Import Risk Analysis:
Tomato and *Capsicum* seed for sowing from all countries

DRAFT

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MAF Information Bureau
P O Box 2526
WELLINGTON

Telephone: 0800 00 83 33
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Ministry of Agriculture and Forestry
Pastoral House
25 The Terrace
PO Box 2526
Wellington 6140
New Zealand

Tel: 64-4-894 0100
Fax: 64-4-894 0731

Biosecurity Risk Analysis Group
Ministry of Agriculture and Forestry

Import Risk Analysis: Tomato and *Capsicum* seed or sowing from all countries.

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Approved for public consultation

Christine Reed
Manager, Biosecurity Risk Analysis Group
Ministry of Agriculture and Forestry

Contributors to this risk analysis

1. Primary author/s

Dr Sarah Clark	Senior Adviser Risk Analysis, Plants	Ministry of Agriculture and Forestry Wellington
Kim Crook	Adviser Risk Analysis, Plants	Ministry of Agriculture and Forestry Wellington

2. Secondary contributors

Deb Anthony	Adviser Risk Analysis, Plants	Ministry of Agriculture and Forestry Wellington
Dr Catherine Duthie	Adviser Risk Analysis, Plants	Ministry of Agriculture and Forestry Wellington
Dr Nicholas Amponsah	Adviser Risk Analysis, Plants	Ministry of Agriculture and Forestry Wellington
Melanie Newfield	Team manager, Risk Analysis, Plants	Ministry of Agriculture and Forestry Wellington

3. External peer review

Associate Professor Mike Pearson (Plant Pathology)
School of Biological Sciences
The University of Auckland
Private Bag 92019
Auckland.

Associate Professor Francisco M. Ochoa Corona
National Institute for Microbial Forensics & Food and Agricultural Biosecurity (NIMFFAB)
Department of Entomology & Plant Pathology
Oklahoma State University
127 Noble Research Center
Stillwater, OK 74078

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Background
MAF is reviewing the phytosanitary requirements for tomato and *Capsicum* seed for sowing. Currently these seeds are imported into New Zealand under 'basic conditions'. The Risk Analysis Plants team was asked to undertake a risk analysis of viroids and viruses in tomato and capsicum seed, as a first step to determining whether the current phytosanitary requirements are appropriate.

Risk assessments were carried out for viroids and viruses where evidence was found in the scientific literature of seed-to-seedling transmission.

Four viroids were risk-assessed:

- *Potato spindle tuber viroid* (PSTVd)
- *Tomato chlorotic dwarf viroid* (TCDVd)
- *Tomato apical stunt viroid* (TASVd)
- *Pepper chat fruit viroid* (PCFVd)

Three viruses were risk-assessed:

- *Pepino mosaic virus* (PepMV)
- *Pelargonium zonate spot virus* (PZSV)
- *Tobacco mild green mosaic virus* (TMGMV)

It is important to note that the risk assessments account for the risk associated with the total volume of seeds being imported over time (i.e. millions of seeds, over a number of years). The risk assessment values (i.e. low, mod, high) <u>do not</u> refer to the risk associated with any particular individual seed, and its subsequent seedling/plant, that might be chosen at random and followed through the pathways of virus/viroid entry, exposure and establishment.

Key findings
Pospiviroids or viruses <u>known</u> to be transmitted by *Capsicum* seed:

- TMGMV
- PCFVd
- PSTVd (suspected, due to an incursion in New Zealand, thought to have arisen from commercial seed)

Pospiviroids or viruses <u>known</u> to be transmitted by tomato seed:

- PepMV
- PZSV
- PSTVd
- TCDVd
- TASVd

<u>All seven of the viruses and viroids risk-assessed are a non-negligible biosecurity risk to New Zealand on the tomato and/or capsicum seed pathway</u> because they are likely to establish in commercial crops and they are likely to cause economic impacts on commercial producers of tomato and/or capsicum crops. The economic impacts will arise from reduced yield and damage to tomato or capsicum fruit. Another aspect of the risk to New Zealand is that there is potential for some of the pathogens to spread to other crops (e.g. potato), and into ornamental and weedy

species. Establishment in ornamental and weedy species would be considered an establishment of a reservoir of the pathogen in the environment, which is quite distinct from an infection that is limited to a crop which will be destroyed at the end of the season. Refer to Table 1 for a summary of the risk assessments.

<u>The likelihood of environmental establishment is highly uncertain, but considered to be within the range from low to moderate</u>. This is a relatively large range for a likelihood of establishment but it is given because of the compounding uncertainties associated with what transmission mechanisms the infected plants will be subjected to.

<u>The most likely sources of environmental establishment of these pathogens is considered to be imported seeds used in the home garden and outdoor commercial crops.</u> In the home garden, the mechanisms of viroid and virus transmission (mechanical, seed-transmission, and via insects for some; and by a fungal vector in the case of PepMV), and the behaviours and habits of home gardeners who grow tomato and capsicum crops from imported seed, mean that these pathogens have the potential to establish in the environment. This might be in weedy species, in ornamentals, or via perpetual re-occurrence in home-grown crops of tomato, capsicum or potato.

<u>Outdoor commercial tomato crops are likely to be a source of environmental establishment of the viroids and viruses that are transmitted via bumblebees and/or other insects:</u> PepMV is known to be transmitted trans-species by bumblebees; TCDVd and TASVd are assumed to be transmitted trans-species by bumblebees. There is a low likelihood of PSTVd establishing in the environment as a result of aphid transmission of the viroid from outdoor commercial crops to other species. PSTVd establishment is constrained by the requirement for another virus, potato leaf roll virus to be present. PSTVd is unlikely to be transmitted by bumblebees, but it is not known if the other pathogens can be transmitted by bumblebees or other insects in this way at present due to a lack of information. There is only a low likelihood that glasshouse crops could be a source of environmental establishment of the viroids and viruses that are transmitted by bumblebees. The likelihood is low (bordering on negligible) because it requires bumblebees to escape from the commercial glasshouses and then locate and feed on a host-species rather than a non-host species.

<u>The consequence of environmental reservoirs:</u> Environmental reservoirs of these pathogens are unlikely to be a source of infection in commercial *glasshouse* crops because of very limited opportunity for transmission of the pathogens from the environment back into the greenhouse crops. However, environmental reservoirs of the viruses and viroids that are transmitted between species by bumblebees (PepMV, TCDVd and TASVd) or aphids (PSTVd) have the potential to be transmitted back into *outdoor* commercial crops via these insects.

<u>The main source of virus and viroid infections for commercial glasshouse crops is considered to be seed</u>. Consequently, the impacts of viroids and viruses in commercial glasshouse crops have the potential to occur every year simply through the importation and growing of infected seed. Outdoor commercial crops also have the potential to become infected every year simply through the importation and growing of infected seed, but these crops have the added potential of becoming infected from environmental reservoirs via insect activity.

Table 1. Summary of risk assessment of tomato and *Capsicum* seeds approved for importation into New Zealand

Risk Organism	Likelihood of Entry[1]	Likelihood of Exposure	Likelihood of Establishment[2] & Spread[3]	Economic Impact	Environmental Impact	Socio-economic Impact	Health Impact	Overall Risk Estimate	
								Tomato Seed	Capsicum Seed
TCDVd	High	High	Crops: High Environment: Low-Mod Spread (environmental): Low-Mod	Low-Mod	Low	Low	Negligible	Non-negligible	Negligible
PSTVd	High	High	Crops: High Environment: Low-Mod Spread (environmental): Low-Mod	Low-Mod	Low	Low	Negligible	Non-negligible	Non-negligible
TASVd	High	High	Crops: High Environment: Low-Mod Spread (environmental): Low-Mod	Low-Mod	Low	Low	Negligible	Non-negligible	Negligible
PCFVd	Uncertain, but non-negligible	High	Crops: High Environment: Low-Mod Spread (environmental): Low-Mod	Low-Mod	Negligible to Low	Low	Negligible	Non-negligible *[but high uncertainty]*	Non-negligible
PepMV	High	High	Crops: High Environment: Mod Spread (environmental): Low - Mod	Low to High	Negligible to Low	Low	Negligible	Non-negligible	Negligible
PZSV	Uncertain, but non-negligible	High	Crops: High Environment: Low-Mod Spread (environmental): Low-Mod	Low to moderate; *with uncertainty*	Low	Low	Negligible	Non-negligible	Non-negligible *[but high uncertainty]*
TMGMV	Uncertain, but non-negligible	High	Crops: High Environment: Low-Mod Spread (environmental): Low-Mod	Low	Low	Low	Negligible	Negligible	Non-negligible

Mod = Moderate
1 The likelihood of entry is assessed in the context of millions of seeds being imported over periods of time. However, the proportion of seeds infected is highly uncertain.
2 Two assessments are provided for 'likelihood of establishment', one is labelled 'Crops', the other is labelled 'Environment'; 'Crops:' denotes establishment of viroids/viruses throughout crops which have been grown from imported seed (and includes both commercial and/or home garden crops); 'Environment:' denotes establishment of viroids/viruses in the wider NZ environment. These two establishment contexts were assessed separately because the impacts are different and the different impacts may influence risk management options.
3 Likelihood of spread relates to spread of viroid/virus from the initially affected area to other parts of New Zealand.

The biosecurity risks are non-negligible, but it should be noted that key factors influencing the risk have uncertainties associated with them. Furthermore, the biosecurity risk is likely to vary from year to year. For instance:

1. Although the likelihood of entry of many of these pathogens is high, the number of entries over time is uncertain and could be very low for many of them. It is likely to vary from year to year.
2. Although the pathogens are transmitted from seed-to-seedling, the efficiency of transmission appears to be high for some, and very low for others.
3. Whilst exposure is highly likely to occur, the number of exposure events over time influences the biosecurity risk. The frequency of exposure depends on both the frequency of entry (or proportion of seeds that are infected) and the efficiency of seed-to-seedling transmission. There is uncertainty about most of these factors.
4. The impact on commercial tomato or capsicum crops depends on the severity of symptoms in infected plants. Some pathogens have been recorded as causing severe symptoms as well as sometimes having symptomless infections (e.g. PSTVd, PepMV). Symptom severity is thought to be influenced by the strain of the pathogen, the cultivar of host, and environmental factors. Therefore, although severe symptoms are possible with many of these pathogens, and may occur on New Zealand commercial crops, the possibility of mild symptoms or no symptoms occurring for some pathogens can not be ruled out.

Summary of the uncertainties

The uncertainties that limit the accuracy of the findings of this risk assessment are related to:

- the efficiency of seed-to-seedling transmission, and what affects efficiency (see risk management options);
- the frequency of exposure events for each pathogen (dependent on the proportion of seeds that are infected, and the rate of seed-to-seedling transmission);
- the extent of insect transmission for most of the pathogens;
- whether seed-to-seedling transmission occurs in species other than the crop species that seed transmission has been observed in;
- whether experimentally identified hosts can become hosts in a natural environment, under natural circumstances;
- what the crop impacts will be in the New Zealand environment, and to what extent the impacts on crops are influenced by environmental factors such as temperature.

Risk Management Options

Pre-border:

- Seed treatment involves disinfection of seeds and is only effective against organisms that occur on the surface of the seed. Currently, only PepMV is confirmed as a surface contaminant. Therefore, seed treatment is likely to only manage the risk of PepMV but not the other risk viruses and viroids.
- Assurance of pest-free place of production or pest free area is likely to greatly reduce the risk of all the viruses and viroids assessed. This measure would manage the risk associated with all exposure pathways and horticultural practices, including seeds grown in home gardens. However, further analysis would be required to determine whether this is a feasible option.

- Non-destructive seed testing for PepMV and PSTVd is possible, but it is not commercially available in New Zealand and it severely limits the shelf-life of seeds. Further analysis is required to determine whether this measure is practical.

Post-border:

- The New Zealand code of practice for the management of PSTVd is already operating in New Zealand greenhouses that produce export tomato crops. This management option is likely to be effective at reducing the risk of PSTVd and the other virus and viroids assessed, if plants are symptomatic. Further analysis and consultation is required to determine whether the code of practice is effective at managing the risk of viruses and viroids that have an initial symptomless phase, and to what extent it can be applied to other production systems, e.g. field tomatoes, outdoor table tomato crops.
- Weed control and increased crop monitoring may provide some risk reduction for tomato crops grown outdoors.
- It is unlikely that the risk posed by the use of tomato and capsicum seeds in home-gardens can be mitigated post-border.

1 Risk analysis background and process

1.1 Background

This risk analysis was requested by the Plant and Plant Products Import team to support their work in reviewing the seed for sowing IHS (155-02-05) for tomato and *Capsicum* seeds. The IHS is under review to determine whether the current importation under basic conditions provides adequate risk management of viroids and viruses associated with tomato and *Capsicum* seeds.

1.2 Scope of this risk analysis

This Import Risk Analysis covers the importation into New Zealand of tomato seeds-for-sowing and *Capsicum* seeds-for-sowing which have been produced in any other country. For the purposes of this risk analysis "tomato seeds" or "*Capsicum* seeds" is defined as **"seeds that have been extracted from the fruits and processed by a variety of methods and treatments; packaged and transported to New Zealand, for sowing".** The risk analysis assumes that there is no disinfection of seeds prior to transportation to New Zealand.

The scope of the analysis:

- is limited to virus and viroid hazards that are seed-borne and that are transmitted seed-to-seedling via tomato or *Capsicum* seeds;
- includes the virus and viroid hazards identified by Plant and Plant Products Import (PSTVd, TCDVd, PepMV, PZSV);
- includes identification of additional virus and viroid hazards detected in a scan of the literature;
- includes assessment of the risks of the identified hazards; this includes likelihoods of entry, exposure establishment and likely consequences for each hazard organism;
- the scope of the 'economic consequences' section is limited to direct impacts;
- includes some analysis of the identified risks against possible mitigation options;
- includes peer review of the draft analysis;
- does not include other potential entry pathways of viruses and viroids, such as via ornamental plants.

The identified options for measures will form the basis of a Risk Management Proposal and new Import Health Standard for importing tomato and *Capsicum* seeds for sowing into New Zealand.

1.3 The risk analysis process

The following briefly describes the MAF process and methodology for undertaking import risk analyses. For a more detailed description of the process and methodology please refer to the Biosecurity New Zealand Risk Analysis Procedures (Version 1 12 April 2006) which is available on the Ministry of Agriculture and Forestry web site (MAF, 2006).

The risk analysis process leading to the final risk analysis document is summarised in figure 1.

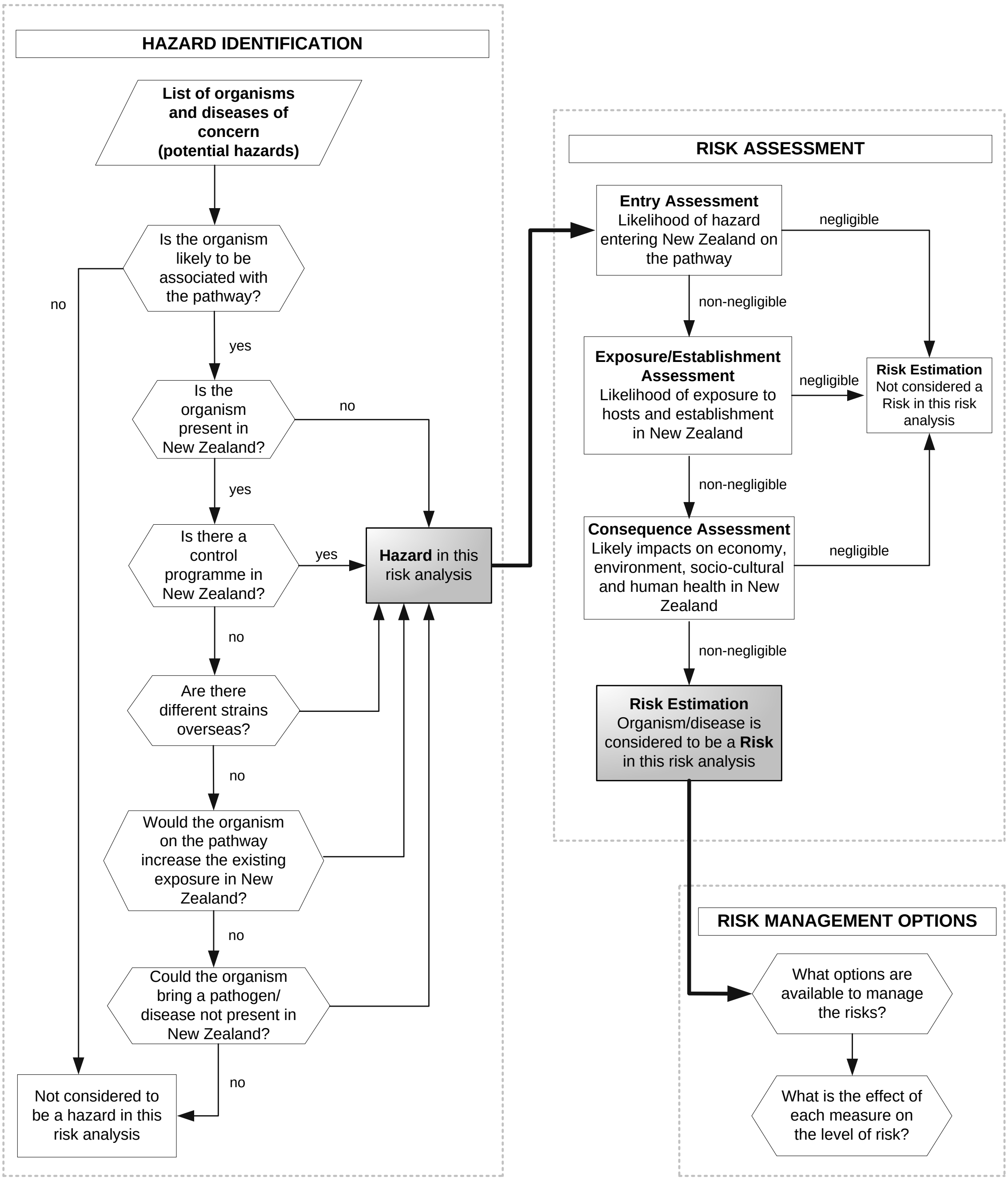

Figure 1. Diagrammatic representation of the risk analysis process

The process outlined in figure 1is further explained in the following sections.

1.3.1 Commodity and pathway description

The first step is to describe the commodity and entry pathway of the commodity. This can include relevant information on:

- the country of origin, including characteristics like climate, relevant agricultural practices, phytosanitary system;
- pre-export processing and transport systems;
- export and transit conditions, including packaging, mode and method of shipping;

- nature and method of transport and storage on arrival in New Zealand;
- characteristics of New Zealand's climate, and relevant agricultural practices.

This information provides context for the assessment of the hazard organisms.

1.3.2 Hazard Identification

Hazard identification is conducted prior to the risk assessment stage. It is basically equivalent to "pest categorisation" in the system used under the International Plant Protection Convention (IPPC) system (FAO 2007). This process begins with the collation of a list of organisms and diseases that might be associated with the commodity in the country of origin and are potentially capable of causing harm (potential hazards). Potential hazards are then screened using the steps listed in the hazard identification section of figure 1 and information on the biology and distribution of the organism or disease.

The hazard identification process identifies the organisms or diseases that are likely to be associated with the commodity from the exporting country, and which have the potential to cause harm to New Zealand (hazards).

Hitchhiker organisms, which have no biological host association with a commodity, are sometimes considered to be hazards where there are other sources of evidence for their likely association with a commodity, and where they meet the other criteria to be considered hazards.

During the hazard identification process, organisms and diseases are sometimes grouped on their biology and likely susceptibility to risk management measures. The groups are not absolute and some organisms fit into more than one group at different life stages.

Chapter 3 describes hazard identification process undertaken for this risk analysis and lists the main information sources.

1.3.3 Assessment of risks

Risk assessments are undertaken for the organisms identified as hazards.

Risk assessment is the evaluation of the likelihood of entry, exposure and establishment of a hazard organism, and the environmental, economic, human and/or animal health and socio-cultural consequences of the entry within New Zealand. The aim of risk assessment is to identify hazards which present an unacceptable level of risk, for which risk management measures may be considered. The risk assessment is qualitative, and descriptors (negligible, low, moderate, high) are used in assessing the likelihood of entry, exposure, establishment and spread, and the economic, environmental, socio-cultural and human health consequences. These descriptors are defined in the Risk Analysis Procedure manual (BNZ 2006).

It is important to note that in this risk analysis, the risk assessments account for the risk associated with the total volume of seeds being imported over time (i.e. millions of seeds). The risk assessments values (i.e. low, mod, high) <u>do not</u> refer to the risk associated with any particular individual seed, and its subsequent seedling/plant, that might be chosen at random and followed through the pathways of virus/viroid entry, exposure and establishment.

1.3.4 Assessment of uncertainties

In this aspect of the risk analysis process the uncertainties and assumptions identified during the preceding hazard identification and risk assessment stages are stated within the text. An analysis of these uncertainties and assumptions can then be completed to identify which are critical to the outcomes of the risk analysis. Critical uncertainties or assumptions can then be considered for further research with the aim of reducing the uncertainty or removing the assumption.

Where the risk assessment has significant uncertainty, this is stated in the conclusion of the risk assessment. In these cases, the Risk Analysis Procedure manual (BNZ 2006) notes a precautionary approach to managing risk may be adopted. In these circumstances the measures should be reviewed as soon as additional information becomes available[4] and be consistent with other measures where equivalent uncertainties exist.

1.3.5 Management options

For each organism classified as a risk, a risk management step is carried out, which identifies the options available for managing the risk and considers the efficacy of those options in relation to the risk. In addition to the options presented, unrestricted entry or prohibition may also be considered for each risk organism. Recommendations for the appropriate phytosanitary measures to achieve the effective management of risks are not made in this document. These will be determined when the Risk Management Proposal (RMP) and Import Health Standard (IHS) are drafted.

As obliged under Article 3.1 of the WTO Agreement on the Application of Sanitary and Phytosanitary Measures (the SPS Agreement), the measures adopted in IHSs will be based on international standards, guidelines and recommendations where they exist, except as otherwise provided for under Article 3.3 (where measures providing a higher level of protection than international standards can be applied if there is scientific justification, or if there is a level of protection that the member country considers is more appropriate following a risk assessment).

1.3.6 Review and consultation

Peer review is a fundamental component of a risk analysis to ensure the analysis is based on the most up to date and credible information available. Each analysis must be submitted to a peer review process involving recognised and relevant experts from New Zealand or overseas. The critique provided by the reviewers is reviewed and where appropriate, incorporated into the analysis. If suggestions arising from the critique are not adopted the rationale must be fully explained and documented.

The conclusions of the risk analysis will be summarised in a risk management proposal that accompanies the draft IHS for consultation. The risk analysis provides additional technical detail should submitters wish to see a more detailed scientific analysis of the biological risks.

All submissions received from stakeholders will be analysed and compiled into a review of submissions. The Risk Analysis, Risk Management Proposal and draft Import Health Standards will be modified where appropriate depending on the outcome of consultation.

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4 Article 5.7 of the SPS Agreement states that “a Member may provisionally adopt sanitary …. measures” and that “Members shall seek to obtain additional information …. within a reasonable period of time.” Since the plural noun “Members” is used in reference to seeking additional information a co-operative arrangement is implied between the importing and exporting country. That is the onus is not just on the importing country to seek additional information.

References for Chapter 1

FAO (2007) International Standards for Phytosanitary Measures (ISPM) no.2: Framework for Pest Risk Analysis. Food and Agriculture Organisation. available online at https://www.ippc.int/file_uploaded/1179929048771_ISPM02_2007_E.pdf

MAF (2006) Biosecurity New Zealand risk analysis procedures. Ministry of Agriculture and Forestry, New Zealand, 201 pp. Available online at http://www.biosecurity.govt.nz/files/pests-diseases/surveillance-review/risk-analysis-procedures.pdf

2 Commodity and pathway description

This chapter provides information on the commodity that is relevant to the analysis of biosecurity risks and common to all organisms or diseases potentially associated with the commodity.

2.1 Commodity description

Taxonomy

Tomato and *Capsicum* species are members of the plant family Solanaceae. As there are many synonyms and varieties of tomato, and many species of the genus *Capsicum*, the species that are included in the commodity descriptions are clarified below in Table 1. The species that are included in the commodity descriptions are derived from the Plants Biosecurity Index (PBI).

- All six tomato species listed in the PBI are synonyms of their accepted scientific name. The accepted scientific name of the synonym *Solanum pimpinellifolium* is *Lycopersicon pimpinellifolium* . The accepted scientific name of all the other tomato synonyms on the PBI is *Solanum lycopersicum*.
- Seven of the nine *Capsicum* species listed in the PBI are the accepted scientific name. The accepted scientific names of the synonyms *Capsicum microcarpum* and *Capsicum pendulum* (on the PBI) are *Capsicum baccatum* var. *baccatum* and *C. baccatum* var. *pendulum* respectively.

Table 2. Taxonomic descriptions of tomato and *Capsicum* seeds approved for importation into New Zealand

Taxonomic Descriptions		
Name as listed in the Plants Biosecurity Index	Scientific Name[5]	Common Name
Tomato seeds for sowing		
Lycopersicon esculentum *Lycopersicon lycopersicum*	*Solanum lycopersicum*	Tomato
L. humboldtii	*S. lycopersicum*	Cherry tomato
L. pimpinellifolium	*S. pimpinellifolium*	Currant tomato
L. piriforme	*S. lycopersicum* (source:SysTax http://www.biologie.uni-ulm.de)	Pear shaped tomato
L. skorospelka	*S. lycopersicum* (source: http://www.tradewindsfruit.com	Tomato
Capsicum seeds for sowing		
Capsicum annuum	*C. annuum*	Capsicum; red pepper
C. baccatum	*C. baccatum*	Pepper
C. cardenasii	*C. cardenasii*	Pepper
C. chinense	*C. chinense*	Pepper
C. eximium	*C. eximium*	Pepper
C. frutescens	*C. frutescens*	Chilli pepper
C. microcarpum	*C. baccatum* var. baccatum	Pepper
C. pendulum	*C. baccatum* var. pendulum	Pepper
C. pubescens	*C. pubescens*	Pepper

Physical Description of tomato and capsicum seeds

Both tomato and *Capsicum* seeds are fairly small; *S. lycopersicum* seeds are 2.5-3.3 mm long, 1.5-2.3 mm wide, 0.5-0.8 mm thick, tear-drop shaped and flat (Natural History Museum, 2011a). *S. pimpinellifolium* seeds can be slightly smaller (2-3 mm long, 1-2.3 mm wide) (Natural History Museum, 2011b). *Capsicum* seeds are also flat and have relatively similar dimensions to tomato seeds, but are rounder in shape.

Processing and Packaging

Processing methods (e.g. washing in bleach and other chemicals) and the impacts of these will be taken into account in the risk management section (Section 6).

Seeds for sowing are packaged in lots of 100, 1,000 or 10,000 seeds. It is understood by MAF that in most cases the packaging is hermetically sealed.

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[5] Unless otherwise stated, the authority used for verification of the scientific names was: for tomatoes, the Natural History Museum; and for capsicum species, GRIN Taxonomy (http://www.ars-grin.gov/cgi-bin/npgs/html/taxgenform.pl).

2.2 Pathway description

2.2.1 Overview of pathway

The steps in the tomato and capsicum seed pathway are outlined in Figures 2 and 3 respectively.

In summary, New Zealand imports tomato and *Capsicum* seeds from many countries. The vast majority of seeds (by volume) are imported from only a few countries, known as the re-exporter countries, such as the Netherlands. Re-exporter countries such as the Netherlands obtain much of their seed from other countries. The seed can be legally repackaged and labelled 'repacked in Netherlands' (P.Reed, Fera, UK, personal communication, 2009; cited in FERA, 2010). The seed is then re-exported to countries such as New Zealand.

- New Zealand imported tomato seeds from 23 countries[6] between 2008 and 2011, but the greatest volume of seeds came from the Netherlands, Australia, China and the USA.
- New Zealand imported capsicum seeds from 25 countries between 2008 and 2011, but the greatest volume of capsicum seeds came from Australia and the USA, followed by the Netherlands.

With regard to tomato seeds, it is estimated that at least 2.75 million seeds are imported per year. This is an extrapolation of knowledge that approximately 2.75 tomato plants are used in New Zealand commercial production systems (K Robertson, personal communication, 2011)

[6] Countries from where tomato seeds are imported: Argentina, Australia, Chile, China, Czech Republic, Denmark, France, Germany, Ghana, Guatemala, Hong Kong, Hungary, India, Israel, Italy, Japan, Korea-South, Malaysia, Mexico, Netherlands, New Zealand, Pakistan, Peru, Philippines, South Africa, Spain, Taiwan, Tanzania, Thailand, United Kingdom, USA, Vietnam, Zambia.

