the pathogens from large seed lots. Similarly, if an application is received to use an alternative to the proposed heat treatment for *Pepino mosaic virus*, the department will require efficacy data that includes sufficient replicates and testing of sufficient numbers of seeds to show that the method will reliably eliminate the virus from large seed lots.

7.5.5 Approved arrangements

The department will consider on a case-by-case basis arrangements where assurance of freedom from the pathogens is obtained using systems of containment and testing. It is envisaged that these arrangements will ensure freedom from the pathogens in a secure containment facility, in germplasm brought into the facility, and in seed that is produced. Smaller sample sizes and other systems of sampling for testing may be considered in these cases.

7.5.6 Visual inspection of seed production crops

Phytosanitary certification of tomato seed lots based on visual inspection of the seed production crop was accepted for a period of time under the emergency measures, but incursions of one of the pathogens continued to occur in Australia (Section 1.2.1). Testing of seed sent to Australia indicated that many seed lots that were certified in this way were contaminated with infected seeds (Section 1.2.1). The department concluded that the measure was insufficient for the listed pathogens.

7.6 Review of policy

The Department of Agriculture and Water Resources reserves the right to review the import policy when it has reason to believe that the pest or phytosanitary status has changed.

8 Conclusion

Australia's reliance on an overseas supply of tomato seeds for sowing presents a risk that seed-transmitted pests will be introduced into the country and affect agricultural production. Seeds for sowing are considered high-risk material in international trade, because they provide a ready pathway for the movement of pests, especially seed-transmitted pathogens.

This draft report has confirmed that *Pepino mosaic virus*, *Columnea latent viroid*, *Pepper chat fruit viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid* and *Tomato planta macho viroid* are pests of quarantine concern that are associated with tomato seeds. These pests are unlikely to be detected during visual inspections on arrival because they do not produce visible symptoms on tomato seeds. The department has therefore proposed additional risk management measures for tomato seeds to reduce the risk of introducing these pests, and to achieve the appropriate level of protection for Australia.

Standard requirements for the importation of seeds for sowing apply to tomato seeds and seeds of wild tomatoes from all sources. The department proposes applying additional risk management measures for the seeds of tomato (*Solanum lycopersicum*), and any hybrid of this species.

This draft report also proposes that seeds of wild tomato species (*Solanum chilense*, *S. chmielewskii*, *S. parviflorum*, *S. peruvianum* and *S. pimpinellifolium*) do not require additional risk management measures, as the listed pathogens are not known to be associated with the seeds of these species.

The ultimate goal of Australia's risk management measures is to protect plant health and prevent the introduction of identified quarantine pests associated with tomato seeds and seeds of wild tomatoes. The department considers that the risk management measures proposed in this draft report of existing import conditions will be adequate for mitigating the risks posed by the identified pathogens.

Appendix A. Australian testing of tomato seeds

A.1 Approved Australian tomato seed testing protocols

A 20,000 seed sample is used for testing tomato seed lots that weigh 300g or more. A sample of 20 per cent of the seed lot may be tested if the lot weighs less than 300 grams. In both cases, the total sample is further divided into subsamples of up to 400 seeds and each subsample is tested independently.

A modified version of the method of Hoshino *et al.* (2006) is used to extract RNA for RT-PCR tests. In this method, a sample of 400 seeds is pulverised to a powder in an extraction bag using a hammer, and the entire sample is heated to 65 degrees Celsius in a high salt buffer that contains sodium dodecyl sulphate (SDS) (Hoshino *et al.* 2006). Potassium acetate is used to precipitate SDS-bound proteins, which is pelleted by centrifugation. Isopropyl alcohol is then added to precipitate and purify RNA from an aqueous sample from the preparation. RNA may be further purified using RNeasy kits (QIAGEN) if needed. Complementary DNA (cDNA) is copied from the RNA and amplified by conventional RT-PCR using appropriate oligonucleotide primers. The PCR amplification products are analysed by agarose gel electrophoresis, and if a product of the target size is found, the product is either directly purified from the remaining PCR reaction or from the excised band, and the DNA is sequenced. Detection of pospiviroids by these methods is confirmed by comparing the sequence of the PCR product to the sequences of viroid species and PepMV variants held in the GenBank database.

The conventional RT-PCR methods of Aguilar *et al.* (2002) and Mansilla *et al.* (2003) are used to detect PepMV with the primer sets MA172(F)/MA173(R) and PepMV(F)/PepMV(R). Pospiviroids are detected using the RT-PCR methods of Spieker (1996), Hailstones *et al.* (2003) and (Verhoeven *et al.* 2004) with the primer sets Posp1, DLH55/DLH56 and CLV4. Appropriate housekeeping-gene controls and positive and negative controls are run and results are recorded.

Details about the Australian protocols are available from the Department of Agriculture and Water Resources. Before other protocols are used advice should be sought from the Department of Agriculture and Water Resources.

A.2 Efficacy of the Australian testing protocol

The Australian testing protocol has been used to test commercially-traded imported seed lots by the diagnostic laboratory at the AgriBio Centre for AgriBioscience for ten years, and at the EMAI diagnostic laboratory for six years. Both laboratories have tested several thousand subsamples and detected a range of pospiviroid species.

Compilation and analysis of the results indicates the diagnostic value of the testing:

- Positive and negative control samples have been tested in parallel with every seed sample, and the controls have produced the expected results.
- More than 95 per cent of the tests done on subsamples by the Australian laboratories using the Australian protocol have not detected a viroid, that is, the tests have given negative results.
- Viroids have been detected in fewer than 10 per cent of the samples tested using the protocol.
- Six pospiviroid species have been detected using the protocol.

- The same viroid species have been detected by the two Australian laboratories.
- Similar proportions of seed lots have been found to be infected by the two Australian laboratories.
- Nearly one hundred positive seed lots have been detected by the laboratories using the protocol.
- Many different PSTVd sequences have been obtained from infected seed lots through the testing regimen.
- Several pospiviroid species detected using the protocol had not been detected previously by Australian laboratories in Australian samples, nor in samples held in quarantine from imports, and no samples of some of the viroids were previously held by the laboratories.

A compilation of test results, including data presented in Table 5.4, has verified the efficacy of the Australian test protocol. The consistency, reliability and accuracy of the test results was supported by (i) the fact that a range of viroid species and viroid sequences were detected, (ii) the similarities between the results from the two laboratories, (iii) the results from positive and negative control samples, and (iv) the great number of negative test results.

The Australian laboratories have detected a greater number of viroid-infected tomato seed lots than has been reported by any other laboratory. This detection rate is probably explained by the success of the Australian protocol, and the sizes of the samples and subsamples mandated for testing.

Glossary

Term or abbreviation	Definition
Additional declaration	A statement that is required by an importing country to be entered on a phytosanitary certificate and which provides specific additional information on a consignment in relation to regulated pests (FAO 2015).
Appropriate level of protection (ALOP)	The level of protection deemed appropriate by the Member establishing a sanitary or phytosanitary measure to protect human, animal or plant life or health within its territory (WTO 1995).
Appropriate level of protection (ALOP) for Australia	The <i>Biosecurity Act 2015</i> defines the appropriate level of protection (or ALOP) for Australia as a high level of sanitary and phytosanitary protection aimed at reducing biosecurity risks to very low, but not to zero.
Area	An officially defined country, part of a country or all or parts of several countries (FAO 2015).
Australian territory	Australian territory as referenced in the <i>Biosecurity Act 2015</i> refers to Australia, Christmas Island and Cocos (Keeling) Islands.
Biosecurity	The prevention of the entry, establishment or spread of unwanted pests and infectious disease agents to protect human, animal or plant health or life, and the environment.
Biosecurity Australia	An agency of the Australian government responsible for biosecurity regulation that was subsumed into the department in 2012.
Biosecurity measures	The <i>Biosecurity Act 2015</i> defines biosecurity measures as measures to manage any of the following: biosecurity risk, the risk of contagion of a listed human disease, the risk of listed human diseases entering, emerging, establishing themselves or spreading in Australian territory, and biosecurity emergencies and human biosecurity emergencies.
Biosecurity import risk analysis (BIRA)	The <i>Biosecurity Act 2015</i> defines a BIRA as an evaluation of the level of biosecurity risk associated with particular goods, or a particular class of goods, that may be imported, or proposed to be imported, into Australian territory, including, if necessary, the identification of conditions that must be met to manage the level of biosecurity risk associated with the goods, or the class of goods, to a level that achieves the ALOP for Australia. The risk analysis process is regulated under legislation.
Biosecurity risk	The <i>Biosecurity Act 2015</i> refers to biosecurity risk as the likelihood of a disease or pest entering, establishing or spreading in Australian territory, and the potential for the disease or pest causing harm to human, animal or plant health, the environment, economic or community activities.
Consignment	A quantity of plants, plant products or other articles being moved from one country to another and covered, when required, by a single phytosanitary certificate (a consignment may be composed of one or more commodities or lots) (FAO 2015).
Control (of a pest)	Suppression, containment or eradication of a pest population (FAO 2015).
Department of Agriculture	The name of the Department of Agriculture and Water Resources prior to 2015
Department of Agriculture, Fisheries and Forestry	The name of the Department of Agriculture and Water Resources prior to 2013
The department	The Australian Government Department of Agriculture and Water Resources.
ELISA	A laboratory test using the Enzyme-linked immunosorbent assay method.
Endangered area	An area where ecological factors favour the establishment of a pest whose presence in the area will result in economically important loss (FAO 2015).
Endemic	Belonging to, native to, or prevalent in a particular geography, area or environment.
Entry (of a pest)	Movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled (FAO 2015).