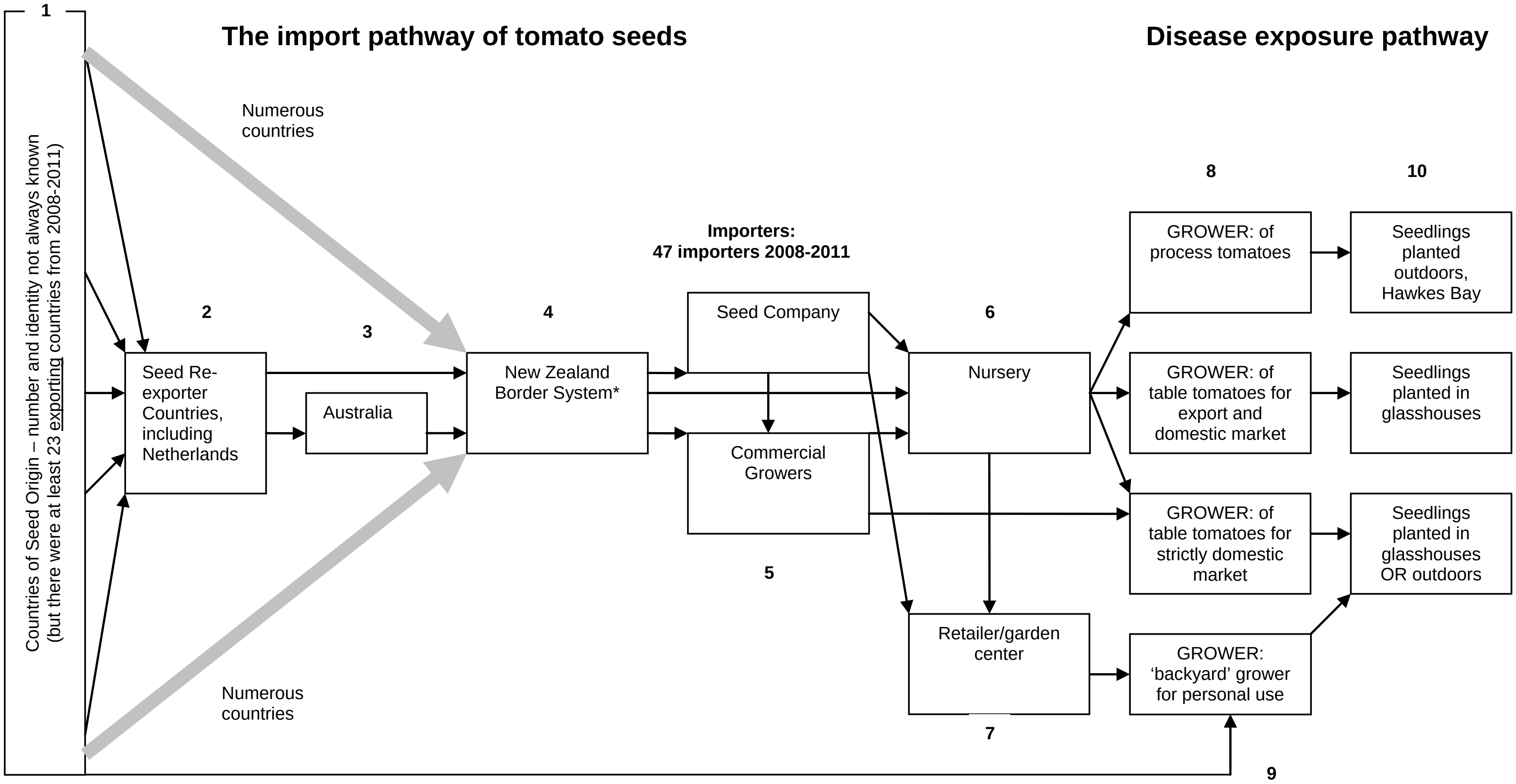

Figure 2. Diagrammatic representation of the import pathway of tomato seeds and of the disease exposure pathway

*NZ Border System = Cargo declaration, paperwork; Seed examined/treated at border; Seed destroyed or re-exported; Seed cleared for entry
Definitions:
Countries of Origin = country where seed was harvested
Exporting countries = may or may not be the country where the seeds were harvested. The exporting country may in fact be a re-exporter.
Seed Re-exporter Countries = countries into which seeds have been imported from around the world, repackaged & labelled, and from where seeds are re-exported

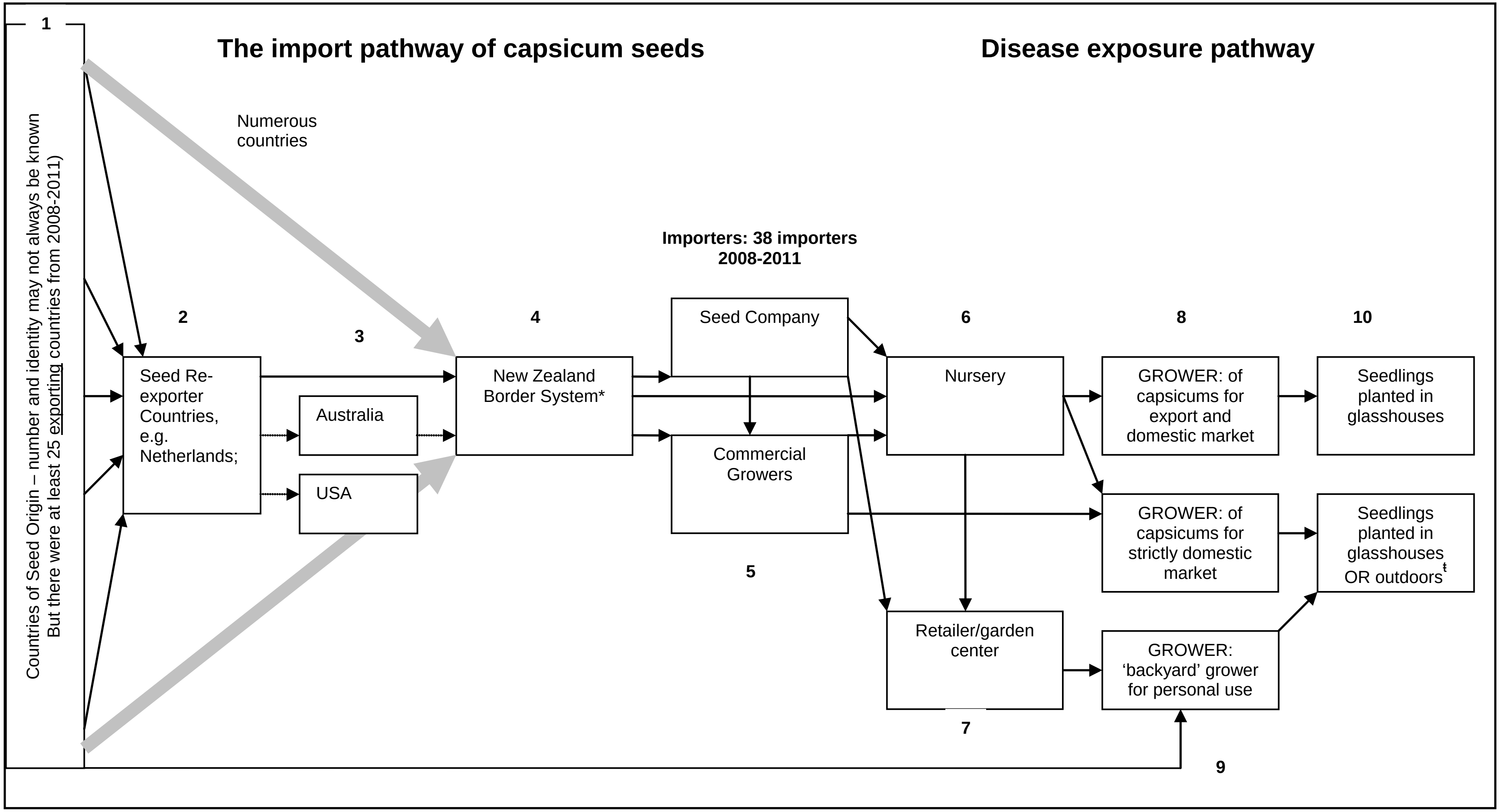

Figure 3. Diagrammatic representation of the import pathway of *Capsicum* seeds and of the disease exposure pathway

*NZ Border System = Cargo declaration, paperwork; Seed examined/treated at border; Seed destroyed or re-exported; Seed cleared for entry
Definitions:
Countries of Origin = country where seed was harvested
Exporting countries = may or may not be the country where the seeds were harvested. The exporting country may in fact be a re-exporter.
Seed Re-exporter Countries = countries into which seeds have been imported from around the world, repackaged & labelled, and from where seeds are re-exported
[ŧ]Home-gardeners are known to grow capsicums outdoors, but commercial growers grow capsicums glasshouses.

2.2.2 Pre-New Zealand Border: the import pathway

Step 1 to 3: Seeds are produced in a country of origin. From there they may be exported directly to New Zealand, or they may be exported first to a ‘re-exporter’ country before being exported to New Zealand, either directly or occasionally via Australia.

Step 4: Seeds arrive at the New Zealand border and are processed through the New Zealand Border System. This involves a cargo declaration and associated phytosanitary paperwork. Currently tomato and *Capsicum* seeds can enter New Zealand under the Basic phytosanitary requirements described in the IHS 155-02-05[7]. Seeds are examined at the border in accord with the Basic regulations. They are either cleared for entry, or if import requirements have not been met they are either: treated, destroyed or re-exported.

2.2.3 Post New Zealand Border: the disease exposure pathway

Step 5 to 8: The seeds are delivered to the seed importer. Although there were 47 importers of tomato seed, and 38 importers of *Capsicum* seed between 2008 and 2011, these can be broadly characterised as seed companies, commercial growers, or nurseries. Members of the public can also import seeds.

From there, the number of disease exposure pathways become numerous and varied, depending on who the end user (the grower) is and what their crop management practices are. Potential disease exposure pathways are:

1. Seed company to nursery to retailer/garden center to backyard grower
2. Seed company to nursery to commercial grower
3. Seed company to commercial grower to nursery to commercial grower
4. Seed company to commercial grower
5. Commercial grower to nursery to commercial grower
6. Commercial grower
7. Nursery to retailer/garden center to backyard grower
8. Nursery to commercial grower
9. Seed exporter to member of the public

Step 10: The imported seeds or seedlings derived from imported seeds are planted either in glasshouses, or outdoors, depending on the cultivar (particularly for tomatoes), and the characteristics of the grower.

In regard to contributors to the biosecurity risks, some key aspects of the pathway are:

- the country of seed origin (as distinct from the country of export), and
- the way in which the plant derived from the imported seed is grown (e.g. in glasshouses or outdoors; under what crop and disease management practices).

[7] IHS 155-02-05 http://www.biosecurity.govt.nz/imports/plants/seeds-sowing.htm

2.2.4 Country of Seed Origin

The country of seed origin (as distinct form the country of seed export) is not always known to MAF when seeds are at Step 4 in the pathway. A random selection of MAF QuanCargo records of tomato and capsicum seed imports, from 2008 to 2011, were reviewed to gain an understanding of what is known about country of origin of imported seeds. The three main findings were:

1. Some consignments from the Netherlands do declare the country of origin in the paper work that is attached with the consignment and available on QuanCargo (e.g. C2010/340739; C2011/61347).
2. The country of origin data may or may not be recorded in QuanCargo under the 'country of origin' data field; sometimes that information remains in the attached documents and is not entered into the country of origin data field. For instance in the consignment example given above, the QuanCargo data field 'country of origin' stated Australia, yet the paperwork lists the country of origin as Thailand, Holland, France, and Guatemala. The 'Australia' record is presumably because the order for the Netherlands consignment was processed through the Australian office.
3. For most consignments there remains uncertainty about the actual country of seed origin. For most of consignment records that were reviewed, the recorded 'country of origin' was the same as the country of export. In the absence of more detailed documentation (e.g. attachments) there was no way of knowing whether the recorded 'country of origin' in QuanCargo was the actual country of seed origin. Uncertainty about country of origin is particularly high for consignments that have arrived from countries that are known 're-exporters', such as the Netherlands (e.g. C2009/115282). All Australian consignments that were checked had a country of origin recorded in QuanCargo as Australia (e.g. C2011/61361; C2011/29610; C2008/188082). As none of these consignments arrived with specific detailing of the country of origin, the records in QuanCargo may simply reflect an assumption that the country of origin is the same as the country of export.

Implications for the risk analysis

In consideration of what is known about the pathway, particularly the uncertainties that exist about the country of seed-origin, this risk analysis has been carried out assuming that tomato and *Capsicum* seeds come from all countries of the world, and that the country of seed-origin is not necessarily known for any given consignment.

2.3 Tomato and capsicum production in New Zealand

2.3.1 An Overview

Tomato and *Capsicum* seed is imported into New Zealand to enable production of tomatoes and capsicums in this country, whether it is for commercial production or for back-yard production. This section describes the production practices of commercial growers, and of home gardeners, with a particular focus on whether they are grown outdoors or indoors. This information is particularly relevant to the assessment of whether viroids and viruses are likely to establish and spread in New Zealand. This information will be referred to in the individual pest risk assessments in following sections.

As at September 2011, Horticulture New Zealand (HortNZ) had 250 commercial tomato growers and 85 commercial capsicum growers in their database. Approximately 30 growers produce both tomato and capsicums; this reflects the practise of some growers who are primarily tomato producers, choosing to also dedicate some of their space to capsicum production. There are additional producers of tomato and capsicum who are not members of HortNZ.

The commercial growers can be categorised in the following way:

- Very large ‘corporate’ companies, producing large quantities of fruit;
- Moderately sized family-owned companies;
- Small family-owned and -operated companies.

There are approximately 2.75 million tomato plants used in commercial production systems in New Zealand over the course of a growing season (K. Robertson, personal communication, 2011).

The following sections deal separately with indoor outdoor production systems. Within each of those sections, production is categorised according to whether fruit is produced commercially or in the home garden.

2.3.2 Indoor/Greenhouse production of Tomato and Capsicum

2.3.2.1 Commercial Tomato

The majority of table-tomatoes produced in New Zealand are grown indoors, in greenhouses. Some companies produce primarily for the export market but nevertheless sell some of their produce domestically, whereas some companies produce solely for the domestic market.

Companies that produce fruit for the export market adhere to a “Compliance Programme for Export Truss Tomato Fruit to Australia (PSTVd)”. One of the conditions of the compliance programme is that truss tomatoes are produced in accordance with the “New Zealand Code of Practice for the Management of Potato Spindle Tuber Viroid (PSTVd) in Greenhouse Tomato and Capsicum Crops”. The purpose of the Code of Practice is:

- to eliminate PSTVd from greenhouse tomato and capsicum production,
- to contain and prevent infection build up, or its spread, within crops
- to prevent spread to other crops (units, greenhouses, properties, or species – especially potatoes)
- to facilitate market access, in particular for truss tomatoes.

The key elements of the Code of Practice are early detection through monitoring for diseased plants, isolation of suspect plants, measures to prevent spread of symptomless infections, and breaking the cycle of infection between growing cycles.

Companies that produce green-house tomatoes solely for the domestic market are not required to adhere to the Code of Practice. Whilst it is generally accepted that there are natural incentives to adhere to the Code of Practice, there is uncertainty about whether all growers do adhere to the Code.

Note: within this risk analysis, the risks of viruses and viroids associated with imported tomato and capsicum seeds have been assessed on the basis that there is no code of practice in place. This approach to risk assessment is used because it ensures that all relevant risks are encapsulated in the assessment, rather than just those that remain after a particular risk management measure is in place. This approach ensures that the risk assessment does not become obsolete when changes are made to risk management measures.

However, the risk management decisions will take into consideration the Code of Practice. See the 'Assumptions' section for further details, and see the section on 'Overview of potential risk management options', (Section 6).

2.3.2.2 Commercial Capsicum

It is thought that all commercially-produced capsicum is grown in greenhouses. As with the tomato growers, some capsicum growers focus on producing fruit for export markets, whereas others focus on producing fruit for the domestic market. Capsicum production, whether for domestic or export markets, is not subject to a compliance programme like tomato production is. Nevertheless, most companies that grow capsicums for the export market are likely to do so in accordance with the Code of Practice described above.

Whilst it is generally accepted that there are natural incentives for growers of capsicums for the domestic market to also adhere to the Code of Practice, there is uncertainty about the proportion of growers that do so.

2.3.2.3 Backyard production of Tomato or Capsicum in greenhouses

A small proportion of New Zealand's greenhouse-produced tomatoes and capsicums are grown in backyard glass-houses or tunnel-houses by home gardeners, all over New Zealand. Home gardeners are highly likely to grow other solanaceous crops (potato), and ornamentals (potential alternative hosts for the viruses and viroids) outdoors, which are likely to be in close proximity to the greenhouse where the tomato and/or capsicum plants grow. It is also likely that some home gardeners grow potato crops within the same greenhouse that tomato and/or capsicum plants are grown[8].

Home gardeners are likely to vary in their ability to detect and manage disease symptoms in their tomato plants, and to prevent spread of viral or viroid disease within the glasshouse, and between glasshouse and outdoor plants.

2.3.3 Outdoor production of Tomato and Capsicum

2.3.3.1 Commercial Tomato

The vast majority of New Zealand's outdoor-grown tomatoes are commercially grown for processing, in the Hawkes Bay (about 36,000 tonnes) and Gisborne (about 30,000 tonnes) (Heinz Watties, personal communication 2011). There is also some outdoor commercial production of table tomatoes in areas of New Zealand with a suitably warm climate; around Whangarei, Auckland, Hastings, Horowhenua/ Otaki and Christchurch.

The crop management practices for outdoor-grown table tomatoes are more intensive because fruit quality is critical. Outdoor crops of table-tomatoes are regularly checked and handled for disease symptoms, but there is uncertainty about whether the management practices are consistent with the key elements of the Code of Practice mentioned above.

[8] This practice is known to occur in frost prone regions, and with gardeners who wish to produce an early potato crop.

The disease management practices of process-tomato crops are not as intensive, but the plants are regularly handled by workers; it is highly unlikely that the Code of Practice is implemented in those crops.

2.3.3.2 Commercial Capsicum

There is no commercial production of capsicums outdoors in New Zealand (Seymour & Robertson, personal communication, 2011).

2.3.3.3 Backyard Tomato or Capsicum (outdoors)

A very small proportion of outdoor-produced tomatoes and capsicums are grown in backyards around New Zealand by home gardeners, where the climate is suitably warm. Home gardeners are highly likely to grow other solanaceous crops, and ornamentals (potential alternative hosts for the viruses and viroids) in close proximity to their tomato and/or capsicum plants.

Home gardeners are likely to vary in their ability to detect and manage disease symptoms in their tomato plants and to prevent spread of viral or viroid disease.

2.3.4 Horticultural Practices that impact the likelihood of self-sown tomatoes occurring

The occurrence of self-sown tomatoes is a consideration that can impact the likelihood of establishment of tomato/capsicum viruses and viroids. Accidental production of plants from seed derived from infected plants could occur as a result of fruit drop, and the seed being left in the growing media (or soil), leading to self-sown plant. The following discussion examines this possibility:

- In commercial greenhouse, or in commercial outdoor crops of table tomatoes, the likelihood that self-sown tomatoes occur is negligible, given that growers are likely to burn or bury the plants and fruit, thereby eliminating most progeny seeds. Commercial growers that use soil-free production systems have no likelihood of accidentally growing plants derived from progeny seeds; Commercial growers that use soil are unlikely to use any plants that have been self-sown.
- In commercial outdoor fields where tomato crops are grown, the likelihood that self-sown tomato plants occur is negligible, because the horticultural practices provide no opportunity for self-sown tomatoes to establish. Field tomatoes in New Zealand are grown in a one in three year cropping rotation, with tomatoes grown the first year, two other crops grown in the following two years and pasture grown over the intervening winter and stock grazed. Any seeds left in the field after harvest that immediately germinate will die in frosts if they occur in frost-prone areas. The herbicides used to spray out winter grass prior to the following year's crop kill any spring germinating tomato plants. Then consecutive herbicides used for weed control in subsequent crops would kill tomatoes (Watties, personal communication, 2011).
- In the home garden, self-sown plants can occur as a result of fruit drop, and sprouting of seeds from plants and fruit that have ended up in home compost bins. Whilst there is only a low likelihood of diseased/symptomatic plants being left to grow long enough that fruit/seeds could drop and yield self-sown plants, there is a low to moderate likelihood that home gardeners would keep self-sown plants originating from compost.

2.4 Assumptions used in this risk analysis

- The risk analysis assumes that there is no disinfection of seeds prior to transportation to New Zealand.
- It is assumed that seeds entering New Zealand can potentially originate from any other country. This assumption is made because MAF does not currently have the capacity to identify the country of seed origin (i.e. where the seed was produced, as distinct from the country of export). See Section 2.2 for further discussion.
- For the purposes of this analysis, it is assumed that the industry Code of Practise is not in place[9]. This assumption is made because it ensures that all relevant risks are encapsulated in the assessment, rather than just those that remain after a particular risk management measure is in place. This approach ensures that the risk assessment does not become obsolete when changes are made to risk management measures.
- It is assumed that there is some potential (albeit low) for bumblebees (and aphids, in the case of PSTVd assessment) to escape from commercial greenhouses.

Some assumptions have been made about the way crops and ornamentals are handled in home gardens:

- It is assumed that potato crops are rarely handled, and that there is only a low likelihood that they'll be handled with the same tools as tomatoes have been handled with;
- It is considered that there is a low to moderate likelihood that annuals and/or perennials (which are alternative host species of virus & viroids) are maintained and handled with tools that have been used to handle tomato and/or capsicum plants.

2.5 Uncertainties in this risk analysis

Some of the viroids and viruses are known to be transmitted by bumblebees between tomato plants:

- For those viroids and viruses where there is no mention of bumblebee transmission in the literature, there is uncertainty about whether they too can indeed be transmitted between tomato plants via bumblebees.
- In the case of PSTVd, which is a very well studied viroid, the lack of reports of bumblebee transmission is considered to mean that it is not transmitted by bumblebees.
- Apart from *Pepino Mosaic virus*, it is uncertain whether bumblebees can transmit viruses and viroids between species, e.g. from tomato plants to potato plants; or from tomato plants to ornamentals, or weedy solanaceous plants; or from ornamentals & weedy solanaceous plants (potential reservoirs of virus/viroid) to potato crops.

Potential host plants have been identified in experimental settings:

- It is uncertain whether plant species identified as hosts in experimental settings (typically using carborundum in mechanical inoculations) will become hosts in a non-experimental setting. But nevertheless, it is standard practice in risk assessments to not consider experimental hosts as actual hosts of the pathogen because the experimental conditions are different from field conditions. This stance has been taken in these risk assessments, unless otherwise stated.

[9] But note that The Code of Practice will be taken into consideration when risk management options are decided.

- Related to this, it is uncertain whether the other potential host species are as susceptible as tomato (or *Capsicum*) to mechanical inoculation with the viroid/virus.

Seed-to-seedling transmission is demonstrated in tomatoes and/or *Capsicum*:
- But it is uncertain whether other host species can transmit the virus/viroid from seed-to-seedling.

2.6 Conclusion for Chapter 2

This risk analysis considers the following with regard to the tomato seed pathway:

Pre-border seed pathway issues:
- The country of origin of imported tomato and *Capsicum* seeds is usually not known, so **seeds are considered to orginate from all countries of the world**;
- **Seeds are not considered to be disinfected.**

Post-border seed pathway issues:
- Companies that produce fruit for the export market adhere to a “Compliance Programme for Export Truss Tomato Fruit to Australia (PSTVd)”, which is a risk management measure for PSTVd. **This is not considered in assessing the risk of any of the viroids and viruses**, **but it will be considered in the risk management proposal (see section 2.3.2.1 for reasons);**
- The horticultural practices of commercial indoor and outdoor crops are considered to eliminate likelihood that self-sown tomatoes occur in commercial crops. This will eliminate one pathway of establishment. **This is considered in assessing the risk.**
- The horticultural practices of home gardeners are considered to not eliminate the likelihood of self-sown tomatoes. **This is considered in assessing the risk.**

References for Chapter 2

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3 Hazard identification

3.1 Identification of hazards

A list of four hazard organisms was provided by the MAF Plant and Plant Products Import team (PSTVd, TCDVd, PepMV, PZSV). A literature search was also conducted to identify other potential seed-borne hazards of tomato and *Capsicum*. Hazard Identification was narrowly focused upon only the viruses and viroids for which there was evidence of seed-to-seedling transmission. Information was derived from:

- Literature searches of databases including, but not limited to: CAB Abstracts, Google Scholar;
- A European Food Safety Authority report on solanaceous pospiviroids (EFSA, 2011);
- Other publications available on the internet.

Table 3 provides a summary of the results of the hazard identification process. The list of organisms in the table is not considered exhaustive; it fairly reflects the relatively limited amount of time spent searching for additional hazards. Organisms on the hazard identification list were screened and were classified as hazards if they were:

- not known to be established in New Zealand
- known to be seed-to-seedling transmitted by either tomato or *Capsicum*.

In addition, any hazard oganisms present in New Zealand were given further consideration if they they met any of the following criteria:

- vectors of pathogens or parasites that are not present in New Zealand
- known to have strains that do not occur in New Zealand
- of restricted distribution in New Zealand
- under official control in New Zealand
- differ genetically from those that occur in New Zealand in a way that may present a potential for greater consequences in New Zealand, either from the organism itself or through interactions with existing organisms in New Zealand
- the nature of the imports would significantly increase the risk.

Four viroids were classified as hazards:

Potato spindle tuber viroid (PSTVd)
Tomato chlorotic dwarf viroid (TCDVd)
Tomato apical stunt viroid (TASVd)
Pepper chat fruit viroid (PCFVd)

Three viruses were classified as hazards:

Pepino mosaic virus (PepMV)
Pelargonium zonate spot virus (PZSV)
Tobacco mild green mosaic virus (TMGMV)

[Note: Table 3 does not report all of the information scanned and collected during the hazard identification process].

Table 3. Findings of the Hazard identification for pospiviroids and viruses of tomato and *Capsicum* seed

Organism Name	**Infects capsicum plant?**	**Ref for seed transmission capsicum**	**Infects tomato plant?**	**Ref for seed transmission tomato**	**In NZ?**	**Hazard**	**Notes on Hazard Identification**	**References**
PepMV	experimentally	-	Y	Y	N	Y	RA done	Seed transmission tomato (Cordoba-Selles et al 2007; Hanssen et al 2010.) Experimental infection capsicum (Hanssen & Thomma, 2010; Fakhro et al., 2011)
PZSV	Y	no reports yet	Y	Y	N	Y	RA done	Infection capsicum (Lapidot et al., 2010; Escriu et al., 2009) Seed transmission tomato (Lapidot et al 2010)
TMGMV	Y	Y	no reports yet	-	N	Y	RA done	Seed transmission capsicum (Cordoba et al. 2006)
PSTVd	Y	Y (suspected transmission)	Y	Y	Dis-puted	Y	RA done	Seed transmission capsicum (Lebas et al 2005, NZ occurrence), tomato (Benson & Singh 1964)
TCDVd	experimentally	-	Y	Y	N	Y	RA done	Exptal infection capsicum (Matsushita et al 2009) Seed transmission tomato (Singh et al 1999)
TASVd	no reports yet	-	Y	Y	N	Y	RA done	Seed transmission tomato (Antignus et al 2007)
PCFVd	Y	Y	Y	no reports yet	N	Y	RA done	Seed transmission capsicum (Verhoeven et al 2009) Tomato infection (Reanwarakorn et al., 2011
CLVd	no reports yet	-	Y	? Not proven	N	?	RA not done;	Infection tomato (numerous, Verhoeven et al 2004; EFSA, 2011) Suspected seed transmission tomato (Fox & Monger 2011 (Ref not publically available; expt flawed, see EFSA 2011) Also, several outbreaks in commercial crops; seed origin suspected (Sansford & Morris 2010)
TPMVd	no reports yet	-	Y	? Not proven	N	?	RA not done;	Infection tomato (Galindo et al 1982; EFSA 2011) Suspected seed transmission tomato (Galindo 1987 - a book ref).
CSVd	no reports yet	-	Y	Y	Y	N		Infection tomato (Niblett et al 1978; EFSA 2011) Seed transmission tomato (Chung & Pak 2008)
CEVd	no reports yet	-	Y	no reports yet	Y	N	Seed transmission occurs in ornamentals (Singh et al., 2009)	Infection tomato (Mishra et al 1991; Verhoeven et al 2004)

PepMV=pepino mosaic virus; PZSV=Pelargonium zonate spot virus; TMGMV=Tobacco mild green mosaic virus; PSTVd=potato spindle tuber viroid; TCDVd=tomato chlorotic dwarf viroid; TASVd=Tomato apical stunt viroid; PCFVd=Pepper chat fruit viroid; CLVd=Columnea latent viroid; TPMVd=Tomato planta macho viroid; CSVd=Chrysanthemum stunt viroid; CEVd=Citrus exocortis viroid.