Term or abbreviation	Definition
Establishment (of a pest)	Perpetuation, for the foreseeable future, of a pest within an area after entry (FAO 2012, 2015).
Fresh	Living; not dried, deep-frozen or otherwise conserved (FAO 2015).
Fumigation	A method of pest control that completely fills an area with gaseous pesticides to suffocate or poison the pests within.
Genus	A taxonomic category ranking below a family and above a species and generally consisting of a group of species exhibiting similar characteristics. In taxonomic nomenclature the genus name is used, either alone or followed by a Latin adjective or epithet, to form the name of a species.
Goods	The <i>Biosecurity Act 2015</i> defines goods as an animal, a plant (whether moveable or not), a sample or specimen of a disease agent, a pest, mail or any other article, substance or thing (including, but not limited to, any kind of moveable property).
Host	An organism that harbours a parasite, mutual partner, or commensal partner, typically providing nourishment and shelter.
Host range	Species capable, under natural conditions, of sustaining a specific pest or other organism (FAO 2015).
IGB	Inspector General Biosecurity
IIGB	Interim Inspector General Biosecurity
Import permit	Official document authorising importation of a commodity in accordance with specified phytosanitary import requirements (FAO 2012, 2015).
Incursion	An isolated population of a pest recently detected in an area, not known to be established, but expected to survive for the immediate future (FAO 2012, 2015).
Infection	The internal 'endophytic' colonisation of a plant, or plant organ, and is generally associated with the development of disease symptoms as the integrity of cells and/or biological processes are disrupted.
Infestation (of a commodity)	Presence in a commodity of a living pest of the plant or plant product concerned. Infestation includes infection (FAO 2015).
Inspection	Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations (FAO 2015).
Intended use	Declared purpose for which plants, plant products, or other regulated articles are imported, produced or used (FAO 2015).
Interception (of a pest)	The detection of a pest during inspection or testing of an imported consignment (FAO 2015).
International Plant Protection Convention (IPPC)	The IPPC is an international plant health agreement, established in 1952, that aims to protect cultivated and wild plants by preventing the introduction and spread of pests. The IPPC provides an international framework for plant protection that includes developing International Standards for Phytosanitary Measures (ISPMs) for safeguarding plant resources.
International Standards for Phytosanitary Measures (ISPM)	An international standard adopted by the Conference of the Food and Agriculture Organization, the Interim Commission on Phytosanitary Measures or the Commission on Phytosanitary Measures, established under the IPPC (FAO 2015).
Introduction (of a pest)	The entry of a pest resulting in its establishment (FAO 2015).
ISTA	International Seed Testing Association
Lot	A number of units of a single commodity, identifiable by its homogeneity of composition, origin et cetera, forming part of a consignment (FAO 2015). Within this report a 'lot' refers to a quantity of seed of a single variety, harvested from a single production site during a season and packed at one time.