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4 Risk analysis of viroids in tomato and/or capsicum seeds

4.1 Tomato chlorotic dwarf viroid (TCDVd)

Scientific name: *Tomato chlorotic dwarf viroid* Singh 1999
Family/Genus: *Pospiviroidae/Pospiviroid*
Acronym TCDVd

4.1.1 Hazard identification

4.1.1.1 Description

Tomato chlorotic dwarf viroid (TCDVd) is a plant pathogen that causes tomato plants to develop chlorotic leaves, become severely dwarfed and bunchy. TCDVd is a circular, single stranded RNA molecule lacking a capsid (a shell of protein).

4.1.1.2 Taxonomy

Several variants of TCDVd have been isolated from natural infections, and additional variants of TCDVd have been isolated under experimental conditions (Singh et al., 2010). They are distinguishable at the genetic level by variations in nucleotide sequence. The length of TCDVd RNA ranges from 356 to 361 nucleotides (Singh et al., 2010). There is 85-89% genetic similarity across all the known variants of TCDVd to *Potato spindle tuber viroid* (PSTVd), the type species of the *Pospiviroid* genus.

4.1.1.3 New Zealand status

TCDVd is not known to be present in New Zealand. In PPIN it is recorded as not present in NZ, and it is not recorded in the latest review of plant virus, viroid and mollicute records for New Zealand (Pearson et al., 2006).

4.1.1.4 Geographic distribution

As at November 2011, the known worldwide distribution of TCDVd is limited but has been expanding in recent years (see below for evidence).

According to CABI (2009) and the other references noted below, TCDVd is present in:
Asia: Israel (James et al., 2008), India
North America: USA (in glasshouses only)
Central America: Mexico (Ling & Zhang, 2009, abstract only)
Europe: Slovenia (Marn & Plesko, 2010), Portugal (James et al., 2008), few occurrences in France (Tassus et al., 2009 abstract only; Candresse et al., 2010), Czech Republic and Finland.

TCDVd has been eradicated from Japan (Matsuura et al., 2010b) and Canada (CABI, 2009). It is absent from the Netherlands (but seeds are traded through this country) and the United Kingdom, although it has been intercepted in these countries (e.g. infected plants supplied by Israel and Portugal were intercepted in the UK) (CABI 2009).

4.1.1.5 Commodity association

Tomato seed: TCDVd occurs in tomato plants (Singh et al., 1999; Verhoeven et al., 2004; Matsushita et al., 2008), and can be transmitted by tomato seed (Candresse et al., 2010; Singh & Dilworth, 2009). TCDVd has been detected in at least one commercial seed lot (source unknown) (Candresse et al., 2010), and the first tomato plants ever reported with TCDVd are suspected to have originated from an infected commercial seed lot from the Netherlands (Singh et al., 1999).

Capsicum seed: Natural TCDVd infections in *Capsicum* have not been reported and no natural infections were detected in a survey of *C. annuum* and *C. frutescens* from a Danish botanical garden (EUPHRESCO, 2009; Nielsen 2011, pers comm). *Capsicum annuum* has only been experimentally identified as a systemic host of TCDVd (Matsushita et al., 2009). The infected plants were symptomless. Therefore, natural infections are highly unlikely to be detectable without performing specific molecular tests (Matsushita et al., 2009). Given that no natural TCDVd infections have yet been reported in *Capsicum* species, it is assumed that TCDVd is not currently associated with imported *Capsicum* seeds.

4.1.1.6 Plant associations

This section describes natural hosts of TCDVd. Other hosts have been experimentally identified, but these are discussed in the Biology section.

Solanaceous hosts:
The most predominant natural host reports are for:

Petunia hybrida (petunia); symptomless; an annual plant (Verhoeven et al., 2007)
Solanum lycopersicum (tomato); symptomatic (Singh et al., 1999)

Other solanaceous natural hosts are:

Calibrachoa species; short-lived perennials (EUPHRESCO, 2009)
Brugmansia sanguinea; a perennial plant (Verhoeven, 2010).

Non solanaceous hosts:

Vinca minor (periwinkle); symptomless; a perennial plant (Singh & Dilworth, 2009).
Pittosporum tobira; a perennial plant (Verhoeven, 2010)
Verbena x *hybrida;* an annual ornamental plant (Singh et al., 2006); naturalised in New Zealand (PPIN, 2011).

4.1.1.7 Potential for establishment and impact

TCDVd is found in countries with similar tomato growing conditions as New Zealand, e.g. tomato production systems in both glass-house crops and outdoors. Therefore, TCDVd could potentially establish in the New Zealand under local conditions. The viroid causes damage to infected tomato plants, and possibly other infected species, and so can potentially cause unwanted impacts in New Zealand.

4.1.1.8 Hazard identification conclusion

Given that TCDVd

- Is associated with tomato seed, but there is no evidence of it being associated with capsicum seed;

- Is present in countries that export tomato and capsicum seeds to New Zealand;
- Is not recorded from NZ;
- Can potentially establish in New Zealand;
- Causes damage to infected tomato plants, and so can potentially cause unwanted impacts in New Zealand;

Tomato chlorotic dwarf viroid is therefore considered a hazard on tomato seed in this risk analysis. It is not considered a hazard on imported *Capsicum* seed[10].

4.1.2 Risk assessment

4.1.2.1 Biology

Host plants

In addition to the naturally occurring host species (refer to Plant Associations above) other plants have been experimentally identified as having the ability to be systemic hosts.

Twenty two solanaceous species have been experimentally host tested; 95% tested positive11 but only one was symptomatic (*S. tuberosum*, potato). Most species were ornamentals, but the host list did include the vegetable crop species *Solanum tuberosum* (potato) and *Capsicum anuum* (capsicum) (Matsushita et al., 2009; Singh et al., 1999).

As experimental transmission to hosts was achieved using methods that do not occur in nature, there is uncertainty about whether the experimentally identified host species are likely to become infected with the viroid in a natural environment. For this reason it is common in risk analyses to exclude experimental hosts from host lists. However, as TCDVd has only recently been described and is closely related to PSTVd, it is assumed that TCDVd has capability in nature to infect PSTVd natural hosts. Therefore, any experimental hosts of TCDVd (Matsushita et al 2009) that are natural hosts of PSTVd (EFSA Panel on Plant Health, 2011) will be considered as hosts of TCDVd in this risk analysis. These hosts are:

Solanum tuberosum (potato);
Capsicum annuum, (capsicum);
Datura stramonium (weedy ground cover plant; naturalised in New Zealand (Landcare NZ plants database, 2011));
Chrysanthemum coronarium (an annual leafy herb; naturalised in New Zealand (Landcare NZ plants database, 2011));

Symptoms of TCDVd infection

Tomato: Commercially grown tomato plants infected with TCDVd have chlorotic leaves, reduced leaf size, smaller and sometimes deformed fruit, veinal and petiolar necrosis, and overall bunchiness and dwarfing (Sing et al., 1999; Singh & Dilworth 2009; Matsushita et al., 2008). Whilst there are no reports of infected tomato plants being symptomless, this does not necessarily mean that

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10 However, as discussed in the biology and establishment sections, as the capsicum plant can be infected with the viroid via mechanical means in an experimental setting it is considered a potential host plant in New Zealand.

11 Capsicum anuum, Datura stramonium, Nicandra physaloides, Nicotiana benthamiana, N.clevelandii, N. debneyi, N. glutinosa, N. occidentalis, N. physaloides, N. rustica, N. tabacum cv. *Samsun, N. tabacum Xanthi-nc, Physalis angulata, P. floridana, Scopolia sinensis, Solanum carolinense, S. demissum, S. melongena, S. mammosum, S. nigrum, S. tuberosum* symptomless infections do not occur. In this risk analysis it is assumed that there is likely to be a proportion of infected tomato plants that are symptomless, given that the related pospiviroid PSTVd can occur symptomless in tomato plants.

Potato: Under experimental conditions potato plants had symptoms of severe dwarfing and upright growth; severe cracking of potato tubers (Singh et al., 1999).

Capsicum annuum and other plants: Most other species infected by TCDVd (either experimentally or naturally) are symptomless, including *Capsicum annuum* (Matsushita et al., 2009). Petunia plants infected with TCDVd are symptomless (Verhoeven et al., 2007; James et al., 2008).

Transmission

TCDVd can be transmitted in four ways: mechanically, via seed, via insect transmission, and via vegetative propagation (e.g. cuttings and tubers).

Seed-transmission: TCDVd can be transmitted by tomato seed, but the rate of seed-to-seedling transmission[12] is highly uncertain, given that extreme variations of transmission rate have been observed (0 to 80%) (Singh & Dilworth, 2009; Matsushita et al., 2011). Due to conflicting evidence, it is unclear whether TCDVd occurs as a contaminant on the surface of the seed coat[13], or whether it is incorporated systemically within seed tissue[14].

Vegetative propagation: Once an infection is established in a plant, it persists; consequently the new plants that are propagated from an infected source also become infected; e.g. via tubers (Singh et al., 2010).

Mechanical transmission: TCDVd is easily transmitted and spread mechanically, e.g. by contact with contaminated tools and fingers during routine crop management such as when removing lateral shoots (Singh & Dilworth, 2009; Matsushita et al., 2009). Symptoms appear by 3-4 weeks after mechanical inoculation of tomato seedlings in an experimental setting (Matsushita et al., 2008).

Bumblebee transmission: A glasshouse-based experiment has demonstrated that bumblebees (*Bombus ignitus*) transmit the viroid between tomato plants via their pollination activities, with a transmission efficiency ranging from 20-50% (Matsuura et al 2010a). Bumblebee transmission of another Pospiviroid, *Tomato apical stunt viroid* (TASVd), between tomato plants is also recorded (Antignus et al., 2007). The specific mechanism of the transmission by bumblebees is yet to be determined, but evidence from Matsuura et al. (2010a) suggests that TCDVd is mechanically transmitted when the bumblebees grasp the anthers with their mandibles. This mechanism would enable trans-species transmission of TCDVd by bumblebees. Transmission through viroid-contaminated pollen carried by bumblebees is another possibility to consider, but no TCDVd data exists for this mechanism.

12 The 'rate of seed-to-seedling transmission' is the proportion of infected seedlings that arise from a batch of seeds.
13 Evidence from Matsushita et al (2011) suggests that TCDVd is a surface contaminant. TCDVd was not detected in ovules in the samples tested with in-situ hybridisation. Number of samples tested not known.
14 Evidence from Singh & Dilworth (2009) suggests that TCDVd is not a surface contaminant, but rather occurs within the seed. Treatment with hypochlorite was unable to reduce seed-to-seedling transmission rate.

In this risk analysis, it is assumed that bumblebees can transmit the viroid in field conditions at the same transmission efficiency as was demonstrated experimentally, and that the approximate foraging range per hive is 2000 m^2 (Matsuura et al., 2010a).

No further studies of insect transmission of TCDVd were found. Transmission of the related viroid PSTVd, from infected plants to non-infected plants, was not demonstrated with honeybees (*Apis mellifera*) or the thrips species *Frankliniella occidentalis* and *Thrips tabaci* (EUPHRESCO, 2009). PSTVd can be transmitted by aphids, but it requires encapsidation in potato leaf roll virus (Querci et al., 1997).

Viroid stability:
TCDVd has strong resistance to heating and drying; the thermal inactivation time is 40 min at 100°C, and infectivity persists throughout a 50 day test period after drying (Matsushita et al., 2009).

4.1.2.2 Entry assessment - TCDVd on tomato seed

TCDVd is associated with tomato seeds. There is conflicting evidence about whether the viroid occurs as a contaminant on the surface of the seed, or whether it occurs within the seed tissue.

Not all tomato seed-lots entering New Zealand can be traced to a known place of origin. Due to the nature of the seed-trade industry (described in Section 2.2), it is assumed in this risk analysis that seed-lots entering New Zealand originate from all over the world, including from countries that are known to have TCDVd in tomatoes.

It seems likely that tomato plants with obvious disease symptoms would be detected and removed from crops, thereby preventing seeds from infected fruit being incorporated into commercial seed production systems. Yet, commercial seed-lots do contain infected seeds capable of transmitting TCDVd (Candresse et al., 2010). This may occur if some infected tomatoes can be symptomless, or if the assumption that plants with symptoms are removed from seed crops, is incorrect. The prevalence of TCDVd-transmissible seed was approximately 1% in the seed lot tested by Candresse et al. (2010) study[15]. In reality, the proportion of seeds infected with TCDVd is likely to be variable to from consignment to consignment, and over time is highly uncertain.

A visual inspection of a consignment of tomato seeds will not enable detection of seeds contaminated with TCDVd.

Given that:

- TCDVd is associated with tomato seeds;
- It is assumed that tomato seed-lots entering New Zealand originate from countries known to have TCDVd;
- There is evidence that the prevalence of TCDVd-transmissible tomato seed in commercial consignments has reached 1% in the past;
- The occurrence of symptomless infections can not be ruled out;
- Infected seeds are undetectable by visual inspection at the border;

[15] Candresse et al. (2010): In a grow-out test involving 2,500 seeds from the original seed lot from which the symptomatic plants were derived, 2 of the 250 pools of 10 plants tested positive for TCDVd infection using PCR testing. These findings establish that the prevalence of TCDVd in that commercial seed-lot was approximately 1%.

In the context of millions of seeds being imported into New Zealand over time, the likelihood of tomato seed infected with TCDVd entering New Zealand is considered to be high; but the proportion of those seeds that are infected with TCDVd is highly uncertain.

4.1.2.3 Exposure assessment

This assessment is made on the basis that contaminated seeds have entered New Zealand. Exposure of the TCDVd to a host plant simply requires seed-to-seedling transmission to occur, i.e. that a tomato plant derived from a seed is infected.

The imported tomato seed would be planted in glasshouses or outside, and a new plant would be generated. The percentage of those plants that would be infected with TCDVd, depends on the seed-to-seedling transmission rate. There is a high degree of uncertainty about the transmission rate of TCDVd in seeds, given that reported transmission rates range from zero to 80% (Matshushita et al., 2011; Singh et al., 2009). But in this risk assessment it is assumed that up to 80% of any of the contaminated tomato seeds that enter New Zealand are likely to transmit the viroid to the seedling.

In the context of millions of seeds being planted in New Zealand over time, the likelihood of exposure of TCDVd to a host plant is considered to be high.

4.1.2.4 Assessment of establishment and spread

There are multiple establishment pathways to be considered in this section because there are four different production systems (commercial greenhouse; commercial outdoor table tomato production; outdoor, commercial field tomatoes; home-garden tomato production), multiple potential hosts of TCDVd, and four methods of viroid transmission.

This section assesses establishment on the basis that TCDVd has already been exposed to (or infected) a new host plant. TCDVd establishment in the environment is affected by the number of tomato plants exposed to (infected with) TCDVd and whether that number is above the threshold for concern. As TCDVd can readily spread within a tomato crop as a result of routine crop handling and maintenance, **even if there is only one initial exposure (infection) event, it is likely to result in tens to hundreds of additional exposures in the tomato crop**, depending on what setting the initial exposure has occurred in (i.e. potentially hundreds of exposures in a commercial greenhouse, or in a commercial outdoor crop of table tomatoes, or in an outdoor, commercial field crop; and potentially tens of exposures in a home garden setting). These numbers of exposures are considered to be above the threshold for concern.

Establishment of TCDVd in New Zealand requires the following scenarios, some of which depend on viroid transmission to other host species:

- Persistence of the infected tomato plant and/or infected crop, or;
- Contaminated seeds derived from the original host plant must generate infected new tomato plants in subsequent seasons, or;
- The viroid must be transmitted to other host species that can either persist longer than one season (e.g. perennials), or generate seeds or tubers contaminated with the viroid.

The following paragraphs will deal with these scenarios.

<u>Persistence of the infected plant or crop:</u> Once TCDVd has infected a tomato plant, the viroid persists systemically within the plant and is considered established in the environment until the plant dies. Infected plants are assumed to have a symptomless phase, and as TCDVd is easily transmitted

mechanically during routine crop handling and maintenance, it is highly likely that TCDVd will spread throughout a tomato crop before symptoms are noticed. This has already occurred in Canada (Singh et al., 1999), Japan (Matsushita et al., 2008) and France (Candresse et al., 2007).

However, tomato plants are annuals so they die after fruiting at the end of the season; or in the case of a commercially grown plant, the plant is destroyed at the end of a tomato production season. Therefore, regardless of how many plants within a crop become infected, the viroid is unlikely to establish in New Zealand beyond one growing season, unless it has already spread to other crops, or unless the infected plant produces contaminated seeds that germinate the following season, and produce a new crop of infected plants.

Can infected seeds derived from the original host plant generate new infected tomato plants in subsequent seasons?: This could happen accidentally as a result of self-sown tomato plants growing, or as a result of seeds being deliberately kept and sown the following season.

Most commercial growers, whether they grow indoors or outdoors, are unlikely to deliberately retain seed from one season for the purposes of generating next season's plants. So any deliberate retention of tomato seeds is likely to be limited to the home gardener. It is common practice for home gardeners to retain tomato seed from their own crops and reuse them the following season. It is conceivable that the fruit selected for seed extraction happens to be infected with TCDVd without the grower knowing, particularly if the plant was asymptomatic.

Accidental production of plants from seed derived from infected plants could occur as a result of fruit drop, and the seed being left in the growing media (or soil), leading to self-sown plants. This is unlikely to occur in commercial greenhouses, commercial outdoor crops of table tomatoes, or in commercial outdoor fields of 'process' tomatoes, because of commercial horticultural practices (see section 2.3.4 for detailed reasoning). However, some home gardeners do not destroy self-sown tomato plants. Accordingly, this analysis considers that there is a low to moderate likelihood of home gardeners keeping self-sown plants, which might originate from composted tomato fruit, or from fruit that has dropped directly into the garden. The seed-to-seedling transmission rate is not 100% (it ranges widely from zero to 80%), which somewhat moderates the likelihood of establishment via self-sown seeds to a range of low to moderate.

Can the viroid establish in New Zealand by being transmitted to other host species?
As described in the Plant Associations and Biology sections (section 4.1.1.6 & 4.1.2.1), TCDVd has a number of other host plants. Many of these occur in the New Zealand horticultural settings, in NZ home gardens, or in the wild (*Capsicum annuum*[16], *Solanum tuberosum*, annuals such as *Petunia* and *Verbena*, perennials such as *Vinca minor*, and weedy *Solanum* species).

Crop management and plant maintenance practices offer an opportunity for mechanical transmission of the viroid from tomato to another species in both commercial greenhouses (e.g. those that grow both tomatoes and *C. annuum*) and in the home garden (where potatoes, capsicum, *Petunia* & *Verbena* are often grown). The opportunities for cross-species viroid transmission vary and depend on the requirements for crop/plant handling of the recipient species[17].

16 Despite the fact that imported *Capsicum* seeds are not considered likely to have TCDVd associated with them, primarily because TCDVd has not yet been detected in *Capsicum annuum*, *C. annuum* plants are nevertheless considered potential hosts of TCDVd in New Zealand, for reasons explained in the Biology section

17 Refer to 'TCDVd Appendix' at the end of this pest risk assessment for more detailed explanations.

It is uncertain whether TCDVd can be spread from tomatoes to other species by bumblebees because the mechanism of TCDVd transmission by bumblebees is not understood. This risk assessment assumes that bumblebees can transmit TCDVd trans-species; therefore tomatoes grown in any of the production systems can be a source of viroid that can be transmitted to other host species (including commercial greenhouses, as the escape of bumblebees from greenhouses can not be ruled out). But the tomato crops grown outdoors, either commercially or in home gardens, are likely to present the greatest opportunity for this, because bees can move freely from species to species in the outdoor setting, and the likelihood is considered to be low to moderate, but with moderate uncertainty.

A persistent establishment of TCDVd in the environment (i.e. sustainable beyond one season) within annual plants would require the annuals to produce contaminated seed, for the seed to be accidentally or deliberately germinated, and for seed-to-seedling transmission to occur. The likelihood of this pathway is uncertain, not least because it is unknown if seed-to-seedling transmission occurs in species other than tomato. Considering all the factors required in the pathway for establishment of TCDVd in annual plants, the likelihood is considered to range from low to moderate (see appendix for more detailed explanation).

In the case of potatoes, if a potato plant becomes infected (e.g. via bumblebees) there is potential for establishment of TCDVd in crops and the environment via infected tubers yielding plants in subsequent seasons; the likelihood of this is considered low to moderate. Conflicting factors taken into consideration are a) that tubers are expected to have obvious symptoms making them undesirable for use as seed-potatoes, and b) on the other hand, tubers often sprout in compost piles and they can be infected with TCDVd.

Establishment of TCDVd in the environment within perennials (e.g. *Brugmansia sanguinea, Vinca minor, Datura stramonium*) could occur via bumblebee transmission (with uncertainty) or by mechanical transmission (see appendix for more detailed reasoning). Considering all the factors required in the pathway for establishment of TCDVd in perennials, the likelihood is considered to range from low to moderate (see appendix for more details).

Spread of the viroid further afield could occur with the movement of cuttings from perennials; given that this can be a common means of propagating perennial species, the likelihood of this is considered to range from low to moderate. A spread mechanism with uncertain likelihood is via the foraging activities of bumblebees on weedy species or infected perennials; spread in this way is expected to be slow, and would likely require years for the viroid to move into other regions.

Given that:

- TCDVd can easily spread throughout a tomato crop; but the tomato plants are annuals and, regardless of whether the plants are infected or not, they do not persist beyond one season;
- TCDVd could establish in New Zealand via self-sown tomato seeds from infected fruit, or from deliberate seed harvesting and growing; the likelihood of TCDVd establishing in this way is considered to range from low to moderate, and is restricted to home garden situations;
- New Zealand has numerous other TCDVd host species (e.g. perennial ornamentals, potato, and weedy *Solanum* species) and there are mechanisms of spread from tomatoes to these species that could result in TCDVd persisting in the environment, with uncertain likelihood, ranging from low to moderate.
- The uncertainties in the establishment pathways relate to whether trans-species transmission of TCDVd by bumblebees occurs (and with what efficiency), and whether seed-to-seedling transmission of TCDVd occurs in species other than tomato;

- There is a low to moderate likelihood that TCDVd will spread beyond a local area of establishment.
- The production systems most likely to be a source of viroid for environmental establishment pathways are considered to be tomato crops grown in home gardens; this is followed by commercial tomato crops grown outdoors, assuming that bumblebees can transmit TCDVd trans-species.

The likelihood of TCDVd establishing and spreading within tomato crops where the initial infection occurs, and for that first season, is considered to be high.

The likelihood of TCDVd establishing in the New Zealand environment (beyond one season) is uncertain but considered to range from low to moderate, and is most likely to originate from the occurrence of TCDVd in backyard tomato crops or in outdoor commercial crops.

The likelihood of TCDVd establishing in New Zealand via transmission from commercial greenhouse tomato crops is low and dependent on bumblebees escaping from greenhouses and spreading the viroid.

The likelihood of TCDVd spreading in New Zealand beyond the initially infected area is considered to be low to moderate.

4.1.2.5 Consequence assessment

Economic consequences

Tomatoes, capsicums and potatoes are important crops in New Zealand. Domestic sales of capsicums in 2008/2010 were worth $29 million, and exports in 2010 were worth $34 million (Plant and Food Research, 2010). Domestic sales of tomatoes in 2008/2010 were worth $113 million, and exports worth $14 million (Plant and Food Research, 2010). The total retail and export value of the potato industry, including what householders and restaurants buy, what is bought by local processors and what is exported, is estimated at $382 million a year (Potatoes New Zealand, 2011).

Tomato plants infected with TCDVd have symptoms: dwarfism, chlorotic, yellowing leaves and small or deformed fruit (see section 'Symptoms of TCDVd Infection' for more detail). Therefore, TCDVd infection in tomato crops would result in financial losses due to reduced production of saleable product. Damage in tomato crops has been recorded from other countries:

- Japan: more than 3,000 of 66,000 (4.5%) tomato plants in a greenhouse had symptoms, which sequentially appeared along the plant rows corresponding with the direction of daily maintenance (Matsushita et al 2008).
- France: 20 to 25% of tomato plants within a group of greenhouse were infected with TCDVd and showed symptoms (Candresse et al, 2010).
- Canada: 2000 greenhouse tomato plants with viroid symptoms had TCDVd (Singh et al 1999). The total number of plants in the greenhouse was not reported. Losses were not reported.

It is expected that once symptoms appear in a commercial tomato crop, steps would be taken to obtain a diagnosis and prevent the further spread and impact of disease. The speed with which this is done will vary from grower to grower, depending on their crop and disease management procedures. Using the crop damage data from Japan and France as an indicator of possible losses, the prevalence of infected plants within a greenhouse will vary but could be up to 25% (as recorded in France (Candresse et al., 2010)). Some of the affected plants may still produce saleable fruit, so the production losses overall are likely to be less than 25%. The economic losses to individual growers and impact to their business will depend on the profit margins and what % loss of fruit can be sustained by the business.

No reports were found of crops of potato being infected with TCDVd. However, symptoms from experimentally infected potato include severe splitting of tubers (Singh et al., 1999), and this analysis assumes that experimental symptoms are likely to be similar if the disease occurred naturally in a crop. Crop management practices dictate that there would not be the same potential for mechanical spread of the viroid disease as occurs in tomato crops; the spread of the disease is likely to be more dependent on bumblebees. The efficiency of this is uncertain, but unlikely to occur more efficiently than within tomato crops, which was reported to be 20-50% by Matsuura et al. (2010a). Consequently, the economic impact on potato crops is likely to be less than in tomato crops.

Capsicum annuum plants experimentally infected with TCDVd are symptomless, and it is assumed that naturally infected crops of *C. annuum* would also be symptomless. Therefore, the impact of TCDVd on *C. annuum* is likely to be negligible.

The potential economic consequences within New Zealand are considered to be low to moderate for individual tomato growers, but considered to be low from a national economic perspective.

Environmental consequences

When 22 solanaceous species were experimentally host-tested, 95% were positive against TCDVd, including five *Solanum* species (Matsushita et al., 2011). This is evidence that other species of the Solanaceae family are potential hosts of TCDVd. There are three species in the Solanaceae that are native to New Zealand: *Solanum aviculare* (poroporo), *S. laciniatum* (poroporo, bullibulli) and *S. nodiflorum* (small flowered nightshade) (Landcare NZ plants database, 2011). As these particular species have not been host-tested it is uncertain whether these plants would become hosts of TCDVd. It is also unknown whether these species would be symptomatic. However, based on the large proportion of asymptomatic species identified in experimental settings, it is moderately likely that these species would be symptomless.

The potential environmental consequences within New Zealand are considered to be very low.

Human health consequences

There are no known human health consequences of TCDVd.
The potential human health consequences within New Zealand are considered to be negligible.

Socio-cultural consequences

Many New Zealanders are avid gardeners and enjoy growing their own crops of tomato, potato and *C. annuum*. *C. annuum* is unlikely to be impacted, but fruit and tubers from tomato and potato crops respectively are likely to be lower in quality and possibly quantity. This would be frustrating for growers, but is unlikely to change a way of life. Impacts are likely to vary from year to year.

For some iwi in New Zealand, the poroporo plant is an important source of food and medicine (The Encyclopedia of New Zealand, 2011). It is uncertain whether poroporo is capable of being a host of TCDVd. If it can be a host it is likely that only a portion of the poroporo population becomes infected. The consequences of infection in poroporo are likely to range from negligible to low.

The potential socio-cultural consequences within New Zealand are considered to be low.

4.1.2.6 Risk estimation

The likelihood of tomato seed infected with TCDVd entering New Zealand is considered to be high, but the proportion of infected seeds in uncertain. The likelihood of TCDVd being exposed to a host plant is considered to be high because all it requires is seed-to-seedling transmission. However, the frequency of initial exposure events is uncertain because the proportion of infected seeds is not known, and the seed-to-seedling transmission rate is uncertain. If new information showed with certainty that the seed-to-seedling transmission rate is much lower than the reported 80%, then the frequency of exposure events would be lower.

TCDVd is highly likely to establish and spread throughout a tomato crop if exposure occurs because it is easily spread mechanically during crop management activities and is also spread between tomato plants by bumblebees. This has the effect of increasing one initial exposure event to tens or hundreds more exposure events, taking the number of exposure events above the threshold of concern. However, the likelihood of TCDVd establishing beyond one growing season, (i.e. in the New Zealand environment in self-sown tomatoes and/or other species) is somewhat uncertain and could range from low to moderate. There is more than one mechanism for environmental establishment, but the highest risk mechanisms involve viroid originating from tomatoes grown in home gardens and in outdoor crops. Furthermore, much of the establishment will depend on how efficiently the viroid spreads trans-species via bumblebees, and whether seed-to-seedling transmission occurs in other species, both of which are currently unknown.

The likelihood of TCDVd spreading beyond the initial infection area is considered to be low to moderate and depends on the efficacy of spread via bumblebee activity and the movement of infected cuttings.

TCDVd is likely to have a low impact on the national economy. However, to individual growers the economic impact is likely to range from low to moderate. **Consequences for commercial tomato and capsicum growers are mostly independent of environmental establishment of TCDVd, and could arise every year simply through the importation and growing of infected seed.**

It is likely that the socio-cultural impact of TCDVd establishing in New Zealand would be low, the environmental impact is likely to be very low, and there are negligible health impacts.

See the table below for a visual summary of the risk estimation.

Risk estimation table: *Tomato chlorotic dwarf viroid* on tomato seeds from all countries				
	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry				
Exposure				
Establishment		(Environmental) ?	(Environmental) ?	(Within a crop)
Spread		(Environmental) ?	(Environmental) ?	(Within a crop)
Consequences of establishment				
Economic		?	?	
Environmental				
Socio-cultural				
Human Health				

? denotes uncertainty

In consideration of these assessments, TCDVd *is considered to be a non-negligible risk on imported tomato seeds. The risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.*

4.1.3 Risk management – efficacy of risk management options

Risk Management for all the viruses and viroids assessed is considered in Section 6.

4.1.4 References for TCDVd

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4.1.5 Appendix for TCDVd

Regarding Establishment and Spread: Detailed explanation of opportunities for transmission of TCDVd between species:

- *Capsicum anuum*: It is considered likely that *Capsicum* plants can become hosts in New Zealand via mechanical spread within commercial operations that grow both tomato and capsicum, and within some backyards. But as these are annual plants, establishment of the viroid depends on seeds being infected and used to produce next season's plants. Commercial growers are highly unlikely to retain seed from their own fruit. Some backyard growers of *C. annuum* are likely to retain capsicum seed but about it is not known whether *C. anuum* seeds transmit the viroid.

  Therefore, *the likelihood of TCDVd establishing via* C. annuum *is considered to be low and note that this pathway is likely to only occur in backyards.*

- *Solanum tuberosum* (potato): TCDVd is unlikely to be transmitted to potato plants via mechanical spread because potato crops are not handled intensively; [but note that transmission may be possible via bumblebees (with uncertain likelihood) particularly from outdoor commercial or backyard tomato plants]. This is an annual plant so establishment of the viroid depends on tubers being infected and being used as seed-potato to grow next season's crop. As potato plants have dwarfing symptoms and severe cracking of tubers, it is highly unlikely that infected potato tubers would be used as seed-potatoes. However, there remains a moderate likelihood that infected tubers may sprout in compost piles, or from gardens that were insufficiently harvested, thereby enabling establishment of the viroid.

  Taking into account the uncertain likelihood of the viroid spreading to this host in the first instance, *the likelihood of TCDVd establishing via* S. tuberosum *is considered to be low to moderate. This pathway is most likely to originate from backyard tomato crops or from outdoor commercial tomato crops, and is based on an assumption that bumblebees can transmit TCDVd trans-species.*

- Solanaceous ornamental annuals, e.g. petunia: There is a negligible likelihood of spread from commercial greenhouse tomato plants via mechanical means; but spread is possible with low to moderate likelihood from backyard tomato plants via mechanical means (plant maintenance activities); transmission/spread to other species may be possible via bumblebees (with uncertain likelihood) particularly from outdoor commercial or backyard tomato plants. These plants are annuals so establishment of the viroid depends on seeds being self-sown, or retained and used the following season, and for seed-to-seedling transmission of TCDVd to occur. This is uncertain. It is expected that gardeners are more likely to obtain their new season's ornamental annuals from a garden centre.

  The likelihood of TCDVd establishing via solanaceous ornamental annuals is considered to be low.

- Perennials (ornamental or naturalised), e.g. *Brugmansia sanguinea*, *Vinca minor*, *Datura stramonium*: There is a negligible likelihood of transmission from commercial greenhouse

tomato plants via mechanical means; but transmission may be possible via bumblebee activity (with uncertain likelihood), particularly from backyard tomato plants or outdoor commercial tomato plants. Transmission could occur with a low to moderate likelihood via mechanical means during plant handling and maintenance in home gardens. These plants persist vegetatively for more than two years, so could become reservoirs of the viroid if infected. Therefore, this is a pathway with potential for TCDVd to establish. *B. sanguinea, V. minor* and *D. stramonium* grow wild in all regions of New Zealand and are considered fully naturalised (Landcare NZ plants database, 2011).
The likelihood of TCDVd establishing via perennials is somewhat uncertain but is considered to be low to moderate. This pathway is most likely to originate from backyard tomato crops; or from outdoor commercial tomato crops, and is based on an assumption that bumblebees can transmit TCDVd trans-species.

- Weedy *Solanum* species that have fully naturalised in New Zealand and grow wild (there are at least 10 species; (Landcare NZ plants database, 2011): weedy *Solanum* plants are likely to be found in some backyards where tomatoes are grown, or nearby. As 95% of the solanaceous plants that have been experimentally host-tested (n=22) are capable of being systemic hosts of TCDVd, there remains a possibility that weedy *Solanum* species can be host plants. There is a negligible likelihood of spread to these plants via mechanical spread; but spread is possible with uncertain likelihood, particularly from backyard tomato plants or outdoor commercial tomato plants via bumblebee activity. These are annual plants so establishment of the viroid depends on seed-transmission. This is highly uncertain. Being a weedy species, the seeds are highly likely to generate new plants the next season, which would enable the viroid to persist if seed-to-seedling transmission of TCDVd occurs in these species. Taking into consideration that spread to these plants requires bumblebee transmission, (occurring with 20-50% efficiency in tomatoes), whereby a bumblebee visits an infected tomato, and then moves to one of these weedy species rather than an annual, or a non-host of TCDVd:

  the likelihood of TCDVd establishing via weedy Solanum *species is uncertain, but is considered to range from low to moderate. This pathway is most likely to originate from either backyard tomato crops or from outdoor commercial crops, and is based on an assumption that bumblebees transmit TCDVd trans-species.*

4.2 Potato spindle tuber viroid (PSTVd)

Scientific name: *Potato spindle tuber viroid*
Family/Genus: *Pospiviroidae* / *Pospiviroid*
Acronym: PSTVd

4.2.1 Hazard identification

4.2.1.1 Description

Potato spindle tuber viroid (PSTVd) is a plant pathogen that causes a disease that primarily affects potato and tomato plants. PSTVd is a small circular single stranded RNA molecule lacking a capsid (a shell of protein).

4.2.1.2 New Zealand status

PSTVd remains an unwanted organism and regulated pest under the Biosecurity Act 1993 as it is not considered established in New Zealand (MAF response close out report, 2009). PSTVd has been detected on a few occasions in New Zealand in tomato and capsicum glasshouse plants, but was eradicated each time (Ward et al., 2010). PSTVd was first detected in New Zealand in glasshouse tomatoes in 2000. A subsequent delimiting survey of 50 New Zealand tomato glasshouses in 2001 found PSTVd at a further two sites in South Auckland and one site in Nelson (Elliot et al., 2001; MAF response close out report, 2009). It has also been isolated from capsicum samples collected from a commercial glasshouse at Auckland (Lebas et al., 2005; MAF response close out report, 2009). PSTVd was found in cape gooseberry and tobacco plants in Christchurch and Auckland respectively in 2009; infected plants and seeds from the supplier were destroyed (MAF response close out report, 2009).

4.2.1.3 Geographic distribution

As at November 2011, PSTVd is found in Afghanistan, Belarus, Canada, Chile, China, Costa Rica, Czech Republic, Egypt, Germany, India, Iran, Italy, Netherlands, Nigeria, Peru, Poland, Russian Federation, Slovenia, Turkey, Ukraine, United Kingdom, USA, and Venezuela (CPC, 2010). It has also been reported from Greece (Malandraki et al., 2010), and Belgium (Verhoeven et al., 2007).

4.2.1.4 Commodity association

PSTVd is present in seed and pollen of infected tomato plants at varying rates depending on the cultivar, the presence of PSTVd in one or both of the botanical parents and the strain of PSTVd (Salazar, 1989). It can be transmitted by both seed and pollen (Singh, 1970; Lebas et al., 2005; Benson and Singh, 1964; EUPHRESCO, 2009). Capsicum seeds are considered to be the most likely source of infection of capsicum plants recorded in New Zealand (Lebas et al., 2005) therefore for the purposes of this assessment PSTVd is considered likely to be seed transmitted in capsicum.

Seed-lots of tomato and capsicum entering New Zealand are considered to originate from all over the world, including from the countries that are known to have PSTVd.

4.2.1.5 Plant associations

PSTVd is primarily a pathogen of potato and may cause significant yield losses in this crop, however, it naturally infects other solanaceous crops such as tomato (*Solanum lycopersicum*), aubergine (*Solanum melongena*), pepino (*Solanum muricatum*), capsicum (*Capsicum annuum*) (Lebas et al., 2005), and cape gooseberry (*Physalis peruviana*) (Verhoeven et al., 2009; Ward et al., 2010). PSTVd also naturally infects avocado (*Persea americana*), sweet potato (*Ipomoea batatas*) (CPC, 2010), and the ornamentals *Brugmansia suaveolens* and *Solanum jasminoides* (Verhoeven et al., 2008a) and *Streptosolen jamesonii* 'Yellow' (Verhoeven et al., 2008b). Experimentally it has been shown to infect at least 39 species in 4 families, with many of them showing few or no symptoms (Singh et al. 2003).

4.2.1.6 Potential for establishment and impact

PSTVd is found in countries with a similar climate to parts of New Zealand and can potentially establish here. Some strains can cause significant yield losses, particularly in potatoes.

4.2.1.7 Hazard identification conclusion

Given that *Potato spindle tuber viroid*

- Is associated with tomato seed and is considered to be associated with capsicum seed;
- Is present in many countries that export tomato and capsicum seed to New Zealand;
- Is not established in New Zealand;
- Can potentially establish in New Zealand;
- Can potentially cause unwanted impacts;
- Causes disease to plant species of economic importance to New Zealand, e.g. potatoes, tomatoes;

Potato spindle tuber viroid is therefore considered a hazard on tomato and capsicum seed in this risk analysis.

4.2.2 Risk assessment

4.2.2.1 Biology

Viroids are small, low molecular weight ribonucleic acids that can infect plant cells, replicate themselves, and cause disease. They are circular, single stranded RNA molecules with extensive base pairing in parts of the RNA strand (Agrios, 2005). Like all known Pospiviriods, PSTVd replicates in the nucleus of the host plant (for details see Verhoeven, 2010). PSTVd usually consists of 359 nucleotides.

Transmission

The viroid is highly contagious and is transmitted between plants by touch, either by plants rubbing against each other or people touching plants. The use of cutting or pruning tools or contaminated machinery or any form of physical contact between plants can result in disease transmission. In potatoes it is spread primarily by knives used to cut healthy and infected potato tubers used as seed-potatoes[18] and during handling and planting of the potato crop. After inoculation of a tuber with PSTVd, the viroid is replicated and spreads systemically throughout the plant (Agrios, 2005).

18 Note that these are not true botanical seeds.

PSTVd is transmitted by seed and pollen of infected tomato and potato plants (Singh, 1970; Lebas et al., 2005; Grasmick and Slack, 1986; Benson and Singh, 1964; EUPHRESCO, 2009), and likely to be seed transmitted in capsicum seeds (Lebas et al., 2005). In tomato seeds, PSTVd occurs within the seed rather than as a contaminant on the surface, as demonstrated by in-situ hybridisation (Matsushita et al., 2011). The percentage of infected seedlings, grown from seeds from plants infected with PSTVd ranged from 2 to 31% in tomato (Grasmick and Slack, 1986; Singh, 1970; Benson and Singh, 1964) and 6-12% in potato (using true potato seed) (Singh, 1970). The viroid can persist and retain viability for many years in potato seeds: when 11 seedlings of a seed lot kept in storage for 12 years were tested, 100% were shown to be infected with PSTVd (Grasmick & Slack, 1986).

PSTVd is not usually spread by insect vectors (Schumann et al., 1980; Querci et al., 1997; Verhoeven, 2010), however, PSTVd transmission by aphids (*Myzus persicae*) has been confirmed from plants co-infected *Potato leafroll virus* (PLRV; Querci et al., 1997; Syller et al., 1997; Verhoeven, 2010). Bokx and Piron (1981) reported that PSTVd could be spread by the aphid *Macrosiphum euphorbiae* without PLRV involved, but Verhoeven (2010) states that this needs confirmation.

Although other viroids (*Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid*) and the virus *Pepino mosaic virus* can be transmitted by bumblebees, there are no reports of PSTVd being transmitted by bumblebees, despite the fact that PSTVd is a comparatively well-studied viroid.

Symptomatology

Tomato: Symptoms of PSTVd infection in tomato plants can range from no symptoms to severe symptoms of purpling and chlorosis of leaves, shortening of the leaf internodes, leaf epinasty, thickening and brittleness, and occasional plant death. As stunting begins, fruit may be dark green or flowers may abort and fruit initiation stopped (Owens and Verhoeven, 2009). Symptomless plants have been reported from infected seed, but plants mechanically inoculated or pollen-infected have not been reported as symptomless (Kryczynski et al., 1988).

Capsicum: Capsicums have been recorded showing only mild symptoms, a slight waviness of the leaf edge (Lebas et al., 2005). However, Verhoeven et al. (2009) experimentally inoculated capsicum plants with PSTVd and recorded that maximum fruit size was reduced.

Potato: PSTVd attacks all varieties of potatoes and spreads rapidly (Agrios, 2005). Symptoms depend on strain, variety and environment and can vary from severe to mild and symptomless expression (EPPO, 2004). Infected potato plants showing symptoms appear erect, spindly, and dwarfed. The leaves are small and erect, and the leaflets are darker green and sometimes show rolling and twisting. The tubers are elongated, with tapering ends. Tubers are smoother, but tuber eyes are more numerous and more conspicuous (Agrios, 2005). Tubers can be cracked (Singh et al., 1971).

Ornamentals: Infection in ornamentals appears to be symptomless, e.g. in *Brugmansia suaveolens* and *Solanum jasminoides* (Verhoeven, 2010).

4.2.2.2 Entry assessment

This organism can enter New Zealand with imports of tomato or capsicum seed. It has been demonstrated to be seed transmitted in tomatoes (Singh, 1970; Kryczynski et al., 1988) and is likely to be transmitted by capsicum seed (Lebas et al., 2005). It seems unlikely that obviously symptomatic plants would be used for high value commercial seed. But some infected tomato plants can be symptomless, so infected seeds are likely to be unknowingly included in commercial seed lots.

Overall, the proportion of imported seeds that are infected with PSTVd is likely to be low. However, large volumes of seed are imported and infected seeds are likely to have been the source of the infected tomato and capsicum plants previously detected in New Zealand (Elliot et al., 2001; Lebas et al., 2005; Ward et al., 2010). Furthermore, seed-lots entering New Zealand are considered to originate from all over the world, including from countries that are known to have PSTVd in tomatoes. In 2011, PSTVd was detected in seed consignments from China, Kenya, the Netherlands and USA (EPPO, 2011).

As a visual inspection of a consignment of tomato or capsicum seeds will not enable detection of contaminated seeds, it is considered highly likely that if tomato or capsicum seeds exported to New Zealand are infected with PSTVd, then PSTVd will enter New Zealand.

In the context of millions of seeds being imported into New Zealand over time, the likelihood of PSTVd entering New Zealand on tomato or capsicum seed is considered to be high; but the proportion of those seeds that are infected with PSTVd is highly uncertain.

4.2.2.3 Exposure assessment

This assessment is made on the basis that contaminated seeds have entered New Zealand. Imported seeds for sowing would be planted in glasshouses or outside. PSTVd is transmitted from seeds to seedlings. The percentage of infected seedlings, grown from seeds from plants infected with PSTVd ranged from 2 to 31% in tomato. It is not known if all of the seeds that came from the infected plant were themselves infected with PSTVd. But, it is certain that a proportion of plants growing from infected seeds are likely to be infected because the viroid is transmitted from seeds to seedlings.

In the context of millions of seeds being planted in New Zealand over time, the likelihood of PSTVd exposure to a tomato seedling or a capsicum seedling is considered to be high.

4.2.2.4 Assessment of establishment and spread

As described in section 2.3.2.1, there is management of PSTVd in place on some pathways. However, this risk assessment considers the risk in the absence of current management for reasons explained elsewhere.[19] The efficacy of the current PSTVd management practices are examined in the management section, section 6.

This section assesses establishment on the basis that PSTVd has already been exposed to (infected) a new host plant. PSTVd establishment in the environment is affected by the number of tomato or capsicum plants exposed to (infected with) PSTVd and whether that number is above the threshold for concern. As PSTVd can readily spread within a tomato or capsicum crop as a result of routine

[19] This approach to risk assessment is used because it ensures that all relevant risks are encapsulated in the assessment, rather than just those that remain after a particular risk management measure is in place. This approach ensures that the risk assessment does not become obsolete when changes are made to risk management measures. See Section 2.3.2.1 for further explanation of this approach.

crop handling and maintenance, **even if there is only one initial exposure (infection) event, it is likely to result in tens to hundreds of additional exposures in the tomato/capsicum crop**. These numbers of exposures are considered to be above the threshold for concern.

For the viroid to establish in New Zealand, infection needs to persist in the plant and the plant needs to remain living. There is no evidence to suggest that infection does not persist in the plant, and the only way to get rid of the infection is to destroy the plant. Occasionally infected tomato plants die from the infection (Agrios, 2005) but this is not usual.

For establishment to last beyond the first season either

- The infected plant must persist; or
- Seeds from the infected plant must be used to produce new infected plants; or
- Infection must spread to other host plants that persist or can spread the infection further.

Spread of PSTVd within tomato and capsicum crops, and the possibility of the infected plant persisting beyond one season

Once PSTVd has infected a plant, it persists systemically within the plant, until the plant dies.

The viroid is highly likely to spread throughout a tomato crop or capsicum crop, via mechanical transmission, as a result of routine crop handling procedures and from direct plant-to-plant contact. Infected tomatoes and capsicums grown from infected seed can be symptomless (Kryczynski et al., 1988) or infected tomatoes can show symptoms late in plant development (Lebas et al., 2005), meaning that PSTVd could easily spread throughout a glasshouse before symptoms are observed (Lebas et al., 2005). There is also a low likelihood that infection could spread from one infected glasshouse to another glasshouse by mechanical transmission by workers.

There is a small possibility that PSTVd could also be spread throughout an indoor or outdoor tomato crop by aphids (and only if the original host plant is also infected with PLRV) – refer to the PSTVd appendix for details of this mechanism. However, in New Zealand aphid transmission is unlikely to be a major contributor to the spread of PSTVd within tomato crops when compared to the ease with which it is spread mechanically.

Although PSTVd can easily spread through a tomato or capsicum crop, tomato and capsicum plants are annuals so they die after fruiting; or in the case of plants grown for commercial production of tomato and capsicum fruit, the plant is destroyed at the end of a tomato or capsicum production season. It is expected that this will lead to elimination of PSTVd from glasshouses in most cases, but there have been reports of the viroid reoccurring due to ineffective glasshouse sanitisation at the end of the previous season (L. Ward pers comm. 2011; Owens and Verhoeven 2009).

Apart from occasional re-occurrence of infection in glasshouses, it is not possible for the viroid to establish in New Zealand beyond one growing season, unless it has already spread to other crops, or unless seed from infected plants are germinated the following season, and yield a new crop of infected plants.

Seeds from the infected plant being used to produce new infected plants

<u>Accidentally[20] vs intentionally germinated plants.</u>

Most commercial growers of tomato and capsicum plants, whether they grow indoor or outdoors, are unlikely to intentionally retain seed from one season for the purposes of generating next season's

[20] Plants that grow on their own after the previous season.

plants. However, some home gardeners do collect seed for use for the following year, and share it with others. Seeds collected from infected plants could be moved around the country and provide a source of new PSTVd infections. Seed transmission rates of tomato seed have been recorded at 2-31% (Singh, 1970). PSTVd has been recorded inside the seed of tomatoes (Matsushita et al 2011). While most seed from infected tomato plants will not be kept because the plants are likely to show obvious symptoms of infection, some infected tomato plants can be symptomless, and infections in capsicum show only mild symptoms which could be missed.

Accidental production of plants from seed derived from infected plants could occur as a result of fruit drop and the seed being left in the growing media (or soil), leading to self-sown plants. This is unlikely to occur in commercial greenhouses, commercial outdoor crops of tomato table tomatoes, or in commercial outdoor fields of ‘process’ tomatoes, because of commercial horticultural practices (see background information and assumptions for detailed reasoning). However, it is considered that there is a low to moderate likelihood of home gardeners keeping self-sown plants, which might originate from composted tomato fruit, or from fruit that has dropped directly into the garden.

In summary, there are opportunities in home gardens for seeds from the originally PSTVd-infected tomato plant to be used to produce newly infected tomato plants, both intentionally or accidentally. It is considered that there is a low to moderate likelihood that PSTVd can establish in the New Zealand environment via progeny seed.

Spread of PSTVd to other plant species

<u>Methods of spread</u>

PSTVd can spread to other species in two ways: mechanically (by touch) or by aphids when the plant is co-infected with PSTVd and PLRV (Verhoeven, 2010), [or possibly *Tomato yellow top virus* (TYTV), a strain of PLRV which occurs in New Zealand. (J D Fletcher, pers comm. 2011)].

<u>Spread by aphid vectors</u>

As there are low levels of incidence of the viruses TYTV and PLRV[21], which are necessary as co-infections for aphid transmission of PSTVd to be effective, and as tomato is a poor host of PLRV it seems there is a low likelihood of aphids finding a plant infected with both PSTVd and PLRV or TYTV, then moving to a new plant and infecting it.

<u>Local spread:</u>

Spread from crops of tomatoes and capsicums to potatoes: In the Hawkes Bay area potatoes and tomatoes are grown in neighbouring fields. PSTVd can spread between species either mechanically or by vector transmission. It is unlikely that people working in outdoor capsicum or tomato crops would also be working in neighbouring potato crops, thereby the likelihood of mechanically spreading the viroid between these crops is low. Aphid transmission of PSTVd requires the plant to be co-infected with PLRV. While tomato and capsicum have been recorded as hosts of PLRV, in New Zealand the prevalence of PLRV in outdoor tomato crops is thought to be around 4% and in potatoes around 1-2%. So, although there is a possibility of aphid transmission between crop species, the likelihood is considered to be low.

However, in the home garden, there is greater potential for PSTVd to be spread from tomato and capsicum crops to potato crops as a result of crop handling. As the viroid can occur in potatoes without symptoms, the viroid could unknowlingly be spread further afield when home gardeners share infected tubers for the purpose of ‘seeding’ next seasons crop. The frequency of home

[21] Refer to the Appendix for PSTVd at the end of section 4.2 for further details about aphid transmission of PSTVd.

gardeners using their own potatoes as ‘seed’ is unknown, but is considered likely to contribute to the PSTVd spreading. Overall, the likelihood of PSTVd establishing in potato crops in home gardens and being spread further afield is considered to be low to moderate.

Spread to and from ornamentals: Ornamentals such as *Brugmansia suaveolens* and *Solanum jasminoides*, which are grown in gardens as well as being naturalised species in New Zealand (PPIN, 2011), can be infected with PSTVd without showing symptoms (Verhoeven, 2010). There is a very low likelihood that PSTVd infection could be spread from infected tomato or capsicum plants from glasshouses, or from tomato plants growing in fields, to ornamental plants (growing inside glasshouses, in fields or in gardens), as it is most likely to rely on transmission via aphids. However, there is greater potential for PSTVd to be spread to these species in home gardens, as a result of mechanical transmission with contaminated tools. If PSTVd is spread to these species, these plants could become a reservoir of PSTVd in the environment. However, the likelihood of the viroid being transmitted from these species back into tomato and or capsicum plants is considered to be low.

<u>Long distance spread:</u>
This could occur through the movement of infected seed or symptomless plant material, e.g. potato tubers or solanaceous or ornamental plants distributed via commercial nurseries/garden centres or by home gardeners; often infected plants show no symptoms which means they are more likely to be moved to new areas.

Fruit from infected symptomless tomato or capsicum plants may be sold for consumption, and what isn’t consumed may be discarded. There is a possibility of spread this way, but the likelihood is low.

Given that:

- PSTVd in capsicums or tomatoes could spread through a crop in a single season, but the plants die at the end of the season;
- PSTVd is unlikely to spread beyond the commercial glasshouse to other host species where it will survive several seasons;
- PSTVd can be spread by aphid vectors in the presence of PLRV, but in New Zealand the likelihood of this resulting in spread of PSTVd to other plant species is considered to be low;
- The likelihood of PSTVd establishing in commercial potato crops is considered to be low, but in home gardens likelihood is considered to be low to moderate.
- PSTVd could establish in New Zealand via self-sown tomato seeds from infected fruit, or from deliberate seed harvesting and growing; the likelihood of PSTVd establishing in this way is considered to range from low to moderate, and is restricted to home garden situations;
- There is a low to moderate likelihood that PSTVd could be spread further afield from infected plant material being distributed by home gardeners, or by garden centres.

The likelihood of PSTVd establishing and spreading within tomato and capsicum where the initial infection occurs, and for that first season, is considered to be high.

The likelihood of PSTVd establishing in the New Zealand environment (beyond one season) is uncertain but considered to range from low to moderate, and is most likely to originate from the occurrence of PSTVd in home garden crops of tomato or capsicum.

The likelihood of PSTVd establishing in New Zealand via transmission from any commercial tomato or capsicum crop is considered to be low as it is considered to be dependent on spread by aphids (and only if the original host is also infected with PLRV).

The likelihood of PSTVd spreading in New Zealand beyond the initially infected area is considered to be low to moderate if that initial area is a home garden.

4.2.2.5 Consequence assessment

Economic consequences

Tomatoes and capsicums and potatoes are all important crops in New Zealand. There is a possibility that PSTVd could spread to potatoes, but the likelihood is very low based on the need for PLRV to be present in a tomato crop for PSTVd to be transmissible by aphids.

<u>Tomato crops:</u> Domestic sales of tomatoes in 2008/2010 were worth $113 million, and exports worth $14 million (Plant and Food Research, 2010). If PSTVd did establish in New Zealand crops, financial losses due to PSTVd are likely to be greater in potato and tomato crops. Reports of the significance of PSTVd on tomato are contradictory. PSTVd has been described as of little economic importance to that crop (Agrios, 2005), but yield losses of 43 to 80% have been reported (Kryczynski et al., 1995). Yield loss in tomatoes has been reported as significant, due to reduced fruit size (Ling and Sfetcu, 2010), and flowers can abort resulting in no fruit, and the plant can be totally malformed in severe cases.

<u>Capsicum crops:</u> Domestic sales of capsicums in 2008/2010 were worth $29 million, and exports in 2010 were worth $34 million (Plant and Food Research, 2010). Capsicums display only very mild symptoms in response to PSTVd infection and are unlikely to have much yield loss. The only symptom recorded from naturally infected capsicums has been a certain "waviness" or distortion of the leaf margins near the top of infected plants (Lebas et al., 2005; Owens and Verhoeven, 2009). Artificially infected capsicums have been recorded to have reduced fruit size (Verhoeven et al., 2009). However, there may be financial losses due to the costs of testing for and removing asymptomatic infected plants. and these costs can be considerable. In the Netherlands in 2006-2007 ornamental plants infected with PSTVd were traced (costing the government 700,000 Euros) and destroyed (costing industry 3 to 5 million Euros) (Verhoeven, 2010). The plants did not show any noticeable symptoms, but were destroyed to prevent infection spreading to tomato and potato crops.

<u>Potato crops:</u> The total retail and export value of the potato industry, including what householders and restaurants buy, what is bought by local processors and what is exported, is estimated at $382 million a year (Potatoes New Zealand, 2011). PSTVd symptoms on potato tubers vary from severe to mild and symptomless. The tubers can be elongated, with tapering ends (Agrios, 2005); they can have more numerous and more conspicuous eyes; they can also be cracked (Singh et al., 1971). There is uncertainty about the impact of PSTVd on potato crops under current agricultural conditions. Many of the yield-loss reports are prior to 1970, and impacts in those reports were confounded by the association of PSTVd with other potato viruses (EFSA, 2011). Further, they were recorded under conditions that are unlikely to be comparable to current agricultural practices in New Zealand. The most recent crop-loss report is from China in 1992; there was a 20-30% yield loss (Cui et al., 1992; cited in EFSA, 2011).

<u>Other crops:</u> Avocado is a host of PSTVd but it is symptomless (Owens and Verhoeven, 2009), so, as with capsicum, the main costs associated with PSTVd infection of avocado trees or seedlings

would be costs of testing for the disease and removing infected plants. Similarly, sweet potato (*Ipomoea batatas*) has been recorded as a host of PSTVd with no symptoms observed (Salazar, 1989).

If PSTVd infection of crops is detected early, there may only be a localised impact and the associated costs may not be too great. However if PSTVd infection was not detected immediately, which is likely as some infected plants are symptomless, infection may spread to a wider area, increasing costs of detection and eradication and possible yield losses.

The potential economic consequences within New Zealand are considered to be low to moderate (but could be moderate to high for individual crop producers).