Term or abbreviation	Definition
National Plant Protection Organization (NPPO)	Official service established by a government to discharge the functions specified by the IPPC (FAO 2015).
Non-regulated risk analysis	Refers to the process for conducting a risk analysis that is not regulated under legislation (Department of Agriculture and Water Resources 2016)
Official control	The active enforcement of mandatory phytosanitary regulations and the application of mandatory phytosanitary procedures with the objective of eradication or containment of quarantine pests or for the management of regulated non-quarantine pests (FAO 2015).
Outbreak	A recently detected pest population, including an incursion, or a sudden significant increase of an established pest population in an area (FAO 2012, 2015).
Pathogen	A biological agent that can cause disease to its host.
Pathway	Any means that allows the entry or spread of a pest (FAO 2015).
Pest	Any species, strain or biotype of plant, animal, or pathogenic agent injurious to plants or plant products (FAO 2015).
Pest categorisation	The process for determining whether a pest has or has not the characteristics of a quarantine pest or those of a regulated non-quarantine pest (FAO 2015).
Pest free area (PFA)	An area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (FAO 2015).
Pest free place of production	Place of production in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period (FAO 2015).
Pest free production site	A defined portion of a place of production in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained for a defined period and that is managed as a separate unit in the same way as a pest free place of production (FAO 2015).
Pest risk analysis (PRA)	The process of evaluating biological or other scientific and economic evidence to determine whether an organism is a pest, whether it should be regulated, and the strength of any phytosanitary measures to be taken against it (FAO 2015).
Pest risk assessment (for quarantine pests)	Evaluation of the probability of the introduction and spread of a pest and of the magnitude of the associated potential economic consequences (FAO 2015).
Pest risk assessment (for regulated non-quarantine pests)	Evaluation of the probability that a pest in plants for planting affects the intended use of those plants with an economically unacceptable impact (FAO 2015).
Pest risk management (for quarantine pests)	Evaluation and selection of options to reduce the risk of introduction and spread of a pest (FAO 2015).
Pest risk management (for regulated non-quarantine pests)	Evaluation and selection of options to reduce the risk that a pest in plants for planting causes an economically unacceptable impact on the intended use of those plants (FAO 2015).
Pest status (in an area)	Presence or absence, at the present time, of a pest in an area, including where appropriate its distribution, as officially determined using expert judgement on the basis of current and historical pest records and other information (FAO 2015).
Phytosanitary Certificate	An official paper document or its official electronic equivalent, consistent with the model of certificates of the IPPC, attesting that a consignment meets phytosanitary import requirements (FAO 2015).
Phytosanitary certification	Use of phytosanitary procedures leading to the issue of a phytosanitary certificate (FAO 2015).
Phytosanitary measure	Phytosanitary relates to the health of plants. Any legislation, regulation or official procedure having the purpose to prevent the introduction and/or

Term or abbreviation	Definition
	spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests (FAO 2015). In this risk analysis the term 'phytosanitary measure' and 'risk management measure' may be used interchangeably.
Phytosanitary procedure	Any official method for implementing phytosanitary measures including the performance of inspections, tests, surveillance or treatments in connection with regulated pests (FAO 2015).
Phytosanitary regulation	Official rule to prevent the introduction and/or spread of quarantine pests, or to limit the economic impact of regulated non-quarantine pests, including establishment of procedures for phytosanitary certification (FAO 2015).
PRA area	Area in relation to which a pest risk analysis is conducted (FAO 2015).
Production site	In this report, a production site is a continuous planting of tomato plants treated as a single unit for pest management purposes. If a growing area is subdivided into one or more units for pest management purposes, then each unit is a production site. If the growing area is not subdivided, then it is also the production site.
Quarantine	Official confinement of regulated articles for observation and research or for further inspection, testing or treatment (FAO 2015).
Quarantine pest	A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled (FAO 2015).
Regulated article	Any plant, plant product, storage place, packaging, conveyance, container, soil and any other organism, object or material capable of harbouring or spreading pests, deemed to require phytosanitary measures, particularly where international transportation is involved (FAO 2015).
Regulated non-quarantine pest	A non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party (FAO 2015).
Regulated pest	A quarantine pest or a regulated non-quarantine pest (FAO 2015).
Restricted risk	Restricted risk is the risk estimate when risk management measures are applied.
RT-PCR	A laboratory test using the reverse transcription polymerase chain reaction method.
SPS Agreement	The WTO Agreement on the Application of Sanitary and Phytosanitary Measures.
Ware potato	Potatoes and potato crops grown for consumption rather than propagation from seed.
WTO	World Trade Organisation

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