Environmental consequences

No records have been found of native New Zealand species infected by PSTVd. Some of the known hosts are in the families Solanaceae, Lauraceae, and Convolvulaceae. Experimentally it has been shown to infect at least 39 species in the families Amaranthaceae, Campanulaceae, Compositae and Solanaceae (Singh et al. 2003). There are New Zealand native species in the Solanaceae (e.g. poroporo), the Lauraceae (e.g. Taraire, Tawa), and the Convolvulaceae (some native bindweed species) (NZ Plants Database 2012). Amaranthaceae, Campanulaceae and Compositae have endemic species (NZ Plants Database 2012). The potential impact to native plants is currently unknown, but it is unlikely that native plants will become infected because there would need to be mechanical transmission from infected plants (unless the aphid and PLRV could also infect them, but this has a low likelihood as levels of PLRV are low in New Zealand).

The potential environmental consequences within New Zealand are considered to be low.

Human health consequences

There are no known human health consequences.

Socio-cultural consequences

PSTVd has been reported from sweet potato or kumara (*Ipomoea batatas*) but with no symptoms (Salazar, 1989). Sweet potato is a crop of importance to Maori. PSTVd shows no symptoms on this crop, however infected plants may have to be removed so that they do not act as a source of infection for potato and tomato plants. Poroporo has an edible fruit eaten by Maori. There is a very low probability that it could be infected with PSTVd, and plants could be stunted or yield of berries could be reduced, but there is very high uncertainty about this. Therefore this disease is likely to have a low impact on Maori.

Home gardeners grow potatoes, tomatoes, capsicums, cape gooseberry and ornamentals. PSTVd infected plants may lead to decreased yield for home gardeners or lead to them having to destroy their plants to try to remove the infection and stop it spreading.

The potential socio-cultural consequences within New Zealand are considered to be low.

4.2.2.6 Risk estimation

The likelihood of PSTVd entering New Zealand on tomato or capsicum seed is considered to be high; but the proportion of infected seeds is uncertain. The likelihood of exposure is high. The likelihood of PSTVd establishing and spreading within tomato and capsicum crops within a single season is considered to be high. The likelihood of PSTVd establishing in the New Zealand environment (beyond one season) is uncertain but considered to range from low to moderate, and is most likely to originate from the occurrence of PSTVd in home garden crops of tomato or capsicum. The likelihood of PSTVd establishing in New Zealand via transmission from any commercial tomato or capsicum crop is considered to be low as it is considered to be dependent on spread by aphids (and only if the original host is also infected with PLRV). The likelihood of PSTVd spreading in New Zealand beyond the initially infected area is considered to be low to moderate if that initial area is a home garden.

The potential economic consequences are considered to range from low to moderate. The potential environmental consequences are low, the potential health consequences are negligible, and the potential socio-cultural consequences are moderate.

Consequences for commercial growers are mostly independent of environmental establishment of PSTVd, and could arise every year simply through the importation and growing of infected seed.

Risk estimation table: *Potato spindle tuber viroid* on tomato and capsicum seed from all countries				
	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry				
Exposure				
Establishment		(Environmental) ?	(Environmental) ?	(Within a crop)
Spread		(Environmental) ?	(Environmental) ?	(Within a crop)
Consequences of establishment				
Economic		?	?	
Environmental				
Socio-cultural				
Human Health				

In consideration of these assessments, PSTVd is considered to be a non-negligible risk on both imported tomato and imported capsicum seed. The risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.

4.2.3 Risk management– efficacy of risk management options

Risk Management for all the viruses and viroids assessed is considered in section 6.

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4.2.5 Appendix for PSTVd

Discussion about spread of PSTVd by aphids

PSTVd can be spread by aphids when the plant is co-infected with PSTVd and PLRV. PSTVd RNA becomes encapsidated by the coat protein of PLRV, thereby enabling the viroid to escape digestion in the gut of the aphid (Querci et al., 1997; Verhoeven, 2010). Although PLRV infects tomato plants (Makovcova et al., 1975; Hassan et al., 1985; Hassan et al., 1995)), tomato is a poor host of PLRV (Syller et al., 1997; Syller and Marczewski, 2001), and symptoms can be only weak or non-existent (Syller et al., 1997). PLRV also infects capsicum (Sertkaya and Sertkaya, 2005) in other countries.

PLRV is present in New Zealand, recorded on potato and tomato (Pearson et al., 2006) and is transmitted by aphids (which are present in New Zealand). PLRV has not been recorded in capsicum crops in New Zealand, but since it is known to infect capsicum (Hanafi et al., 1995), in this risk analysis it is assumed to be in capsicum crops in New Zealand. Tomato plants in New Zealand fields and glasshouses can be infected with the *Tomato yellow top virus* (TYTV) strain of PLRV, and its capsid protein may possibly encapsidate PSTVd in plants co-infected with PSTVd and TYTV in glasshouses (pers comm. John Fletcher) which would prevent the PSTVd being digested by the aphid and allow the aphid to spread PSTVd. In glasshouse environments if PSTVd and TYTV were both present at high levels and there was a high level of infestation of the aphids then there may be the distinct possibility of vectoring PSTVd (pers comm. John Fletcher). However, current evidence suggests that the prevalence of TYRV and PLRV is low:

- TYTV prevalence: It is not known how common TYTV is in glasshouse tomatoes in New Zealand, but when process tomato plants showing symptoms of virus infection were tested in Hawkes Bay and Poverty Bay, 4% of those showing symptoms were infected with TYTV (Tate et al., 1991).
- PLRV prevalence: Fletcher (2011) surveyed potato viruses in thirty five New Zealand crops in 2010-2011. PLRV was present in 4 process and one fresh potato crop, and not detected in surveyed seed potato crops. The incidences recorded were 1-2%. It is thought that the effective use of systemic insecticides for a number of years has contributed to the low prevalence of PLRV in potatoes because the aphid vectors were kept at low levels.

4.3 Tomato apical stunt viroid (TASVd)

Scientific name: *Tomato apical stunt viroid*
Family/Genus: *Pospiviroidae/Pospiviroid*
Acronym: TASVd

4.3.1 Hazard identification

4.3.1.1 Description

Tomato apical stunt viroid (TASVd) is a plant pathogen that causes disease in tomatoes. TASVd is a small, naked, circular, highly structured single-stranded RNA molecule, lacking a capsid (a shell of protein).

4.3.1.2 New Zealand status

TASVd is not known to be present in New Zealand. Not recorded in Pearson *et al*. (2006), or PPIN (2011).

4.3.1.3 Geographic distribution

As at November 2011, TASVd has a wide geographical distribution as there are records of it in Europe, Middle East, Africa, SE Asia, and the USA (EPPO 2010): TASVd is recorded from Austria (Grausgruber-Gröger and Gottsberger, 2011), Belgium (Verhoeven et al., 2008a), Italy (Luigi et al., 2011), Finland, Germany, the Netherlands, Indonesia, Israel, Ivory Coast, Senegal, and Tunisia (CABI/EPPO, 2008; EPPO, 2011). Its presence in some countries has only been reported recently, and it is likely to be present in other countries where it has not yet been discovered.

4.3.1.4 Commodity association

TASVd is present in tomato seed and is known to be seed transmitted to its seedlings (Antignus et al., 2007). TASVd was intercepted in 2010 on a consignment of tomato seeds exported from the USA (EPPO, 2010).

TASVd is not known to be associated with capsicum seeds.

4.3.1.5 Plant associations

Natural hosts include *Solanum lycopersicon* (tomato) (Antignus et al., 2007), *S. jasminoides* (Verhoeven et al., 2008a), *Brugmansia* sp. (Olivier et al., 2011), *Cestrum* sp. (Verhoeven et al., 2008b), *Lycianthes rantonnetii* and *Streptosolen jamesonii* (Verhoeven et al., 2010). Some of these hosts have only been recorded recently, and it is likely that there are more undiscovered hosts.

Experimentally, TASVd can be transmitted to potato (*S. tuberosum*) (Verhoeven and Roenhorst, 2010).

4.3.1.6 Potential for establishment and impact

TASVd is found in countries with similar climatic and tomato growing conditions to New Zealand, e.g. climate and tomato production systems in both glass-house crops and outdoors. Therefore, the viroid could potentially establish in New Zealand. TASVd is likely to have unwanted impacts if it becomes established here as some strains are known to cause severe symptoms and crop losses in tomato (Antignus et al., 2002).

4.3.1.7 Hazard identification conclusion

Given that *Tomato apical stunt viroid*

- Is associated with tomato seed;
- Is not established in New Zealand;
- Could potentially establish in New Zealand;
- Can potentially cause unwanted impacts;
- Is present in a country that exports tomato seed to New Zealand;
- Is recorded causing a disease to a plant species of economic importance to New Zealand

Tomato apical stunt viroid is therefore considered a hazard on tomato seed in this risk analysis. It is not considered a hazard on imported *Capsicum* seed.

4.3.2 Risk assessment

4.3.2.1 Biology

TASVd has a circular, single-stranded, low molecular weight ribonucleic acid (RNA) of 360 to 363 nucleotides depending on the strain, that can infect plant cells, replicate themselves, and cause disease(Antignus et al., 2002). Viroid RNA molecules have extensive base pairing in parts of the RNA strand (Agrios, 2005) making them highly structured. They lack capsid protein and detectable messenger RNA activity (Antignus et al., 2002 and 2007). Pospiviroids including TASVd replicate in the nucleus of their host plant (Verhoeven, 2010).

<u>Host symptoms</u>
Leaves and the whole above ground parts of the plant are affected, including the fruits. The symptoms of TASVd in tomatoes include stunting, apical proliferation, apical leaf narrowing and yellowing, leaf crinkling, tissue brittleness, and necrosis (Antignus et al., 2002 and 2007). The fruits can be considerably reduced in size and have pale red discolouration (Antignus et al., 2002).

There are several strains of TASVd:

- TASVd was first found in Ivory Coast in tomato plants grown in small gardens, sometimes referred to as TASVd-Ivory Coast, and this is considered the type-strain of this viroid (Antignus et al., 2002 and 2007);
- A strain eliciting severe symptoms in tomato was identified in Indonesia, this strain shared 91.5% identity with the nucleotide sequence of the Ivory Coast strain;
- A third distinct strain, TASVd-S, was isolated from the ornamental plant *Solanum pseudocapsicum* and shared 92.1% sequence identity with the Ivory Coast strain. TASVd-S has not been found on tomato in nature but tomato plants can be infected experimentally by mechanical inoculation (Antignus et al., 2002 and 2007).
- Another TASVd isolate, TASVd-Is, was identified in Israel and shared 92%, 99%, and 87% sequence identity with the Ivory Coast strain, the Indonesian strain, and TASVd-S, respectively. TASVd-Is, has been found consistently in glasshouse-tomato crops in Israel, causing severe symptoms and losses (Antignus et al., 2002 and 2007). The experimental host range of TASVd-Is differs significantly from that of the type strain TASVd-Ivory Coast

(Antignus et al., 2002 and 2007) but its biology has yet to be compared to the closely related Indonesian strain.

Transmission

TASVd can be transmitted via seed, by bumble bees, and has been transmitted mechanically under experimental conditions using sap from infected plants, and by workers' infested hands and tools (Antignus et al., 2007). A transmission experiment conducted by Antignus et al. (2007) showed that 24 out of 30 tomato seedlings propagated from seeds taken from TASVd-infected tomato plants tested positive for the viroid. This represents an 80% infection rate.

It is not certain whether bumble bees transmit the viroid through the movement of pollen or mechanical transmission by wounding of flowers (Matsuura et al., 2010; Verhoeven, 2010). If it is by wounding of flowers, they could also transfer the viroid to other susceptible plant species.

Low efficiency transmission of TASVd from tomato to tomato by the aphid *Aphis craccivora* was reported but not proven and might have been due to mechanical transmission by the insect rather than true feeding-associated transmission (Singh et al., 2003). TASVd does not appear to be transmitted by the aphid *Myzus persicae* or the whitefly *Bemisia tabaci* (Antignus et al., 2007). Although a related viroid, *Potato spindle tuber viroid* (PSTVd), can be transmitted by aphids after feeding on plants doubly infected with *Potato leaf roll virus* and PSTVd, it is not known whether TASVd can be transmitted in the same manner (Antignus et al., 2007).

4.3.2.2 Entry assessment

TASVd could enter New Zealand with imports of tomato seed for sowing. It has been demonstrated to be seed transmitted in tomatoes (Antignus et al., 2007).

TASVd has been recorded in commercial glasshouses and has been recorded in commercial seed lots, having been intercepted once in tomato seed being exported from the USA to Israel (EPPO 2010). TASVd-Is has been consistently found in tomato glasshouses in Israel causing severe losses (Antignus et al., 2007). TASVd has also been recorded in commercial tomato production facilities in Tunisia (Verhoeven et al., 2006). Furthermore, although TASVd has only been reported from a few countries, some of these reports are recent (2007-2010) and it is likely that TASVd is present in other countries where it has not yet been detected or reported.

Seed-lots entering New Zealand are considered to originate from all over the world, including from countries that are known to have TASVd in tomatoes (e.g. Israel, USA (Quancargo, 2011))

It seems reasonably unlikely that obviously symptomatic plants would be used for high value commercial seed production, but asymptomatic infections could result in infected seeds contaminating seed consignments. In reality, the proportion of seeds infected with TASVd is likely to be variable from consignment to consignment and over time is highly uncertain. As large volumes of tomato seed are imported into New Zealand, TASVd may enter New Zealand through this pathway.

As a visual inspection of a consignment of tomato seeds will not enable detection of contaminated seeds, it is considered highly likely that if tomato seeds exported to New Zealand are infected with TASVd, then TASVd will enter New Zealand.

Given that:

- TASVd has been recorded in commercial tomato seed lots;
- TASVd has been recorded from commercially grown tomato facilities;
- TASVd has a wide geographic distribution and has been reported in countries from which New Zealand sources tomato seed;
- It is unlikely symptomatic plants would be used for the production of commercial seed but infected plants may be asymptomatic;

In the context of millions of seeds being imported into New Zealand over time, the likelihood of tomato seed infected with TASVd entering New Zealand is considered to be high; but the proportion of those seeds that are infected with TASVd is highly uncertain.

4.3.2.3 Exposure assessment

If TASVd entered New Zealand in imported tomato seed, most plants arising from the infected seed are likely to be infected because the seed-to-seedling transmission rate is high. A transmission experiment conducted by Antignus and others (2007) showed that 24 out of 30 tomato seedlings propagated from seeds taken from TASVd-infected tomato plants tested positive for the viroid. This represents 80% infection. The generation of an infected plant from an infected seed is considered to be completion of the exposure pathway.

In the context of millions of seeds being planted in New Zealand over time, the likelihood of TASVd exposure to a tomato seedling is considered to be high.

4.3.2.4 Assessment of establishment and spread

This section assesses establishment on the basis that TASVd has already been exposed to (or infected) a new host plant. TASVd establishment in the environment is affected by the number of tomato plants exposed to (infected with) TASVd and whether that number is above the threshold for concern. As TASVd can readily spread within a tomato crop as a result of routine crop handling and maintenance, **even if there is only one initial exposure (infection) event, it is likely to result in tens to hundreds of additional exposures in the tomato crop**, depending on what setting the initial exposure has occurred in (i.e. potentially hundreds of exposures in a commercial greenhouse, or in a commercial outdoor crop of table tomatoes, or in an outdoor, commercial field crop; and potentially tens of exposures in a home garden setting). These numbers of exposures are considered to be above the threshold for concern.

The discussion below focuses firstly on infected seeds/seedlings occurring in commercial crops of tomato. This is followed by a discussion about infected seeds/seedlings occurring in the home garden.

<u>Establishment and spread of TASVd in commercial tomato crops</u>
Tomato seeds will be planted in commercial glasshouses and open fields.

In commercial glasshouses in New Zealand, TASVd in plants grown from infected seeds is likely to be spread through the glasshouse mechanically by contaminated workers' hands or equipment, or by bumble bees. In New Zealand bumble bees are used to pollinate commercially grown tomato plants (W. Zwart, pers. comm. 2011). Outbreaks in other countries demonstrate that TASVd can spread quickly through a glasshouse. For example, in an outbreak of TASVd in Tunisia initially only 5% of plants in a production facility showed symptoms but the number of symptomatic plants increased quickly to 100% as temperatures increased in the facility (Verhoeven et al., 2006). It is not known if TASVd is ever symptomless in tomatoes but possibly it is similar to *Potato spindle tuber viroid* (PSTVd). Symptomless infection of PSTVd has been observed in some plants grown from infected seed (Kryczynski et al., 1988), or symptoms have developed following a lag period from initial infection so allowing the infection to spread through a glasshouse unnoticed (Lebas, 2005).

The viroid could establish for a season in New Zealand glasshouses, but is highly likely to be destroyed when plants are destroyed and the glasshouse disinfected at the end of the season. If there was an outbreak of TASVd in a glasshouse tomato crop, there is a low likelihood that infection could be moved from one glasshouse tomato crop to another glasshouse tomato crop, or to tomatoes growing outdoors. The primary mechanism of spread would be by mechanical transmission by sap, on the hands or equipment of workers. To persist outside of a commercial glasshouse for longer than one season, TASVd would have to establish in host plants that do not die at the end of a growing season (e.g. a perennial host species), or it must infect 'annual' hosts regularly (see discussion below or possible mechanisms).

For outdoor crops of commercially grown field tomato plants, there is a high probability of spreading throughout the field, through a combination of bumblebee activity, and on workers hands or equipment because field tomatoes will be checked by workers on a regular basis and leaves are touched for nearly all plants during crop scouting (checking for insect pests and looking for symptoms due to pathogens or nutrient deficiency). Also, the viroid could be spread from machinery operated in the field i.e. during spraying. However, these outdoor crops would not provide hosts for multiple seasons because at the end of the season, tomato plants are destroyed and usually the fields are replanted in a three year rotation with other crops grown the following two years, and with grass planted for grazing in the intervening winters (Heinz Watties, personal communication, 2011). Any viroid-infected tomato plants in the field would be destroyed.

The likelihood that TASVd would be spread to alternative host plants (such as *Solanum jasminoides*, *Brugmansia* sp, and *Cestrum* sp. which are described as weeds in New Zealand (Ogle and Lovelock, 1989; Popay et al., 2010; Bay of Plenty Regional Council, 2011) before crops are destroyed at the end of the growing season is uncertain. While spread to new host species through mechanical means is considered to be negligible[22], it is assumed in this risk analysis that bumblebees can cause trans-species viroid transmission. Infections of TASVd in perennial or weedy annual species could establish if bumblebees visit flowers of suitable host plants, rather than non-host plants. If populations of these plants are infected they could act as a reservoir for TASVd. Infection is symptomless in all of these hosts (Verhoeven et al., 2008a and 2008b; Olivier et al., 2011).

It is assumed that seed is not collected by commercial growers either from glasshouse or field crops for growing new plants, so persistence of the viroid in New Zealand via seed transmission in tomatoes is highly unlikely in the commercial setting.

<u>Establishment and spread of TASVd in home gardens</u>
Home gardeners growing plants from infected seed or seedlings could spread TASVd throughout their own tomato crop mechanically, or it could spread via bumble bee activity.

22 It is not expected that people working in the commercial tomato crops either in the field or in glasshouses would then be in contact or work with those species.

Home gardeners often save seed and distribute it. Seeds collected from infected plants could be moved around the country and provide a source of infection, this is the major way that infection could travel long distances within New Zealand.

Seed may not be collected from obviously infected plants, but there is a possibility that infected plants may be symptomless (like some plants grown from seeds infected with PSTVd). Furthermore, home gardeners may not be aware of symptoms of viroids, or be aware that they shouldn't collect seeds from diseased plants. The likelihood of semi-wild tomatoes growing from seed of fallen fruit (including bird spreading) in home gardens or of fruit disposed in compost, and establishing TASVd in this manner is considered low.

As with commercial crops of tomatoes, it is assumed in this risk analysis that TASVd can be spread from tomatoes in the home garden to other host plants (such as *Solanum jasminoides*, *Brugmansia* sp, and *Cestrum* sp.) by bumblebees. It is conceivable that these plants may occur in home gardens, and they may be trimmed and maintained using tools that have become contaminated with TASVd through exposure to tomato plants. Therefore, there is a low-moderate likelihood that TASVd could be spread from tomato plants in home gardens to other host plants.

Given that:

- TASVd infections in glasshouse or field crops of tomatoes are likely to be destroyed at the end of the growing season;
- It is assumed that bumblebees can spread TASVd from tomatoes to other hosts (based on extrapolation of information from TCDVd studies with bumblebees);
- There is potential for TASVd to get into weed species or ornamental plants via mechanical spread from tomatoes in home gardens, which would result in establishment of a reservoir of TASVd in the New Zealand environment.
- Home gardeners may collect and distribute infected tomato seed;

The likelihood of TASVd establishing in tomato crops where the initial infection occurs, and for that season, is considered to be high.

The likelihood of TASVd establishing in the New Zealand environment (beyond one season) is uncertain but considered to range from low to moderate, and is most likely to originate from the occurrence of TASVd in home garden tomato crops; and from outdoor commercial crops assuming that bumblebees can transmit TASVd trans-species.

The likelihood of TASVd establishing in the New Zealand environment via transmission from commercial greenhouse tomato crops is low and dependent on bumblebees escaping from greenhouse and spreading the viroid.

The likelihood of TASVd spreading in New Zealand beyond the initially infected area is considered to be low to moderate.

4.3.2.5 Consequence assessment

Economic consequences

Tomatoes are an important crop in New Zealand. Domestic sales of tomatoes in 2008/2010 were worth $113 million, and exports worth $14 million (Plant and Food Research, 2010). The

Indonesian strain of TASVd and the Israeli isolate (TASVd-Is) are reported to cause severe symptoms and crop losses in tomato (Antignus et al., 2002 and 2007). The outbreak of TASVd in a commercial glasshouse in the Netherlands in May 2011 resulted in heavy damage on plants (EPPO, 2011b). Reports detailing the percentage yield losses on tomato crops were not found during the preparation of the risk analysis. However, based on the description of damage caused by TASVd (e.g. reduced fruit size, pale red discolouration), it is estimated that if TASVd became established in New Zealand crops, the yield losses are likely to be similar to those caused by other viroids such as PSTVd, or TCDVd. Other direct costs to the tomato industry may include costs of detection and eradication of the viroid from crops.

The potential economic consequences within New Zealand are considered to be low to moderate.

Environmental consequences

All of the known hosts are in the family Solanaceae. There are New Zealand native species in the Solanaceae (e.g. poroporo), and it may be possible for some of these to become infected by TASVd. However this isn't particularly likely, as there would need to be mechanical transmission from infected plants, or possibly transmission by bumble bees. It is expected that the likelihood of transmission to native plants by bumble bees is low, as this would only happen if bumble bees visit the flowers of native plants (it is not known that they do), and only if bumble bee transmission of the viroid is by wounding of flowers rather than movement of pollen.

The potential environmental consequences within New Zealand are considered to be low.

Human health consequences

There are no known human health consequences.

Socio-cultural consequences

Poroporo has an edible fruit eaten by Maori. There is a very low probability that it could be infected with TASVd, and plants could be stunted or yield of berries could be reduced, but there is very high uncertainty about this. There is also a possibility that home gardener's tomato or capsicum plants become infected with TASVd which could lead to reduced yield of fruit or having to destroy the plants to prevent further spread of the viroid.
The potential socio-cultural consequences within New Zealand are considered to be low.

4.3.2.6 Risk estimation

The likelihood of entry is high, but the proportion of seeds infected with TASVd is highly uncertain. The likelihood of exposure is high. The likelihood of TASVd establishing and spreading within crops is high, but is only low to moderate for environmental establishment and spread. The potential economic consequences within New Zealand are considered to be low to moderate. The potential environmental consequences within New Zealand are considered to be low. The human health consequences are considered to be negligible. The potential socio-cultural consequences are considered to be very low.

Consequences for commercial growers are mostly independent of environmental establishment of TASVd, and could arise every year simply through the importation and growing of infected seed.

Risk estimation table: *Tomato apical stunt viroid* on tomato seed from all countries				
	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry				
Exposure				
Establishment		(Environmental) ?	(Environmental) ?	(Within a crop)
Spread		(Environmental) ?	(Environmental) ?	(Within a crop)
Consequences of establishment				
Economic		?	?	
Environmental				
Socio-cultural				
Human Health				

In consideration of these assessments, TASVd is considered to be a non-negligible risk on imported tomato seed. The risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.

4.3.3 Risk management – efficacy of risk management options

Risk Management for all the viruses and viroids assessed is considered in section 6.

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4.4 Pepper chat fruit viroid (PCFVd)

Scientific name: *Pepper chat fruit viroid* Verhoeven 2009
Family/Genus: *Pospiviroidae/Pospiviroid*
Acronym: PCFVd

4.4.1 Hazard identification

4.4.1.1 Description

Pepper chat fruit viroid (PCFVd) is a plant pathogen of *Capsicum annuum* and tomato plants. In capsicum plants it causes fruit size to be reduced by up to 50% and plant growth to be slightly reduced. In tomato plants it causes plant stunting and leaf symptoms. PCFVd is a circular, single stranded RNA molecule lacking a capsid (a shell of protein).

4.4.1.2 Taxonomy

PCFVd was discovered for the first time in 2006. The classification of this viroid as a new species was justified on the basis of its molecular properties: it has less than 66% sequence homology with nucleotide sequences of other Pospiviroids , which results in the primary structure of the PCFVd being different from all other viroids (Verhoeven et al., 2009).

4.4.1.3 New Zealand status

PCFVd is not known to be present in New Zealand. It is not recorded in PPIN (2011).[23]

4.4.1.4 Geographic distribution

As at November 2011, PCFVd is reported from just three countries (the Netherlands, Canada, Thailand), but these are in three different continents (Europe, North America, Asia) (Verhoeven et al., 2009; Verhoeven et al., 2011; Reanwarakorn et al., 2011). As this is a newly described viroid, and is reported in the Netherlands where tomato and *Capsicum* seeds are processed from different geographical origins and distributed worldwide, it is likely that the PCFVd occurs in other countries but is yet to be officially identified and reported.

4.4.1.5 Commodity association

<u>Tomato seed:</u> PCFVd affects tomato plants (Reanwarakorn et al., 2011) but it is unknown if it is associated with tomato seeds.

<u>Capsicum seed:</u> PCFVd is seed-borne in *C. annuum* (Verhoeven et al., 2011).

4.4.1.6 Plant associations

<u>Natural hosts:</u> *C. annuum* (capsicum) (Verhoeven et al., 2009; Verhoeven et al., 2011); and *Solanum lycopersicum* (tomato) (Reanwarakorn et al., 2011).

23 The latest review of plant virus, viroid and mollicute records for New Zealand (Pearson et al., 2006) pre-dates the discovery of PCFVd, so it is not a relevant reference to use for determination of NZ status.

Experimental hosts: *Solanum tuberosum* 'Nicola'(potato) (Verhoeven et al., 2011).

The known plant associations are currently limited. As this is a newly described viroid, there are likely to be other natural hosts that have not yet been discovered.

4.4.1.7 Potential for establishment and impact

PCFVd is found in countries with similar tomato growing conditions as New Zealand, e.g. tomato production systems in both glass-house crops and outdoors. Therefore, PCFVd could potentially establish in the New Zealand under local conditions. The viroid causes damage to infected tomato and capsicum plants, and possibly other infected species, and so can potentially cause unwanted impacts in New Zealand.

4.4.1.8 Hazard identification conclusion

Given that PCFVd

- Is associated with capsicum seed, but there is not yet evidence of it being associated with tomato seed;
- Is present in countries that export tomato and capsicum seeds to New Zealand;
- Is not recorded from NZ;
- Can potentially establish in New Zealand;
- Causes damage to infected tomato plants, and so can potentially cause unwanted impacts in New Zealand;

Pepper chat fruit viroid is therefore considered a hazard on imported *Capsicum* seed in this risk analysis. An association of PCFVd with tomato seed cannot be ruled out, so PCFVd is also considered a hazard on tomato seed.

4.4.2 Risk assessment

4.4.2.1 Biology

Host plants

As described in Plant Associations.

Symptoms of PCFVd infection

Tomato: Field grown tomato plants infected with PCFVd in Thailand were stunted and had leaf symptoms including necrosis, distortion and discoloration (Reanwarakorn et al., 2011).

Capsicum: In glasshouse-grown capsicum, fruit size of the affected plants was reduced by as much as half of the normal size. Plant growth was slightly reduced, and the young leaves of the infected plants were slightly smaller and paler than those of healthy plants (Verhoeven et al., 2009; Verhoeven et al., 2011).

Potato: Under experimental conditions, PCFVd occasionally caused necrotic lesions on the leaves and petioles 2-3 weeks after inoculation. No further leaf symptoms appeared on potato. However, the tubers appeared misshapen: they only measured one fourth to half of the normal size, were elongated, and produced lateral extensions along the main tubers (Verhoeven et al., 2009).

Transmission

Seed-transmission: PCFVd is transmitted by seed in capsicum (19% seed-to-seedling transmission rate) (Verhoeven et al., 2009). It is not known whether the viroid occurs inside or outside of the seed.

Mechanical transmission: PCFVd is transmitted by mechanical inoculation in experimental settings (Verhoeven et al., 2009), and it is assumed that it can be mechanically transmitted during crop maintenance and handling activities, as occurs with other Pospiviroids. The occurrence of PCFVd along rows in commercial crops (Verhoeven et al., 2009) is indicative of the viroid being spread in this manner.

Insect transmission: It is not known if PCFVd is transmitted by insects.

4.4.2.2 Entry assessment - PCFVd on tomato or capsicum seed

PCFVd is transmitted by capsicum seeds and transmission by tomato seeds cannot be ruled out at this stage. This viroid has not yet been reported in commercial seed consignments but it is not known if commercial seeds are tested for the presence of this viroid. The source of the three known PCFVd infections (in Netherlands, Thailand and Canada) is uncertain.

Very few of the tomato and capsicum seed-lots entering New Zealand can be traced to a known country of origin. Due to the nature of the seed-trade industry (described in section 2.2), it is assumed in this risk analysis that seed-lots entering New Zealand originate from all over the world, including from countries that are known to have PCFVd in tomatoes and capsicums.

Symptoms of PCFVd on tomato plants are obvious, and obviously infected plants are likely to be removed from production systems. But, the occurrence of asymptomatic infections in tomato cannot be ruled out (as another pospiviroid, PSTVd, can occur asymptomatically in tomato). Furthermore, the symptoms on capsicum plants are not necessarily obvious (fruit size reduced, but not misshapen or miscoloured, and slight stunting of plant). Given that other viroids that can cause obvious symptoms have been detected in commercial seeds (e.g. TCDVd), it is clear that the seed production systems are not yet able to eliminate the occurrence of viroids from seeds.

A visual inspection of a consignment of tomato or capsicum seeds will not enable detection of seeds contaminated with PCFVd.

Given that:

- Seed transmission of PCFVd occurs in capsicum seeds, and cannot be ruled out in tomato seeds;
- It is assumed that tomato and capsicum seed-lots entering New Zealand originate from countries known to have PCFVd, but it is only recorded from three countries to date;
- The fact that viroids can cause obvious symptoms in plants is no guarantee that seed producers can eliminate viroids such as PCFVd from their seeds;
- PCFVd has not yet been reported in commercial tomato or capsicum seeds, but it is not known to what extent commercial seed has been tested for presence of PCFVd;
- Infected seeds are undetectable by visual inspection at the border;

The likelihood of Capsicum *or tomato seed infected with PCFVd entering New Zealand is uncertain (due to a lack of relevant information), but considered to be non-negligible.*

4.4.2.3 Exposure assessment

This section assumes that PCFVd-contaminated seed has already entered New Zealand. Exposure of the PCFVd to a host plant simply requires seed-to-seedling transmission to occur, i.e. that a seedling derived from a seed is infected.

The imported tomato or capsicum seed would be planted in glasshouses or outside, and a new plant would be generated. The percentage of those plants that would be infected with PCFVd, depends on the seed-to-seedling transmission rate. In capsicum the transmission rate is nearly 20%. In tomatoes, it is unknown if seed-to-seedling transmission occurs or what the transmission rate would be. But given that other pospiviroids that infect tomatoes are transmitted by tomato seed, it is assumed that the same would be true for PCFVd.

In the context of millions of seeds being planted in New Zealand over time, the likelihood of exposure of PCFVd to Capsicum *plants is considered to be high.*

In the context of millions of seeds being planted in New Zealand over time, the likelihood of exposure to a tomato plant is considered to be high. ***This rating is made with high uncertainty****.*

4.4.2.4 Assessment of establishment and spread

There are multiple establishment pathways to be considered in this section because there are four different production systems (commercial greenhouse; commercial outdoor table tomato production; outdoor, commercial field tomatoes; home-garden tomato production), multiple potential hosts of PCFVd, and four methods of viroid transmission.

This section assesses establishment on the basis that PCFVd has already been exposed to (or infected) a new host plant. PCFVd establishment in the environment is affected by the number of tomato plants exposed to (infected with) PCFVd and whether that number is above the threshold for concern. As PCFVd is assumed to be spread readily within a capsicum crop as a result of routine crop handling and maintenance, **even if there is only one initial exposure (infection) event, it is likely to result in tens to hundreds of additional exposures in the capsicum crop**, depending on what setting the initial exposure has occurred in (i.e. potentially hundreds of exposures in a commercial greenhouse; potentially tens of exposures in a home garden setting). These numbers of exposures are considered to be above the threshold for concern. The same situation is expected to occur in tomato crops, but note that **in tomato crops, exposure in <u>assumed</u> to occur via seed-to-seedling transmission (based on extrapolation of evidence about other pospiviriods), so there is much greater uncertainty surrounding establishment via tomato seeds.**

Establishment of PCFVd in New Zealand requires the following scenarios, some of which depend on viroid transmission to other host species:

- Persistence of the infected tomato or capsicum plant and/or infected crop, or;
- Contaminated seeds derived from the original host plant must generate infected new tomato or capsicum plants in subsequent seasons, or;
- The viroid must be transmitted to other host species that can either persist longer than one season (e.g. perennials), or generate seeds or tubers contaminated with the viroid.

The following paragraphs will deal with these scenarios.

<u>Persistence of the infected plant or crop:</u> Once PCFVd has infected a tomato or capsicum plant, the viroid persists systemically within the plant and is considered established in the environment until

the plant dies. Infected plants are assumed to have a symptomless phase and symptoms on capsicum are not particularly distinct. Therefore, as PCFVd is transmitted mechanically, it is likely to spread to other plants in a tomato or capsicum crop during routine crop handling and maintenance, before symptoms are noticed. In the infected capsicum crops in Canada and the Netherlands the viroid was observed spreading along rows (Verhoeven et al., 2009; Verhoeven et al., 2011), which is indicative of mechanical transmission.

Tomato and capsicum plants are annuals so they die after fruiting at the end of the season; or in the case of a commercially grown plant, the plant is destroyed at the end of a production season, which can be expected to destroy the viroid as well. However, an infection of PCFVd reoccurred in the Netherlands in the same locations in the glasshouse as the previous years' infection. The scientists that studied these infections suspected that the second infection occurred as a result of the glasshouse not being disinfected thoroughly during crop rotation. The quality of glasshouse disinfection is likely to vary in New Zealand, so a re-occurrence of PCFVd in a New Zealand glasshouse cannot be ruled out, thus providing a mechanism for PCFVd to re-establish in glasshouses in subsequent years.

In glasshouses where sanitation between crops is thorough enough to eliminate the likelihood of PCFVd re-occurring, the viroid is unlikely to establish in New Zealand beyond one growing season, unless other establishment pathways exist. For instance, if PCFVd has already spread to other crops, or if the infected plant produces contaminated seeds that germinate the following season, and produce a new crop of infected plants.

<u>Can infected seeds derived from the original host plant generate new infected tomato plants in subsequent seasons?:</u> This could happen <u>accidentally</u> as a result of self-sown tomato or capsicum plants growing, or as a result of seeds being <u>deliberately</u> kept and sown the following season.

Most commercial growers, whether they grow indoors or outdoors, are unlikely to deliberately retain seed from one season for the purposes of generating next season's plants. So any deliberate retention of tomato or capsicum seeds is likely to be limited to the home gardener. It is common practice for home gardeners to retain seed from their own crops and reuse them the following season. It is conceivable that the fruit selected for seed extraction happens to be infected with PCFVd without the grower knowing, particularly if the plant was asymptomatic, and particularly in capsicum where the symptoms are not particularly obvious.

Accidental production of plants from seed derived from infected plants could occur as a result of fruit drop, and the seed being left in the growing media (or soil), leading to self-sown plants. This is unlikely to occur in commercial greenhouses, commercial outdoor crops of table tomatoes, or in commercial outdoor fields of 'process' tomatoes, because of commercial horticultural practices (see section 2.3.4 for detailed reasoning). However, it is considered that there is a low to moderate likelihood of home gardeners keeping self-sown plants, which might originate from composted fruit (especially so for tomato), or from fruit that has dropped directly into the garden. The seed-to-seedling transmission rate is not 100% (it is almost 20% in capsicum, and unknown in tomato), which somewhat moderates the likelihood of establishment via self-sown seeds to a range of low to moderate.

<u>Can the viroid establish in New Zealand by being transmitted to other host species?</u>
Very little is known about the host range of PCFVd. The known natural host range is currently limited to just tomato and capsicum, and the experimental host range is currently limited to potato.

As more studies of PCFVd are reported in the future, it is expected that the known host range will expand. A consistent finding from studies of other tomato pospiviroids is that the host range includes a number of other solanaceous species including weedy species, ornamental annuals and perennials. It is not unreasonable to expect that PCFVd will also be found to be capable of infecting other solanaceous species.

Crop management and plant maintenance practices offer an opportunity for mechanical transmission of the viroid from capsicum or tomato to another species in the home garden (where potatoes, tomato, capsicum, and solanaceous annuals are often grown). The transmission opportunity occurs to a greater or lesser extent depending on the requirements for crop/plant handling of the recipient species[24].

It is unknown whether PCFVd can be transmitted by insects, such as bumblebees. However, two other pospiviroids (TASVd and TCDVd) are known to be transmitted between tomato plants by bumblebees. Given that PCFVd is a newly described pospiviroid and not well studied, transmission by bumblebees from tomato or capsicum to other species cannot be ruled out. If bumblebees can indeed transmit PCFVd trans-species, tomatoes and capsicums grown in any of the production systems can be a source of viroid (including commercial greenhouses, as there is likely to be some potential for bees to escape from the greenhouse). But the tomato and capsicum crops grown outdoors, (including commercial outdoor tomato crops), are likely to present the greatest opportunity for enabling transmission because insects such as bees can move freely from species to species in the outdoor setting.

Establishment of PCFVd in the environment (i.e. sustainable beyond one season) within other annual host plants would require the annuals to produce contaminated seed, for the seed to be accidentally or deliberately germinated, and for seed-to-seedling transmission to occur. The likelihood of this pathway is uncertain, not least because it is unknown what the potential host species of PCFVd are, or if seed-to-seedling transmission occurs in species other than *Capsicum*. Considering all the factors required in the pathway for establishment of PCFVd in annual plants, the likelihood is considered to be the same as for the other pospiviroids, which ranges from low to moderate (see appendix for more detailed explanation). If potatoes prove to be a natural host of PCFVd, there is potential for establishment via the use of infected seed potatoes; the likelihood of this is considered low to moderate. Conflicting factors taken into consideration are that tubers are expected to have obvious symptoms making them undesirable for use as seed, but on the other hand tubers often sprout in compost piles and they could be infected with PCFVd.

<u>Spread:</u> There is insufficient knowledge about the natural hosts of PCFVd, including whether they include perennials (as is the case for other pospiviroids), to assess the likelihood of PCFVd spreading further afield via the movement of infected plant material. It is also unknown whether insect vectors will enable PCFVd to be spread further afield.

Given that:

- PCFVd can spread throughout a tomato or capsicum crop via mechanical transmission;
- Although tomato and capsicums are annuals and they do not persist beyond one season, there is potential for PCFVd to re-establish in glasshouses that are not thoroughly disinfected between crop cycles/seasons;
- PCFVd could establish in New Zealand via self-sown tomato or capsicum seeds from infected fruit, or from deliberate seed harvesting and growing; the likelihood of PCFVd

24 Refer to 'PCFVd Appendix' at the end of this pest risk assessment for more detailed explanations.

establishing in this way is considered to range from low to moderate, and is restricted to home garden situations;
- The host range of PCFVd is unknown, but is expected to include solanaceous species that occur in New Zealand (e.g. ornamentals, potato, and weedy species) and there are mechanisms of spread from tomatoes and capsicum to these species that could result in PCFVd persisting in the environment, with uncertain likelihood ranging from low to moderate.
- The uncertainties in the establishment pathways are numerous – whether PCFVd is seed transmitted in species other than capsicum, whether PCFVd is transmitted by insects (e.g. bumblebees) and if so, whether trans-species transmission of PCFVd by insects occurs (and with what efficiency);
- The likelihood of PCFVd spreading beyond a local area of establishment is uncertain.
- The production systems most likely to be a source of viroid for environmental establishment pathways are considered to be tomato and capsicum crops grown in home gardens, followed by commercial tomato crops grown outdoors.

The likelihood of PCFVd establishing and spreading within tomato and capsicum crops where the initial infection occurs, and for that first season, is considered to be high.

The likelihood of PCFVd establishing in the New Zealand environment (beyond one season) is uncertain but considered to range from low to moderate, and is most likely to originate from the occurrence of PCFVd in backyard capsicum or tomato crops; or outdoor tomato crops (vector dependent).

The likelihood of PCFVd establishing in New Zealand via transmission from commercial greenhouse capsicum or tomato crops is low and would be dependent on insect transmission, and then on insects escaping from greenhouses and spreading the viroid (both of which are uncertain for PCFVd).

The likelihood of PCFVd spreading in New Zealand beyond the initially infected area is uncertain (due to a lack of relevant information).

4.4.2.5 Consequence assessment

Economic consequences

Tomatoes, capsicums and potatoes are important crops in New Zealand. Domestic sales of capsicums in 2008/2010 were worth $29 million, and exports in 2010 were worth $34 million (Plant and Food Research, 2010). Domestic sales of tomatoes in 2008/2010 were worth $113 million, and exports worth $14 million (Plant and Food Research, 2010). The total retail and export value of the potato industry, including what householders and restaurants buy, what is bought by local processors and what is exported, is estimated at $382 million a year (Potatoes New Zealand, 2011).

<u>Tomato:</u> Tomato plants infected with PCFVd have leaf symptoms including necrosis, distortion and discoloration. Impacts of PCFVd on tomato fruit have not yet been reported. As PCFVd is only recently described, there are no records about tomato crop-losses.

<u>Capsicum:</u> Capsicum plants infected with PCFVd have slight stunting and the fruit are reduced in size by up to half. Consequently, yield by weight is likely to be reduced in capsicum crops.

Potato: Potato has only been identified as an experimental host of PCFVd. In experimentally infected potato PCFVd caused misshapen tubers: they only measured one fourth to half of the normal size, were elongated, and produced lateral extensions along the main tubers. It is assumed that experimental symptoms are likely to be similar if the disease occurred naturally in a potato crop. Crop management practices dictate that there would not be the same potential for mechanical spread of the viroid disease as occurs in tomato and capsicum crops; the spread of the disease is likely to be more dependent on bumblebees, which is currently an uncertainty. Consequently, the economic impact on potato crops is likely to be less than in tomato crops.

The potential economic consequences within New Zealand are considered to be low to moderate for individual tomato growers, but considered to be low from a national economic perspective.

Environmental consequences

There are three species in the Solanaceae that are native to New Zealand: *Solanum aviculare* (poroporo), *S. laciniatum* (poroporo, bullibulli) and *S. nodiflorum* (small flowered nightshade) (Landcare NZ plants database, 2011). There is a high degree of uncertainty about whether these plants would become hosts of PCFVd. It is also unknown whether these species would be symptomatic, and whether seed set or seed viability would be affected. At the population level, PCFVd is unlikely to have significant impact.

The potential environmental consequences within New Zealand are considered to be very low.

Human health consequences

There are no known human health consequences of PCFVd, or any of the other pospiviroids.

The potential human health consequences within New Zealand are considered to be negligible.

Socio-cultural consequences

Many New Zealanders are avid gardeners and enjoy growing their own crops of tomato, potato and *C. annuum*. All three of these crops are likely to be impacted in the home garden. This would be frustrating for growers, but is unlikely to change a way of life. Impacts are likely to vary from year to year.

For some iwi in New Zealand, the poroporo plant is an important source of food and medicine (The Encyclopedia of New Zealand, 2011). It is uncertain whether poroporo is capable of being a host of PCFVd. If it can be a host it is likely that only a portion of the poroporo population would become infected. The consequences of infection in poroporo are likely to range from negligible to low.

The potential socio-cultural consequences within New Zealand are considered to be low.

4.4.2.6 Risk estimation

PCFVd is a relatively newly described pospiviroid occurring in tomato and capsicum crops. It has not been well studied, very few reports about it were publically available and there is a lack of scientific information about this specific viroid. Consequently, **this risk estimation is made with a very high degree of uncertainty –** more so than with any of the other viroids.

The likelihood of *Capsicum* or tomato seed infected with PCFVd entering New Zealand is uncertain because relevant information is lacking, but it is considered to be non-negligible. The likelihood of PCFVd being exposed to a host plant is considered to be high because all it requires is seed-to-seedling transmission. However, the frequency of exposure events is uncertain because the proportion of infected seeds is not known. In the case of tomatoes, seed-to-seedling transmission is assumed, rather than proven. The assumption is based on extrapolation of knowledge about other pospiviroids and their transmission via tomato seeds. **Consequently, regarding the risk associated with PCFVd <u>in tomato seeds</u>, there is a very high degree of inherent uncertainty throughout the risk assessment.**

PCFVd is highly likely to establish and spread throughout a tomato or *Capsicum* crop if exposure occurs because it can be spread mechanically during crop management activities. However, the likelihood of PCFVd establishing beyond one growing season, (i.e. in the New Zealand environment in self-sown tomatoes, capsicums and/or other species) is somewhat uncertain and could range from low to moderate. There is more than one mechanism for establishment beyond one season, but the highest risk mechanisms involve viroid originating from tomatoes and capsicums grown in home gardens and in outdoor crops. Furthermore, much of the establishment will depend on how efficiently the viroid can spread trans-species via insects (e.g. bumblebees), and whether seed-to-seedling transmission occurs in other species, both of which are currently uncertain.

The likelihood of PCFVd spreading beyond the initial infection area is uncertain due to a lack of information about hosts and insect vectors. Spread would depend on the efficacy of spread via insects (e.g. bumblebees) and the movement of infected plant material.

PCFVd is likely to have a low impact on the national economy. However, to individual growers the economic impact is likely to range from low to moderate.

It is likely that the socio-cultural impact of PCFVd establishing in New Zealand would be low, the environmental impact is likely to be very low, and there are negligible health impacts.

See the table below for a visual summary of the risk estimation.

Risk estimation table: *Pepper chat fruit viroid* on tomato or capsicum seeds from all countries

	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry		Uncertain; non-negligable	Uncertain; non-negligable	Uncertain; non-negligable
Exposure				?*
Establishment		(Environmental) ?	(Environmental) ?	(Within a crop)
Spread		(Environmental) ?	(Environmental) ?	(Within a crop)
Consequences of establishment				
Economic		?	?	
Environmental				
Socio-cultural				
Human Health				

? denotes uncertainty

* The likelihood of exposure via tomato seed is highly uncertain due to paucity of information about PCFVd; The 'high' exposure rating for tomato is based on assumption from what is known about other pospiviroid seed transmission in tomatoes.

In consideration of these assessments, PCFVd *is considered to be a non-negligible risk on both imported capsicum and imported tomato seeds. The risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.*

4.4.3 Risk management – efficacy of risk management options

Risk Management for all the viruses and viroids assessed is considered in section 6.

4.4.4 References for PCFVd

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4.4.5

4.4.6 Appendix for PCFVd

Regarding Establishment and Spread: Detailed explanation of opportunities for transmission of PCFVd between species:

- *Solanum tuberosum* (potato): PCFVd is unlikely to be transmitted to potato plants via mechanical spread because potato crops are not handled intensively; but transmission may be possible via bumblebees (with uncertain likelihood) particularly from outdoor commercial or backyard tomato plants or capsicum plants. Potato is an annual plant so establishment of the viroid depends on tubers being infected and being used as seed-potato to grow next season's crop. As potato tubers have obvious symptoms when infected with PCFVd (mishappen, one fourth to half the normal size, elongated, lateral extensions along main tuber), it is highly unlikely that infected potato tubers would be used as seed-potatoes. However, there remains a moderate likelihood that infected tubers may sprout in compost piles, or from gardens that were insufficiently harvested, thereby enabling establishment of the viroid.

  Taking into account the uncertain likelihood of the viroid spreading to this host in the first instance, *the likelihood of PCFVd establishing via* S. tuberosum *is considered to be low to moderate. This pathway could originate from backyard tomato or capsicum crops or from outdoor commercial tomato crops.*

- Solanaceous ornamental annuals (hosts currently unknown): There is a negligible likelihood of spread from commercial greenhouse tomato or capsicum plants; but spread is possible with low to moderate likelihood from backyard tomato or capsicum plants via mechanical means (plant maintenance activities); spreading is also possible with uncertain likelihood, particularly via bumblebee activity from outdoor commercial tomato plants and backyard tomato plants. These plants are annuals so establishment of the viroid depends on seeds being self-sown, or retained and used the following season, and for seed-to-seedling transmission of PCFVd to occur in these ornamental species. This is uncertain. It is expected that gardeners are more likely to obtain their new season's ornamental annuals from a garden centre.

  The likelihood of PCFVd establishing via solanaceous ornamental annuals is considered to be low to moderate.

- Weedy *Solanum* species that have fully naturalised in New Zealand and grow wild (there are at least 10 species; (Landcare NZ plants database, 2011) (hosts currently unkown): Other pospiviroids, including *Potato spindle tuber viroid* and *Tomato chlorotic dwarf viroid* infect other solanaceous, including *Solanum* species. Based on this knowledge, a number of *Solanum* species are considered likely to be potential hosts of PCFVd too. One or two weedy *Solanum* plants are likely to be found in some backyards where *Capsicum* or tomatoes are grown, or nearby. There is a negligible likelihood of spread to these plants via mechanical spread; but spread is possible with uncertain likelihood via insect (e.g. bumblebee) activity, particularly from backyard tomato & capsicum plants or outdoor commercial tomato plants. These are annual plants so establishment of PCFVd depends on seed-transmission. This is highly uncertain. Being a weedy species, the seeds are highly likely to generate new plants the next season, which would enable the viroid to persist if seed-to-seedling transmission of

PCFVd occurs in these species. Taking into consideration that spread to these plants requires insect transmission, whereby an insect visits an infected capsicum or tomato plant, and then moves to one of these weedy species rather than a non-host of PCFVd:

the likelihood of PCFVd establishing via weedy Solanum *species is uncertain, but is considered to range from low to moderate. This pathway could originate from either backyard tomato or capsicum crops or from outdoor commercial crops of tomato.*

5 Risk analysis of viruses in tomato and/or capsicum seeds

5.1 Pepino Mosaic Virus (PepMV)

Scientific name: *Pepino mosaic virus*
Family/Genus: *Flexiviridae / potexvirus*
Acronyn: PepMV

5.1.1 Hazard identification

5.1.1.1 Description

Pepino mosaic virus (PepMV) is a plant pathogen that infects tomatoes, and other plants. PepMV symptoms in tomato plants can be very diverse: infected plants can be symptomless, or can display variable symptoms including: a yellow mosaic of varying intensity on the leaves, single yellow spots between veins, green mosaic (Figure 1), leaf bubbling, other leaf malformations, and fruit marbling (Figure 2).

Figure 1: Symptoms on tomato leaf **Figure 2:** Symptoms on tomato fruit

(Source of both photos: Piero Roggero, via CABI, (2011).

5.1.1.2 Taxonomy

PepMV is a definitive species of the genus *Potexvirus*, of which *Potato virus X* (PVX) is the type species. Four variants that infect tomato can be distinguished genetically (Hanssen & Thomma, 2010):

- the original Peruvian genotype (LP), described on pepino by Jones et al. (1980), but found regularly in tomato crops in Europe (Fakhro et al., 2011);
- the so-called European (tomato) genotype (EU) (van der Vlugt et al., 2000);
- the American genotype (US1); first isolated in the USA (Maroon-Lango et al., 2005).
- the Chilean genotype (CH2) occurring tomato crops throughout Europe (Ling, 2007).

5.1.1.3 New Zealand status

PepMV is not known to be present in New Zealand. It is not recorded in PPIN (2011) and it is not recorded in Pearson et al. (2006), the most recent review of plant virus, viroid and mollicute records for New Zealand. PepMV was not detected in a survey of a germplasm collection of 89 accessions of *Solanum muricatum* (pepino) maintained by HortResearch (Ochoa Corona et al., 2006).

5.1.1.4 Geographic distribution

As at November 2011: PepMV is widespread throughout Europe[25], North America[26], and occurs in South America (Chile, Ecuador, Peru), Africa (Madagascar, Morocco, South Africa (Carmichael et al., 2011)) and Asia (China, Syria) (CABI/EPPO, 2011; CABI 2011). Unofficially, the virus has been found in plant material originating from other European and non-European countries. Given its transmission characteristics it is likely that the virus is more widespread than currently officially recognised (CABI, 2011)

5.1.1.5 Commodity association

Tomato seed: PepMV has been detected by other countries in tomato seed consignments every year since at least 2007 (EPPO, various dates). Between 2007 and 2011, the countries of origin of the tomato seed consignments with PepMV were Chile, China, Guatemala, India, Israel, Netherlands, Senegal, Thailand, and USA.

Capsicum seed: No records are known of PepMV association with capsicum seeds, and no natural infections of *Capsicum* have been reported. Several experimental host range studies of PepMV (the tomato isolates) failed to generate systemic infections in *Capsicum annuum* test plants (Gomez et al., 2009; Verhoeven et al., 2003; Salomone & Roggero, 2002). Although weak infection with the EU-tomato isolate was demonstrated recently, this needs to be verified (Fahkro et al., 2011). But the LP-isolate (the pepino isolate), reported to be regularly found in virus infected tomato plants in Europe until 2007 (Fakhro et al., 2011), caused systemic infections in *C. annuum* in two independent host testing experiments (Verhoeven et al., 2003; Fahkro et al., 2011). Symptoms of yellow spots or stripes and chlorotic mottle were observed in one experiment (Fakhro et al., 2011), and in one of the studies the infection of *C. annuum* occurred only occasionally (Verhoeven et al., 2003).

5.1.1.6 Plant associations

PepMV was once thought to have a relatively narrow host range, mostly occurring within the Solanaceae family. But recent surveys of weed species demonstrate that the natural host range of PepMV is much wider (Papayiannis et al., 2012; Cordoba et al., 2004). Unless specific citations have been provided below, the citation for plant associations is the review by Hanssen & Thomma (2010).

Note that many of the hosts referred to in the literature have only been identified by experimental means.

Naturally occurring infections in **crop species**: tomato (*Solanum lycopersicum*); basil (*Ocimum basilicum*) (Davino et al., 2009); potato (*Solanum tuberosum* cv. 'Yungay', occurring in the Andes in Peru; and in 14% of tested accessions in the potato germplasm collection at the Centro Internacional de la Papa in Peru), pepino (*Solanum muricatum*) (CSL, 2005).

Other naturally occurring infections: wild tomato species *S. chilense, S. chmielewskii, S. parviflorum* and *S. peruvianum.*

25 Europe: Austria, Belgium, Bulgaria, Cyprus (Papayiannis et al., 2012), Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Mainland Italy, Sardinia, Sicily, Lithuania, Netherlands, Norway, Poland, Slovakia, Spain, Canary Islands, Mainland Spain, Sweden, Switzerland, UK, England and Wales.
26 North America: Canada, Alberta, British Columbia, Ontario, Quebec, USA, Alabama, Arizona, California, Colorado, Florida, Maryland, Minnesota, Oklahoma, Texas

In a survey of 42 native weed species growing in or around tomato production sites in Spain, PepMV was found in 18 weed species including those belong to the Amaranthaceae (e.g. *Chenopodium murale*), Convolvulaceae (e.g. *Calystegia sepium*), Brassicaceae (e.g. *Diplotaxis erucoides*), Boraginaceae, Asteraceae, Plantaginaceae and Polygonaceae (*Rumex* sp.) (Cordoba et al, 2004). And in Cyprus, a survey of weed species collected from a PepMV-infected greenhouse and a PepMV-infected open field-tomato field identified 20 weed species as hosts of PepMV. The weed species were in the families Amaranthaceae, Chenopodiaceae, Compositae, Convolvulaceae, Malvaceae, Plantaginaceae and Solanaceae (Papayiannis et al., 2012).

Many of the weed species are naturalised in New Zealand (e.g. Solanaceae species, *Chenopodium murale*, *Calystegia sepium*, *Rumex* sp. and *Diplotaxis* sp.) (Landcare, 2011).

<u>Experimental hosts (as determined by mechanical inoculation):</u> **Crop species**: potato (*S. tuberosum*), aubergine (*S. melongena*), capsicum (*Capsicum annuum*), broad bean (*Vicia faba*), cucumber (*Cucumis sativus*) and garlic (*Allium sativum*) (Hanssen & Thomma, 2010; Fakhro et al., 2011).

Species from the genera *Nicotiana*, *Datura* and *Physalis*.

5.1.1.7 Potential for establishment and impact

PepMV is found in countries with similar climatic and tomato growing conditions to New Zealand, e.g. climate and tomato production systems in both glass-house crops and outdoors. Therefore the virus could potentially establish in New Zealand. The virus can potentially caused unwanted impacts in New Zealand because it damages tomato crops, and could potentially damage potato crops.

5.1.1.8 Hazard identification conclusion

Given that *Pepino mosaic virus*
- Is associated with tomato seeds, but <u>is not</u> associated with capsicum seeds;
- Is present in countries that export tomato seeds to New Zealand, and has been intercepted (by other countries) on tomato seed consignments from some of these countries;
- Is not recorded from NZ;
- Can potentially establish in New Zealand;
- Damages tomato crops, so can potentially cause unwanted impacts in New Zealand;

Pepino mosaic virus is therefore considered a hazard on imported tomato seeds in this risk analysis. It is not considered a hazard on imported *Capsicum* seed.

5.1.2 Risk assessment

5.1.2.1 Biology

<u>Symptomatology</u>
PepMV symptomatology has been most extensively studied in cultivated tomato. A wide range of symptoms have been associated with PepMV infection in tomato (reviewed by Hanssen & Thomma, 2010), and ranges from asymptomatic infections to very severe symptomatology:
- Symptomless (Soler-Aleixandre et al., 2005; Pospieszny et al., 2008; Fakhro et al., 2011)

- Fruit marbling
- Fruit discoloration (blotchy ripening or flaming)
- Occurrence of 'open fruit' (fruit that splits shortly after setting, such that seeds become visible)
- Nettle-heads (upper young leaves distorted, serrated and upright with reduced surface)
- Leaf blistering or bubbling
- Chlorosis and yellow angular leaf spots
- Severe leaf mosaics
- Leaf or stem necrosis

It has also been suggested that the so-called 'tomato collapse' disease, a sudden and progressive wilt of tomato in Spain which eventually leads to plant death, is caused by necrosis of the vascular system as a result of PepMV accumulation, but in association with a fungus and/or environmental factors (Soler-Aleixandre et al., 2005). *Olpidium brassicae*, (synonym *Olpidium virulentus*) was detected in most of the roots of collapsed tomato plants (Córdoba et al., 2004, cited in Soler-Aleixandre et al., 2005)

The severity of PepMV symptoms is thought to be dependent on environmental conditions such as temperature and light conditions. But there is inconclusive evidence to indicate whether high or low temperature and light result in severe or mild symptoms. The interplay of environmental factors contributing to PepMV damage appears to be complex and remains to be elucidated (Hanssen & Thomma, 2010).

Symptoms are also influenced by the PepMV isolate and by co-infection with more than one isolate (Hanssen et al., 2009; Pospieszny et al., 2008; and reviewed by Hanssen & Thomma, 2010).

Symptoms of yellow spots or stripes, or chlorotic mottle, on other host species have been reported in an experimental setting (Fakhro et al., 2011).

<u>Mixed populations of PepMV</u>
The population of PepMV is diverse and occurs in European crops as mixed infections of more than one isolate, and in the presence of recombinants, e.g. in Spain combinations of EU and US2-like genotypes, and LP and US2-like genotypes in Spain; and combinations of EU and CH2 genotypes in Belgium (reviewed by Hanssen & Thomma, 2010).

<u>Transmission</u>
PepMV is very efficiently transmitted mechanically (Jones et al, 1980). The virus is highly contagious in tomato, as it is easily spread by the standard crop handling procedures in a glasshouse through contaminated tools, hands and clothing and by direct plant-plant contact (Spence et al., 2006; Wright & Mumford, 1999; cited in Hanssen & Thomma, 2010).

Bumblebees are often used for pollination in commercial tomato production; the bumblebee species *Bombus impatiens* and *B. terrestris* contribute to the spread of the virus between tomato plants(Shipp et al, 2008; Lacasa et al., 2003). Bumblebees can also transmit PepMV between different species: from tomato plants into the weed species *Solanum ptycanthum, S. sarrachoides* and *Datura stramonium*, and from these species back into tomato again (Stobbs et al., 2009).

Seed-to-seedling transmission has been demonstrated in tomato, at variable but low rates of up to ~ 2%, depending on the time of seed harvest, the tomato variety and the seed cleaning or disinfection methods applied (Cordoba-Selles et al., 2007; Ling, 2008; Hanssen et al., 2010; Hanssen &

Thomma, 2010). When seeds infected with PepMV27 were prepared to industry standards[28], the seed-to-seedling transmission rate was estimated at 0.026%, based on a grow-out trial of almost 90,000 tomato seedlings (Hanssen et al, 2010). The low transmission rate is explained by the finding that PepMV is located in the seed coat, but not in the embryo of tomato seeds (Ling, 2008).

PepMV can also be transmitted in hydroponic systems via the irrigation water/nutrient solution, leading to the infection of healthy tomato plants (Alfaro-Fernandez et al., 2010; Schwarz et al., 2009). However, it seems that this requires the co-existence of a fungal vector. Alfaro-Fernandez et al. (2010) carried out highly controlled experiments and showed that PepMV transmission required the fungal vector *Olpidium virulentus*[29] to also be in the irrigation water. Schwarz et al. (2009) did not analyse whether *Olpidium* species zoopores were present in their irrigation system.

It is considered in this risk analysis that *O. virulentus* occurs widely spread in New Zealand, and the fungus has been previously associated with tomato crops in New Zealand[30].

Host range
Refer to Plant Associations, section 5.1.1.6.

Experimental co-inoculation of test plants with EU and CH2 variants extended the host range beyond that of single isolates (Gomez et al., 2009).

5.1.2.2 Entry assessment

Hanssen & Thomma (2010) report in their review article that many tomato growers, especially in the Netherlands, have chosen to inoculate their crops with a mild PepMV isolate in an attempt to protect their crops from the severe damage upon natural infection by an aggressive isolate based on cross-protection.

Given that PepMV has been detected in tomato seed consignments every year since at least 2007 (see commodity association above); that seed-lots entering New Zealand are considered to originate from all over the world, including from countries that are known to have PepMV in tomatoes; and that a visual inspection of a consignment of tomato seeds will not enable detection of contaminated seeds, it is considered highly likely that tomato seed infected with PepMV will enter New Zealand.

The prevalence of PepMV in tomato seed consignments is unknown, but likely to be variable between consignments depending on the symptomatology of tomato plants in the seed production system; specifically, symptomless plants are unlikely to be removed from seed production systems, and are likely to be a source of infected seeds.

27 Seeds were harvested from tomatoes infected with PepMV, and were batches were confirmed to be contaminated with PepMV via ELISA assay.
28 Seeds were separated from the tomato pulp and collected in containers to which an equal volume of tap water was added. Subsequently, citric acid pH 4 (6.7% v/v) and pectinase (0.24% v/v) were added and the pulp incubated for 3 h at 28°C, stirring every 30 min. Next seeds were retrieved by sieving, thoroughly rinsed with tap water and dried for 24 h in an oven at 26°C until the water content was below 6%. Seeds were not disinfected.
29 *Olpidium virulentus,* formally referred to as *O. brassicae,* is a known vector of plant viruses (Alfaro-Fernandez et al., 2010).
30 The taxon *Olpidium brassicae* occurs in New Zealand and is associated with *Lettuce big vein virus* (Landcare Research, 2011), and recent survey evidence suggests it is associated in New Zealand with *Mirafiori lettuce big vein virus* (Fletcher et al., 2005). As the strain of *O. brassicae* that vectors *Mirafiori lettuce big vein virus* was renamed in 2006 to *O. virulentus* (Sasaya & Koganezawa, 2006), it is considered in this risk analysis that the fungus in New Zealand known as *O. brassicae*, is in fact *O. virulentus.* Recent New Zealand surveys of lettuce viruses *Lettuce big vein virus* and *Mirafiori lettuce big vein virus*, which are both associated with *O. virulentus* (Maccarone et al., 2010), indicate that the fungus is widely spread throughout New Zealand (Fletcher et al., 2005). Of further relevance to this risk analysis is that greenhouse tomatoes grown in New Zealand have previously been recorded as having roots heavily infected with *Olpidium brassicae* (synonym *O. virulentus*) (Thomas, 1972)

In the context of millions of seeds being imported into New Zealand over time, the likelihood of tomato seed infected with PepMV entering New Zealand is considered to be high; the proportion of imported seeds infected with PepMV is highly uncertain.

5.1.2.3 Exposure assessment

This section assumes that PepMV-contaminated seed has already entered New Zealand. Exposure of PepMV to a plant occurs if the seedling produced from the seed is infected with PepMV.

The imported infected tomato seed would be planted in glasshouses or outside, and a new plant would be generated. As PepMV is seed-transmitted, albeit at a low rate ranging from 0.026% (from infected seeds prepared to industry standards, but not disinfected) to ~2% (from infected seeds prepared with less rigorous standards), exposure is highly likely.

In New Zealand, at least 2.75 million tomato seeds are planted each year[31]. The frequency of exposure events each year is uncertain because it depends on what proportion of seeds that are contaminated with PepMV, which is itself unknown and likely to be variable. But by way of example, exposure of PepMV to a tomato seedling is highly likely to occur at least once a year in New Zealand if more than approximately 0.14% of imported seeds (1 in ~700) are contaminated with PepMV[32].

Given that:

- The seed-to-seedling transmission rate of PepMV is low, ranging from 0.026% to 2% depending on the method of seed preparation;
- A key factor in the frequency of exposure via seed-to-seedling transmission is the proportion of seeds that are infected with PepMV.
- The proportion of infected seed is uncertain, but if it is >0.14% (at least 1 in 700 seeds infected), seed-to-seedling transmission is likely to occur at least once a year because New Zealand imports more than 2.5 million seeds annually.

In the context of millions of seeds being planted in New Zealand over time, the likelihood of PepMV exposure to a tomato seedling is considered to be high.

5.1.2.4 Assessment of establishment and spread

There are multiple establishment pathways to be considered in this section because there are four different production systems (commercial greenhouse; commercial outdoor table tomato production; outdoor, commercial field tomatoes; home-garden tomato production), multiple potential hosts of TCDVd, and four methods of viroid transmission.

This section assesses establishment on the basis that PepMV has already been exposed to (infected) a new host plant. PepMV establishment in the environment is affected by the number of tomato plants

31 This is an extrapolation of knowledge that approximately 2.75 tomato plants are used in New Zealand commercial production systems (K Robertson, personal communication, 2011).

32 It takes 3846 contaminated seeds to get one seedling infection if the transmission rate is 0.026% [1/0.00026 = 3846]. Therefore, 3846 infected seeds, as a proportion of the amount of seed sown in NZ, ~2.75 million, is ~0.14% [3846 / 2750000 x 100 = 0.13985%]. So a seed contamination rate of >0.14% can be expected to result in at least one exposure per year, if the seed-to-seedling transmission rate is 0.026%. A contamination rate of 0.14% is the same as saying 1 in ~700 seeds being infected [1 / 0.0014]. If all 2.75 million seeds were contaminated with PepMV, it is likely that there would be 715 exposures: [2 750 000 seeds x (0.026/100) = 715 exposures].

exposed to (infected with) PepMV and whether that number is above the threshold for concern. As PepMV can readily spread within a tomato crop as a result of routine crop handling and maintenance, **even if there is only one initial exposure (infection) event, it is likely to result in tens to hundreds of additional exposures in the tomato crop**, depending on what setting the initial exposure has occurred in (i.e. potentially hundreds of exposures in a commercial greenhouse, or in a commercial outdoor crop of table tomatoes, or in an outdoor, commercial field crop; and potentially tens of exposures in a home garden setting). These numbers of exposures are considered to be above the threshold for concern.

Establishment of PepMV in New Zealand requires the following scenarios, some of which depend on virus transmission to other host species:

- Persistence of the infected tomato plant and/or infected crop, or;
- Contaminated seeds derived from the original infected plant must generate infected new tomato plants in subsequent seasons, or;
- The virus must be transmitted to other host species that can either persist longer than one season (e.g. perennials), or generate seeds or tubers contaminated with the virus.

The following paragraphs will deal with these scenarios.

Persistence of the infected plant or crop: Once PepMV has infected a tomato plant, the virus persists systemically within the plant and is considered established in the environment until the plant dies. The virus is highly likely to spread throughout a tomato crop as a result of bumblebee activity, routine crop handling procedures and from direct plant-to-plant contact. Closed recirculation systems for delivering irrigation and nutrient solution to tomato plants can also spread the virus if the fungal vector *Olipidium virulentus* is also present (Alfaro-Fernandes et al., 2010). Infected plants do not always show symptoms; growers with symptomless infections would have no warning that their plants are infected with the virus and so would not know to take precautions to stop the spread of the virus.

With regard to whether PepMV can persist through to the next cropping season, it depends if decaying or decayed tomato material remaining in the soil at the end of a season provides a source of persistent PepMV. Tomato plants are annuals so they die after fruiting at the end of the season; or in the case of a commercially grown plant, the plant is destroyed at the end of a tomato production season. However, PepMV association with the fungus *O. virulentus* is a potential mechanism for PepMV to persist in soil and growing media in New Zealand[33]. The likelihood of this is unknown, but is considered non-negligible because *O. virulentus* resting spores retain the ability to transmit viruses for decades in infested soil (Maccarone et al., 2010).

Other ways that PepMV can establish in New Zealand beyond the first growing season are via spread to other crops, or if the infected plant produces contaminated seeds that germinate the following season, and produce a new crop of infected plants.

Can infected seeds derived from the original infected plant generate new infected tomato plants in subsequent seasons? It is common practice for home gardeners to retain tomato seed from their own crops and reuse them the following season. As PepMV can occur asymptomatically in tomato plants, it is conceivable that the fruit selected for seed extraction happens to be infected with PepMV without the grower knowing. Most commercial growers, whether they grow indoors or outdoors, are

33 The root-infecting fungus *Olpidium virulentus*, (which is considered widespread in New Zealand (Fletcher et al 2005) and has been associated with tomato crops in New Zealand (Thomas, 1972)), can transmit PepMV to tomato plants (Alfaro-Fernandez et al., 2010).

unlikely to deliberately retain seed from one season for the purposes of generating next season's plants.

Accidental production of plants from seed derived from infected plants could occur as a result of fruit drop and the seed being left in the growing media (or soil), leading to self-sown plants. This is unlikely to occur in commercial greenhouses, commercial outdoor crops of tomato table tomatoes, or in commercial outdoor fields of 'process' tomatoes, because of commercial horticultural practices (see section 2.3.4 for detailed reasoning). However, it is considered that there is a low to moderate likelihood of home gardeners keeping self-sown plants, which might originate from composted tomato fruit, or from fruit that has dropped directly into the garden. But given that the seed-to-seedling transmission is low, the likelihood of self-sown plants being infected with the virus is considered to be low.

<u>Can PepMV establish in New Zealand by being transmitted to other host species?</u>
Some potato cultivars have been identified as naturally occurring hosts (CSL, 2005) as well as experimental hosts, so potato (*Solanum tuberosum*) is considered to be a potential host plant in the New Zealand environment. Other potential host species in New Zealand are basil, and weedy species (e.g *Chenopodium murale*, *Calystegia sepium*, *Rumex* sp. and *Diplotaxis* species). Aubergine has been identified as a host only under experimental conditions, using inoculation methods unlikely to occur in the environment. It is highly uncertain whether aubergine is likely to become a host under non-experimental conditions.

Crop management practices offer an opportunity for mechanical transmission of the virus from tomato to another species, in both commercial greenhouses and in the home garden[34]. The transmission opportunity occurs to a greater or lesser extent depending on the recipient crop species and requirements for crop handling. However, even though PepMV is highly contagious and easily transmitted mechanically between tomato plants, it is unknown whether this is true for transmission between species.

The widespread nature of the soil fungus *O. virulentus*, which can vector PepMV in hydroponic systems and is assumed to be capable of vectoring PepMV in non-hydroponic systems, adds to the likelihood that PepMV can establish in the New Zealand environment.

Transmission of PepMV by bumblebees to other plant species is another major factor to consider in environmental establishment. Tomatoes grown in any of the production systems could be a source of virus, including commercial glasshouses because there are likely to be opportunities for bumblebees to occasionally escape these structures. But the tomato crops grown outdoors, either commercially or in home gardens, are likely to present the greatest likelihood because bees can move freely from species to species in the outdoor setting.

Spread of the virus to weed species has the potential to result in a reservoir of the virus in the environment that may persist by continual transmission by bee activity, by mechanical transmission via plant to plant contact, or potentially via seed transmission; (seed transmission has only been reported in tomatoes but can not be ruled out for other host species). In Canada the weed species that contained PepMV did not overwinter, so did not appear to represent a significant role in the epidemiology of this disease in Onatario (Stobbs et al., 2009). However, as the New Zealand environment does not have harsh winters, it is possible for weed species at various stages of their life cycle to occur during winter and so overwintering would be possible.

34 Refer to 'PepMV Appendix' at the end of this pest risk assessment for more detailed explanations.

Finally, since first appearing in protected tomato crops in Europe in 1999, PepMV has displayed a high potential to adapt to diverse environmental conditions. It has become established in tomato-producing regions worldwide (Hanssen & Thomma, 2010). It is unlikely that practices and conditions for tomato production in New Zealand are so markedly different from Europe and the United States that the ability of the virus to establish in this country is significantly different to those countries.

Considering all of these factors, the likelihood of PepMV establishing and spreading in the New Zealand environment is considered to be moderate.

Given that:

- PepMV can easily spread throughout a tomato crop by crop handling. It can also be spread via bumblebee activity, and via the soil fungus *Olpidium virulentus*;
- Tomato plants are annuals and they do not persist beyond one season, but as PepMV is associated with the soil fungus *O. virulentus*, which is widespread in New Zealand, there is a potential mechanism for PepMV to persist in New Zealand soils.
- PepMV could establish in New Zealand via self-sown tomato seeds from infected fruit, or from deliberate seed harvesting and growing; the likelihood of PepMV establishing this way is considered to be low (due to the low seed-to-seedling transmission rate) and is restricted to home garden situations.
- PepMV can be transmitted from tomatoes to other species by bumblebees,
- There is uncertainty about the efficiency of mechanical transmission between crop species;
- Host species that could enable persistence in the environment include basil, weedy species, and possibly potatoes (via infected tubers).
- PepMV has become established in tomato-producing regions worldwide;
- The production systems most likely to be a source of virus for environmental establishment pathways are considered to be tomato crops grown in home gardens (via bumblebees, mechanical transmission, vectoring by *O. virulentus*), and by commercial crops grown outdoors (bumblebees are free to move from species to species; vectoring by *O. virulentus*), followed by commercial greenhouses (potential for bumblebees to escape greenhouse can not be ruled out);

The likelihood of PepMV establishing and spreading in tomato crops where the initial infection occurs, and for that first season, is considered to be high.

The likelihood of PepMV establishing in the New Zealand environment is considered to be moderate, and is most likely to originate from the occurrence of PepMV in home garden tomato crops or in outdoor commercial crops.

The likelihood of PepMV establishing in New Zealand via transmission from commercial greenhouse crops is low because it is dependent on bumblebees escaping from greenhouses.

The likelihood of the organism spreading in New Zealand beyond the initially infected area is considered to be moderate.

5.1.2.5 Consequence assessment

Economic consequences

Tomato is likely to be the main crop affected by PepMV in New Zealand. The symptoms of PepMV on individual tomato plants are highly variable, ranging from symptomless to severe symptoms. Consequently the impact on crops has varied from no impact, to significant reduction in fruit quality. The impacts on tomato plants are influenced by the PepMV strain(s), the tomato cultivar, environmental conditions, presence of the fungus *Olpidium brassicae* (syn *O. virulentus*) and the timing of crop infection (i.e. early or late in season; see Hanssen & Thomma, 2010).

Previously estimated crop losses are as follows:

- An infected crop in Ireland and some in the Netherlands had no noticeable symptoms, nor recognised yield or quality losses (CSL, 2005).
- In a United Kingdom trial, replicating commercial production and done to define the impact of PepMV, bulk yields were not reduced. But quality of fruits harvested was reduced significantly – although the quality was variable from trial to trial. Trial 1 had 6.8% fruit downgraded from class 1, but trial 2 had 38% of class 1 fruit downgraded. Loss of quality was mainly a result of blotchy ripening, gold marbling, gold spot, and symptoms directly attributed to PepMV infection. PepMV infection also affected fruit size (Spence et al., 2006).
- A European Union study did semi commercial-scale trials over two consecutive seasons in four major tomato growing areas (Hungary, the Netherlands, Spain and UK) to assess the economic impact of PepMV under different environmental and cropping conditions. A mild isolate of PepMV had no effect on fruit yield. An aggressive PepMV isolate reduced the yield of Class I fruit by between 10 and 29%. The total fruit yield losses were reduced by between 4 and 11% (DEFRA, 2010).
- In Spain in the 1999/2000 season, the incidence of collapsed plants was variable between infected farms, ranging from 25 to 90%. Collapse of the plants was associated with the presence of both PepMV and the fungus *O. brassicae* (syn *O. virulentus*) (Soler-Aleixandre et al., 2005).

Tomato is an important crop in New Zealand. Domestic sales of tomatoes in 2008/2010 were worth $113 million, and exports worth $14 million (Plant and Food Research, 2010). The evidence presented above shows that the impact involves reduction in both quality and yield of tomatoes. Although growers of both table tomatoes and 'process' tomatoes are likely to be affected, the impact on growers of table tomatoes is likely to be greater because their returns are more dependent on fruit aesthetic. The economic losses to individual growers are likely to vary from year to year depending on factors such as the PepMV strain, the tomato cultivar, and the environmental factors of that season, and on the price differential between the highest quality fruit, and fruit which has reduced quality due to PepMV. Co-infection with the fungus *O. virulentus* has the potential to cause collapse of a high percentage of plants and so could have a high economic impact on individual growers.

Other New Zealand grown crop species that are natural hosts of PepMV are basil and (*Ocimum basilicum*), potato (*S. tuberosum*).

- The impact on basil is uncertain. There is only one report about PepMV infection in basil and it reported symptoms of chlorotic spots and leaf deformation (Davino et al., 2009).
- No data could be found about impacts of PepMV on potato crops in the field. Therefore, it is uncertain what the impact in New Zealand potato cultivars would be in a field setting. Symptoms described in an experimental setting range from 'symptomless infection' for some cultivars, to 'severe systemic necrosis' in other cultivars (Jones et al., 1980). The total retail and export value of the potato industry, including what householders and restaurants buy,

what is bought by local processors and what is exported, is estimated at $382 million a year (Potatoes New Zealand, 2011).

The potential economic consequences for individual growers are likely to vary from season to season and could range from low to high, but considered to be low from a national economic perspective.

Environmental consequences

PepMV primarily infects species of Solanaceae. There are three species of Solanaceae that are native to New Zealand: *Solanum avicular* (poroporo), *S. laciniatium* (poroporo, bullibulli) and *S. nodiflorum* (small flowered nightshade) (Landcare NZ plants database, 2011). As bumblebees can vector PepMV between plant species, this is a mechanism to enable native species to become infected. However, there is a high degree of uncertainty about whether these plants would become hosts of PepMV. Based on the proportion of symptomatic species identified in experimental settings, it is moderately likely that these species would be symptomless if they did become hosts.

PepMV occurs in some weed species in Europe and Canada, but no negative environmental impacts have been reported.

The potential environmental consequences within New Zealand are uncertain, but could range from negligible to low.

Human health consequences

There are no known human health consequences of PepMV.
The potential human health consequences within New Zealand are considered to be negligible.

Socio-cultural consequences

Many New Zealanders are avid gardeners and enjoy growing their own crops of tomato, potato, and aubergine. In any home garden where PepMV has established in potatoes, if the grower retains tubers for seeding the next crop, garden potato crops are likely to be infected year after year. However, there has been no report of symptoms or impacts in potato outside of the laboratory setting. In the laboratory setting the symptoms were cultivar dependent, and ranged from symptomless to severe across a variety of cultivars (Jones et al 1980).

The likelihood of tomato crops in a home garden being infected the following year, as a result of seed-to-seedling transmission from retained seed, is considered to be low because the virus transmission rate is low. In home gardens, the likelihood of PepMV occurring the following year due to the soil fungus *Olpidium virulentus* vectoring PepMV into new tomato crops is unknown. Symptoms on home-grown tomatoes could range from negligible to severe. These impacts are unlikely to change a way of life, and are more likely to be of annoyance.

For some iwi in New Zealand, the poroporo plant is an important source of food and medicine (The Encylcopedia of New Zealand, 2011). It is uncertain whether poroporo is capable of being a host of PepMV. If it can be a host of PepMV it is likely that only a portion of the poroporo population becomes infected. The consequences of infection in poroporo are likely to range from negligible to low.

The potential socio-cultural consequences within New Zealand are somewhat uncertain and considered to be low.

5.1.2.6 Risk estimation

The likelihood of PepMV entry into New Zealand on tomato seed is high, as is the likelihood of exposure, but the frequency of these events is uncertain. PepMV is highly likely to establish and spread throughout a tomato crop if exposure occurs because it is highly contagious and easily spread between tomato plants. However, the likelihood of PepMV establishment beyond one growing season, (i.e. in the New Zealand environment in self-sown tomatoes and/or other species) is considered to be moderate. The virus can be transmitted by the soil fungus *Olpidium virulentus* (synonym of *O. brassicae* which is widespread in New Zealand). The likelihood of PepMV spreading beyond the initial infection area is also considered to range from moderate.

The economic impacts of PepMV are most likely to occur in tomato crops and are likely to vary from very low to high depending on factors such as the PepMV strain, tomato cultivar, environmental factors, and co-infection with *O. virulentus*. **These consequences are mostly independent of environmental establishment of PepMV and could arise every year simply through the importation and growing of infected seed.**

The environmental and socio-cultural impacts could range from negligible to low.

See the table below for a visual summary of the risk estimation.

Risk estimation table: *PepMV* on tomato seeds from all countries				
	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry				
Exposure				
Establishment			(Environmental)	(Within a crop)
Spread			(Environmental)	(Within a crop)
Consequences of establishment				
Economic		?	?	?
Environmental	?	?		
Socio-cultural	?	?		
Human Health				

? denotes uncertainty.

In consideration of these assessments, PepMV is considered to be a non-negligible risk on imported tomato seeds. The risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.

5.1.3 Risk management – efficacy of risk management options

Risk Management for all the viruses and viroids assessed is considered in section 6.

5.1.4 References for PepMV

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5.1.5

5.1.6 Appendix for PepMV

Regarding Establishment and Spread: Detailed explanation of opportunities for mechanical transmission of PepMV between species:

- Aubergines can be mechanically inoculated in experimental conditions, therefore they are possible hosts in gardening/cropping situations. In the home garden, spread could occur to aubergine as a result of routine plant handling (uncertain likelihood); to potato (with low likelihood because potatoes require very little handling); to basil (moderate to high likelihood because it would be common for a home gardener to harvest a tomato and some basil leaves at the same time);
- Aubergines are sometimes grown alongside tomatoes in the same commercial greenhouses (John Seymour, personal communication 2011), so mechanisms for tomato to aubergine transmission are likely to exist with crop handling and maintenance activities. However, as aubergine plants are likely to be destroyed at the end of the harvest season, infected aubergine plants pose no greater likelihood of long-term establishment of PepMV than tomatoes.
- Transmission from commercial greenhouse tomato crops, to commercial potato crops is unlikely because of a lack of mechanical transmission opportunities. But if potatoes did become infected, tubers are likely to become infected and be a source of virus establishment and spread in the environment (Jones et al 1980).
- There are likely to be opportunities for tomato to capsicum mechanical transmission in home gardens, and some commercial greenhouses. But *Capsicum annuum* is a poor host of PepMV, and has only been detected as a host of the LP-strain in an experimental setting. *Capsicum* plants are annuals that do not persist beyond one year, and there is no record of seed transmission. Therefore, the likelihood of spread to capsicum and establishment of a reservoir in capsicum seed is very low, bordering on negligible.

5.2 Pelargonium zonate spot virus (PZSV)

Scientific name: *Pelargonium zonate spot virus*
Family/Genus: *Bromoviridae*/ *Anulavirus*
Acronym: PZSV

5.2.1 Hazard identification

5.2.1.1 Description

Pelargonium zonate spot virus (PZSV) is a plant pathogenic virus that causes concentric chlorotic or necrotic rings and line patterns on leaves, stems, and fruit of tomato and capsicum plants.

5.2.1.2 New Zealand status

PZSV is not known to be present in New Zealand. Not recorded in: Pearson et al., 2006; PPIN, 2011.

5.2.1.3 Geographic distribution

As at November 2011, PZSV has been recorded from Italy, Spain, France, the USA (California), Israel (Lapidot et al., 2010) and Australia (Luo et al., 2010).

5.2.1.4 Commodity association

The virus is seed transmitted in tomato plants, as seed from infected tomato plants gave rise to infected seedlings with a seed-to-seedling transmission rate of 11-29% (Lapidot et al., 2010). PZSV infects capsicum (Lapidot et al., 2010; Escriu et al., 2009) but is not known to be seed transmitted in capsicum although it is seed borne in several hosts (Lapidot et al., 2010).

5.2.1.5 Plant associations

PZSV was originally isolated from *Pelargonium zonale* (common Geranium) plants but has since been found in tomato (*Solanum lycopersicum*) and capsicum (*Capsicum annuum*) plants, and also *Nicotiana glutinosa* and *Diplotaxis erucoides* (an endemic weed in southern Italy that remains symptomless after infection) (Luis-Arteaga & Cambra, 2000; Lapidot et al., 2010; Escriu et al., 2009). It is found on weeds such as *Capsella bursa-pastoris, Chrysanthemum segetum, Picris echioides,* and *Sonchus oleraceus* (Gallitelli et al., 2005).

PZSV has been detected in kiwifruit (*Actinidia deliciosa*) in Italy (C Ratti pers. comm. 2011).

It has also been reported in globe artichoke (*Cynara scolymus*) (ICTVdb, 2011), and *Cakile maritima* (Luo et al., 2010). Experimentally, it has been transmitted via mechanical inoculation to 29 species of plants in nine dicotyledonous families (Gallitelli et al., 2005).

5.2.1.6 Potential for establishment and impact

PZSV is found in countries with similar tomato growing conditions to New Zealand, e.g. tomato and capsicum production systems in both glass-house crops and outdoors. Therefore PZSV could potentially establish in New Zealand under local conditions. PZSV has the potential to have a negative impact in New Zealand as it induces severe disease symptoms in tomato plants.

5.2.1.7 Hazard identification conclusion

Given that *Pelargonium zonate spot virus*

- Is seed transmitted in tomato seed; and seed transmission in *Capsicum* is unknown;
- Is not present in New Zealand;
- Can potentially establish in New Zealand;
- Causes disease to plant species of economic importance to New Zealand, i.e. tomatoes;

Pelargonium zonate spot virus is therefore considered a hazard on tomato seed in this risk analysis, and could possibly be a hazard on capsicum seed.

5.2.2 Risk assessment

5.2.2.1 Biology

Pelargonium zonate spot virus (PZSV) is a plant pathogenic virus that causes concentric chlorotic or necrotic rings and line patterns on leaves, stems, and fruit of tomato and capsicum plants.

<u>Transmission</u>
PZSV is seed transmitted in *D. erucoides, Nicotiana glutinosa* and *S. lycopersicum*, with efficiency in *N. glutinosa* and *D. erucoides* being approximately 5% (Gallitelli et al., 2005; Lapidot et al., 2010). None of the chemical treatments of tomato seed (hydrochloric acid, trisodium phosphate, or sodium hypochlorite) removed PZSV from the seed, suggesting that this virus may occur within the seed, rather than on the seed surface (Lapidot et al., 2010).

PZSV is readily transmitted mechanically from the sap of infected plants to indicator plants using carborundum as an abrasive (Lapidot et al., 2010). None of the scientific articles about PZSV reports whether the virus is easily transmitted between tomato plants as a result of routine plant handling and maintenance activities, but it is assumed that it is.

PZSV is transmitted by tomato pollen. Pollen from infected tomato plants contains PZSV; when it was used in an experiment to pollinate flowers of uninfected mother plants, the mother plants did not become infected with PZSV, but the seeds from the resultant fruit transmitted PZSV to seedlings (Lapidot et al., 2010).

It is uncertain whether PZSV is transmitted by thrips[35].

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35 Vovlas et al. (1989) attempted to investigate transmission of PZSV by the thrips species *Megalothrips spinosus*, and showed that one out of the ten plants tested became infected with the virus. However, because the experiment was uncontrolled and had a very small sample size, this result was inconclusive; the possibility of accidental mechanical inoculation due to insect and plant handling during the experiment set up could not be ruled out (Lapidot et al. 2010).

Symptomatology:
PZSV induces severe disease symptoms in tomato plants, characterised by chlorotic and necrotic ring and line patterns on the leaves and fruit together with plant stunting, leaf malformation, and reduced fruit set, which often result in plant death (Lapidot et al., 2010). The Italian weed *Diplotaxis erucoides* can be infected naturally but remains asymptomatic.

There are no specific reports of asymptomatic infections in tomato and capsicum. But as it is common for viruses and viroids to occur both symptomatically and asymptomatically in tomato, it can not be ruled out that this virus could sometimes occur asymptomatically in tomatoes and capsicum.

5.2.2.2 Entry assessment

PZSV has not been recorded in commercial tomato seed production facilities, but it is seed transmitted in tomato, and it is not known if commercial seeds are tested for presence of PZSV, so it could potentially enter New Zealand through the tomato seed import pathway. Seed-lots entering New Zealand are considered to originate from all over the world, including from countries that are known to have PZSV in tomatoes (Italy, Spain, France, the USA (California), Israel and Australia) (Lapidot et al. 2010, Luo et al., 2010).

It has been described as prevalent in tomato crops for canning in Italy, where field surveys have shown that incidence of the disease is usually below 1% but may reach 30% in rows of plants growing in the vicinity of roads and irrigation canals (Vovlas et al., 1989). If this is typical, and is the same in tomatoes grown for commercial seed, it seems that the incidence of the virus can be relatively high.

It is not known if PZSV is seed borne in capsicum seed. However, as this virus is seed-borne in at least three of its host species (tomato, *N. glutinosa*, *D. erucoides*), PZSV can not be ruled out as a seed-borne virus in capsicum.

As a visual inspection of a consignment of tomato or capsicum seeds will not enable detection of contaminated seeds, it is considered highly likely that if tomato seeds are infected with PZSV they will enter New Zealand.

Given that:

- The presence of PZSV has not been recorded in commercial tomato seed, but it is not known to what extent commercial seed has been tested for presence of PZSV;
- PZSV is seed transmitted in tomato and in at least three of its host species;
- PZSV can not be ruled out as a seed-borne virus in *Capsicum*;
- Tomato seed is imported into New Zealand from countries where PZSV has been recorded;
- Infected seed cannot be detected by visual inspection of the seed;

The likelihood of tomato or Capsicum *seed infected with PZSV entering New Zealand is uncertain (due to a lack of relevant information), but in the context of millions of seeds being imported into New Zealand over time, it is considered to be non-negligable.*

5.2.2.3 Exposure assessment

This section assumes that PZSV-infected seed has already entered New Zealand. Imported seeds for sowing would be planted in glasshouses or outside. Many tomato plants that grow from infected seeds are likely to be infected. Seed from infected tomato plants has been recorded as giving rise to infected seedlings with a seed-transmission rate of PZSV of 11 -29% (Lapidot et al., 2010).

Seed-to-seedling transmission rate in capsicum is not known as it is not known whether PZSV is transmitted in capsicum seed. However, as this virus is transmitted to seedlings in other host species, it cannot be ruled out for capsicum.

In the context of millions of seeds being planted in New Zealand over time, the likelihood of PZSV exposure to a tomato seedling is considered to be is high.

In the context of millions of seeds being planted in New Zealand over time, the likelihood of PZSV exposure to a capsicum seedling is considered to be high. ***This rating is made with high uncertainty.***

5.2.2.4 Assessment of establishment and spread

This section assesses establishment on the basis that PZSV has already been exposed to (infected) a new host plant. PZSV establishment in the environment is affected by the number of tomato plants exposed to (infected with) PZSV and whether that number is above the threshold for concern. As PZSV is assumed to spread within a tomato crop as a result of routine crop handling and maintenance, **even if there is only one initial exposure (infection) event, it is likely to result in tens to hundreds of additional exposures in the tomato crop**, depending on what setting the initial exposure has occurred in (i.e. potentially hundreds of exposures in a commercial greenhouse, or in a commercial outdoor crop of table tomatoes, or in an outdoor, commercial field crop; and potentially tens of exposures in a home garden setting). These numbers of exposures are considered to be above the threshold for concern.

The same is assumed to hold true for capsicum crops, **but it should be noted that initial exposure in capsicum crops is based on an <u>assumption</u> that seed-to-seedling transmission occurs (based on an extrapolation of evidence about seed transmission in its other host plants). But as this is highly uncertain, establishment via capsicums is also highly uncertain.**

For establishment of PZSV to last beyond the first season either
- The infected plant must persist; or
- Seeds from the infected plant must be used to produce new infected plants; or
- Infection must spread to other host plants that persist or can spread the infection further.

Spread of PZSV within tomato and capsicum crops, and the possibility of the infected plant persisting beyond one season
Once a plant is infected with PZSV it persists in the plant until the plant dies. As it is assumed that PZSV is readily transmitted between plants as a result of routine crop handling procedures, or through tomato pollen, the virus is highly likely to spread throughout any tomato or capsicum crop. It is assumed that there can be asymptomatic infections of PZSV in tomato and capsicum, particularly in the early stages of systemic infection. Therefore, there may be a period when infected plants are not detected and this would be a contributing factor to the virus spreading to other plants in the crop before the grower was aware of it.

However, tomato and capsicum plants are annuals so they die after fruiting; or in the case of plants grown for commercial production of tomato and capsicum fruit, the plant is destroyed at the end of a tomato or capsicum production season. Therefore, it is not possible for the virus to establish in New Zealand beyond one growing season, unless it has already spread to other crops, or unless seed from infected plants are germinated the following season, and yield a new crop of infected plants. These other opportunities for establishment of PZSV are described below.

Seeds from the infected plant being used to produce new infected plants
Accidentally[36] vs intentionally germinated plants.
Most commercial growers of tomato and capsicum plants, whether they grow indoors or outdoors, are unlikely to intentionally retain seed from one season for the purposes of generating next season's plants. However, home gardeners are likely to collect seed for use in the following year, and disseminate this seed. The home grower would not necessarily know that the seed was contaminated if the plant is symptomless, particularly if the source of the seed-infection originated from infected pollen (see Biology section for details). Infected seeds could be moved around the country and provide a source of PZSV.

Accidental production of plants from seed derived from infected pollen or infected plants could occur as a result of fruit drop and the seed being left in the growing media (or soil), leading to self-sown plants. This is unlikely to occur in commercial greenhouses, commercial outdoor crops of table tomatoes, or in commercial outdoor fields of 'process' tomatoes, because of commercial horticultural practices (see background information and assumptions for detailed reasoning). However, it is considered that there is a low to moderate likelihood of home gardeners keeping self-sown plants, which might originate from composted tomato fruit, or from fruit that has dropped directly into the garden.

In summary, there are opportunities in home gardens for seeds from the originally PZSV-infected tomato plant to be used to produce newly infected tomato plants, both intentionally and accidentally. It is considered that there is a low to moderate likelihood that PZSV can establish in the New Zealand environment via progeny seed.

Spread of PZSV to other plant species
Other host plants of PZSV that occur in New Zealand include *Pelargonium zonale* (common geranium, found in home gardens throughout New Zealand) and the weeds *Cakile maritima* (occurring in Auckland, Bay of Plenty, East Cape, Gisborne, Hawke's Bay), *Capsella bursa-pastoris, Chrysanthemum segetum, Sonchus oleraceus,* and globe artichoke (*Cynara scolymus*) which is grown as an ornamental or crop plant in home gardens and also occurs as a weed in riverbeds, roadsides, and waste land (Landcare Research, 2011). Kiwifruit (*Actinidia deliciosa*) is a recently reported host of PZSV.

Transmission of PZSV to ornamentals or crop plants in home gardens is conceivable via mechanical means, during plant handling. The likelihood of this is considered to be low to moderate.

As weedy species are not handled by workers (except for the purpose of removal or destruction) transmission of PZSV to weedy plants would be reliant on transmission via insects. Due to a lack of quality scientific evidence about insect transmission of PZSV, it is uncertain whether insects (such as bumblebees or thrips) can transmit the virus. But given that PZSV does occur in a number of weedy

36 Plants that have arisen from self-sown seed

species in Italy, it is assumed that insect transmission was a factor in them being infected. Tomato crops grown outdoors in New Zealand would present the means for insects to transmit the virus from tomatoes to weedy species, with a low to moderate likelihood. If glasshouse crops are not insect-free, and the insects can escape from the glasshouse, then these crops will also be a potential source of PZSV infection into the wider environment.

Long distance spread:
Within New Zealand, the PZSV could be spread beyond the initial area of infection through the movement of infected seed distributed by home gardeners.

If insect transmission of PZSV occurs, this would also be a mechanism for the virus to spread over larger distances, with time.

Fruit from infected tomato or capsicum plants may be sold for consumption, and what isn't consumed may be discarded. There may be some possibility of PZSV spreading this way, but this isn't considered likely to be a major contributor to spread of the virus.

Given that:
- It is considered likely that PZSV in capsicums or tomatoes could spread through a crop in a single season by mechanical means; but they are annuals and do not persist beyond one season;
- PZSV could establish in New Zealand via self-sown tomato seeds from infected fruit, or from deliberate seed harvesting and growing. The likelihood of PZSV establishing in this way is considered to be low to moderate and is restricted to home garden situations.
- There are minimal opportunities for PZSV to be mechanically spread to other host species by workers/gardeners because most of them are weedy, with the exception of globe artichoke;
- There is uncertainty about the ability of insects to transmit PZSV, but it is assumed that insects plays a role in the spread of PZSV;
- The production systems considered most likely to contribute to the establishment of PZSV in the environment are crops grown in the home garden, or grown outdoors (including commercial crops) where insects are free to feed and move between host species.

The likelihood of PZSV establishment and spread is in tomato and/or capsicum crops where the initial infection occurs, and for that first season, is considered to be high.

The likelihood of PZSV establishing in the New Zealand environment (beyond one season) is uncertain but considered to range from low to moderate. It is most likely to originate from the occurrence of PZSV in the home garden or in outdoor commercial crops of tomato.

The likelihood of PZSV establishing in New Zealand via transmission from commercial greenhouse tomato crops or capsicum crops is uncertain, but no higher than low. It would be dependent on insects escaping from greenhouses and spreading the virus, which is an uncertain mechanism.

The likelihood of PZSV spreading in New Zealand beyond the initially infected area is considered to be low to moderate.

5.2.2.5 Consequence assessment

Economic consequences

Tomatoes are important crops in New Zealand. Domestic sales of tomatoes in 2008/2010 were worth $113 million, and exports worth $14 million (Fresh facts 2010). PZSV has been reported as a severe disease of tomatoes, which often results in plant death (Vovlas et al. 1989). In Italy incidence has been reported at usually below 1% (Vovlas et al., 1989) so if this is typical of PZSV then it is unlikely to have major negative impacts on tomato crops.

PZSV has been reported on capsicum crops (Escriu et al., 2009), but there is no information on its impact on capsicum.

In Italy, PZSV has been found on kiwifruit plants which show symptoms on leaves and fruit. Leaves show chlorotic rings and in some cases necrosis. Fruit shows depressed areas and consequently a slight deformation. As no causal link between the virus and the symptoms has yet been shown (C. Ratti pers. comm. 2011), the potential impact of PZSV in New Zealand kiwifruit crops is unknown.

The potential economic consequences for New Zealand tomato and capsicum growers are considered to be low.

The potential economic consequences for New Zealand kiwifruit growers is not known, so a moderate impact cannot be ruled out.

Environmental consequences

Known natural hosts are in the families Geraniaceae, Solanaceae, Cruciferae, and Asteraceae. There are New Zealand native species in the Solanaceae (e.g. poroporo), and Cruciferae (*Pachycladon* spp., *Lepidium* spp., *Notothlaspi* spp.) (Landcare Research, 2011). Consequences for infected plants may include reduced fruit set impacting population recruitment and leaf malformation impacting photosynthetic capacity, and perhaps plant death. There is a very low likelihood that these plants could be infected by PZSV but there is high uncertainty about this.

The potential environmental consequences within New Zealand are considered to be low.

Human health consequences

There are no known human health consequences.

Socio-cultural consequences

Poroporo has an edible fruit eaten by Maori. There is a very low probability that it could be infected with PZSV, and plants could be stunted or the yield of berries could be reduced, but there is very high uncertainty about this. There is also a possibility that home gardener’s tomato or capsicum plants become infected with PZSV which could lead to reduced yield of fruit or having to destroy the plants to prevent further spread of the virus.

The potential socio-cultural consequences within New Zealand are considered to be low.

5.2.2.6 Risk estimation

PZSV is a relatively newly described virus occurring in tomato and capsicum plants. It has not been well studied, so the number of publically available scientific reports about it are relatively few, particularly relating to it’s associated with *Capsicum* seeds. Consequently, **the risk estimation about PZSV in *Capsicum* seeds has a high degree of uncertainty**, compared with the risk estimation about PZSV in tomato seeds.

The likelihood of entry on tomato or capsicum seed is uncertain, but non-negligable. The likelihood of exposure is high because PZSV is transmitted from seed to seedling in tomatoes, and assumed to be in capsicum. The likelihood of PZSV establishment and spread in tomato and/or capsicum crops where the initial infection occurs, and for that first season, is considered to be high. But the likelihood of PZSV establishing in the New Zealand environment (beyond one season) is uncertain - considered to range from low to moderate. It is most likely to originate from the occurrence of PZSV in the home garden or in outdoor crops of tomato. The likelihood of PZSV establishing in New Zealand via transmission from commercial greenhouse tomato or capsicum crops is low and dependent on insects escaping from greenhouses and spreading the virus. The likelihood of PZSV spreading in New Zealand beyond the initially infected area is considered to be low to moderate.

The potential economic consequences for tomato and capsicum growers are low, but unknown for kiwifruit growers; so overall the economic impacts could range from low to moderate. The potential environmental consequences are low, and the potential socio-cultural consequences are low.

Risk estimation table: *Pelargonium zonate spot virus* on tomato or capsicum seeds from all countries

	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry		Uncertain; non-negligable	Uncertain; non-negligable	Uncertain; non-negligable
Exposure				
Establishment		(Environmental) ?	(Environmental) ?	(Within a crop)
Spread		(Environmental) ?	(Environmental) ?	(Within a crop)
Consequences of establishment				
Economic		?	?	
Environmental				
Socio-cultural				
Human Health				

In consideration of these assessments, PZSV *is considered to be a non-negligible risk on both imported capsicum and imported tomato seeds. The risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.*

5.2.3 Risk management – efficacy of risk management options

Risk Management for all the viruses and viroids assessed is considered in section 6.

5.2.4 References for PZSV

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5.3 Tobacco mild green mosaic virus (TMGMV)

Scientific name: *Tobacco mild green mosaic virus*
Family/Genus: *Virgaviridae / Tobamovirus*
Acronym: TMGMV

5.3.1 Hazard identification

5.3.1.1 Description

Tobacco mild green mosaic virus (TMGMV) is a plant pathogen causing disease in a number of hosts including *Capsicum* species.

5.3.1.2 New Zealand status

TMGMV is not known to be present in New Zealand. Not recorded in: Pearson et al. (2006), PPIN (2011).

5.3.1.3 Geographic distribution

As at November 2011: TMGMV probably occurs in all regions where the host *Nicotiana glauca* is distributed: North America, Australia, and many European and African countries including England (Wetter, 1989; Skelton et al., 2010), but a detailed list of countries could not be found. TMGMV is also found in Panama (Herrera-Vasquez et al., 2009), Venezuela (Cordoba et al., 2006), Tunisia (Font et al., 2009) Japan (Miyazaki et al., 2010) and Taiwan (Li and Chang, 2005).

5.3.1.4 Commodity association

TMGMV is seed transmitted in *Capsicum chinense* (e.g. Habanero chillies)(Cordoba et al., 2006), and it is assumed that it is also seed transmitted in the related species *Capsicum annuum*.

5.3.1.5 Plant associations

Tomato is not a host of TMGMV (Ishibashi et al., 2011; Wetter, 1989). Tomato (*Solanum lycopersicum*) has been reported as a host (Alishiri et al., 2011), however this seems to be a case of misidentification of the pathogen because the sequence reported by Alishiri et al. (2011) is more similar to Tomato mosaic virus than TMGMV (K. Ishibashi, pers comm. 2011). Pepper species (*Capsicum annuum*, *Capsicum chinense*) have been reported as hosts (Cordoba et al., 2006; Herrera-Vásquez et al., 2009; Font et al., 2009). *Nicotiana glauca* is a host (Wetter, 1989).
The virus infects many solanaceous species including tobacco (*Nicotiana tabacum*) and also infects species of the families Amaranthaceae, Apocynaceae, Asteraceae (Compositae), Balsaminaceae, Commelinaceae, Gesneriaceae, Labiatae and Umbelliferae (Cohen et al., 2002; Skelton et al., 2010; Wetter, 1989).

5.3.1.6 Potential for establishment and impact

TMGMV may be able to establish in glasshouses or outside in New Zealand. There is some possibility that TMGMV may have negative impacts in New Zealand, as it has been reported causing symptoms on capsicum.

5.3.1.7 Hazard identification conclusion

Given that *Tobacco mild green mosaic virus*

- Is seed transmitted in *Capsicum chinense* seed, and association with seeds of *Capsicum annuum* can not be ruled out;
- Is not established in New Zealand;
- Could potentially establish in New Zealand;
- Is present in countries that export capsicum seed to New Zealand;
- Is recorded causing a disease to a plant species of economic importance to New Zealand (i.e. *Capsicum* spp.);

Tobacco mild green mosaic virus is therefore considered a hazard on *Capsicum chinense* and *Capsicum annuum* seed in this risk analysis. It is not considered a hazard on tomato seed.

5.3.2 Risk assessment

5.3.2.1 Biology

Tobacco mild green mosaic virus is single stranded RNA with tubular rod shaped morphology and particles c. 308 x 18 nm (Wetter 1989). The TMGMV capsid protein content makes up about 95% of the particle weight, with RNA being the remaining 5% (Gibbs 1988).

<u>Transmission</u>

It is transmitted by mechanical inoculation and handling during cultivation, transmitted by grafting (Gibbs, 1988; Wetter, 1989), and transmitted in the seed of *Capsicum chinense* (Cordoba et al. 2006). Transmission by insect vectors has not been recorded[37]. Tobamoviruses are not known to have insect vectors (M. Pearson pers comm. 2011).

<u>Symptomatology</u>

Symptoms on *Capsicum annuum* plants have been reported as mild chlorosis and necrotic lesions, leaf deformations and mild green and chlorotic mosaic (Li and Chang 2005, Font et al. 2009). Symptoms in *C. chinense* have been described as obvious curling and bubbling on leaves of the plants (Cordoba et al. 2006). Symptoms generally on other host plants are described as local lesions or yellow flecks in some hosts. In some hosts infection is non systemic while in others it has been described as systemic. Infection can also be symptomless on some hosts (Wetter, 1989), and although this has not been reported for *Capsicum*, the possibility that it is symptomless on this host can not be ruled out.

5.3.2.2 Entry assessment

TMGMV has not been reported from commercial capsicum seed lots, but it is not known to what extent commercial seeds have been tested for TMGMV. TMGMV is known to be seed transmitted in *C. chinense,* and is assumed to be seed transmitted in *C. annuum* so could be present in commercial seed lots. Capsicum seed-lots entering New Zealand are considered to originate from all over the world including from countries that are known to have TMGMV. The virus would not be detected on capsicum seed entering New Zealand as it can not be detected on visual inspection.

Given that:

- TMGMV has not been recorded from commercial seed lots, but it is not known to what extent commercial seed has been tested for the presence of TMGMV.

[37] Randles (1981) speculated the existence of airborne vector(s) of TMGMV in Australia, but there is no credible evidence to support this speculation.

- Capsicum seed entering New Zealand may originate from countries which have TMGMV;
- TMGMV would not be detected entering New Zealand as seed is not tested for TMGMV;

The likelihood of Capsicum *seed infected with TMGMV entering New Zealand is uncertain (due to a lack of relevant information), but in the context of millions of seeds being imported into New Zealand over time it is considered to be non-negligible.*

5.3.2.3 Exposure assessment

This section assumes that TMGMV-infected seed has already entered New Zealand. Imported seeds for sowing would be planted in glasshouses or outside. Seed-to-seedling transmission is known to occur in *Capsicum chinense*; however the rate has not been reported. Seed-to-seedling transmission can not be ruled out for *Capsicum annuum*. Consequently, some plants growing from infected seeds are likely to be infected.

In the context of millions of seeds being planted in New Zealand over time, the likelihood of TMGMV exposure to a Capsicum *seedling is considered to be high.*

5.3.2.4 Assessment of establishment and spread

This section assesses establishment on the basis that TMGMV has already been exposed to (infected) a new host plant. TMGMV establishment in the environment is affected by the number of capsicum plants exposed to (infected with) TMGMV and whether that number is above the threshold for concern. As TMGMV can readily spread within a capsicum crop as a result of routine crop handling and maintenance, **even if there is only one initial exposure (infection) event, it is likely to result in tens to hundreds of additional exposures in the tomato crop**, depending on what setting the initial exposure has occurred in (i.e. potentially hundreds of exposures in a commercial greenhouse, and potentially tens of exposures in a home garden setting). These numbers of exposures are considered to be above the threshold for concern.

For establishment of TMGMV to last beyond the first season either

- The infected plant must persist; or
- Seeds from the infected plant must be used to produce new infected plants; or
- Infection must spread to other host plants that persist or can spread the infection further.

Spread of TMGMV within capsicum crops, and the possibility of the infected plant persisting beyond one season

Once TMGMV has infected a plant, it persists systemically within the plant, until the plant dies.

The virus is highly likely to spread throughout a capsicum crop, via mechanical transmission, as a result of routine crop handling procedures. There is also a low likelihood that infection could spread from one infected glasshouse to another glasshouse, or to host plants growing outside the glasshouse, by mechanical transmission by workers.

Although TMGMV can easily spread through a capsicum crop, capsicum plants are annuals so they die after fruiting; or in the case of plants grown for commercial production of capsicum fruit, the plant is destroyed at the end of a capsicum production season, and the glasshouses sanitised. It is expected that this will lead to elimination of TMGMV from glasshouses in most cases.

Apart from possible reoccurrence of infection in glasshouses, it is not possible for the virus to establish in New Zealand beyond one growing season, unless it has already spread to other crops, or unless seed from infected plants are germinated the following season, and yield a new crop of infected plants.

Seeds from the infected plant being used to produce new infected plants

Accidentally[38] vs intentionally germinated plants.

Most commercial growers of capsicum plants, whether they grow indoor or outdoors, are unlikely to intentionally retain seed from one season for the purposes of generating next season's plants. However, some home gardeners do collect seed for use for the following year, and share it with others. Seeds collected from infected plants could be moved around the country and provide a source of new TMGMV infections. While seed from severely infected capsicum plants may not be kept because the plants are likely to show obvious symptoms of infection, it may be possible that some infected capsicum plants are symptomless or show only mild symptoms, or home growers may not recognise that they should not keep seeds from diseased plants.

Accidental production of plants from seed derived from infected plants could occur as a result of fruit drop and the seed being left in the growing media (or soil), leading to self-sown plants. This is unlikely to occur in commercial greenhouses because of commercial horticultural practices (see section 2.3.4 for detailed reasoning). *Capsicum anuum* is reported as a naturalised plant in New Zealand (Williams & Randall, undated), but it considered to have a lower propensity for weediness than tomatoes. Therefore, it is considered that there is a low likelihood of home gardeners keeping self-sown plants, which might originate from composted capsicum fruit, or from fruit that has dropped directly into the garden.

In summary, there are opportunities in home gardens for seeds from the originally TMGMV-infected capsicum plant to be used to produce newly infected capsicum plants, intentionally or accidentally. It is considered that there is a low to moderate likelihood that TMGMV can establish in the New Zealand environment via progeny seed.

Spread of TMGMV to other plant species

Methods of spread

TMGMV may be able to be spread to other species mechanically (by touch).

Local spread:

Spread to and from other hosts: *Impatiens* sp. and *Osteospermum* sp. are known to be hosts (Skelton et al. 2010). There are fully naturalised wild exotic species of both of these genera in New Zealand (Landcare New Zealand Plants Database 2011). *Osteospermum* and *Impatiens* species are also ornamentals which may be grown in home gardens. *Nicotiana glauca* and tobacco (*Nicotiana tabacum*) are also hosts (Bodaghi et al. 2000) which are fully naturalised wild exotics in New Zealand (Landcare New Zealand Plants Database). There are likely to be other weedy and ornamental hosts in the known host families and the following argument applies to these also.

There is a negligible likelihood that TMGMV infection could be spread from infected capsicum plants from commercial glasshouses, to other host plants (*Impatiens* sp., *Osteospermum* sp., *N. glauca* or *N. tabacum*) growing outside because Tobamoviruses are not known to have insect vectors, and mechanical transmission is unlikely. It is unlikely that people working on infected

[38] Plants that grow on their own after the previous season.

tomato or capsicum plants in commercial settings would then touch weedy host species with their hands or equipment.

However, in home gardens people could spread TMGMV mechanically from infected capsicum to ornamental *Osteospermum* and *Impatiens* species. As these species have weedy characteristics, if TMGMV is spread to these species, they could become a reservoir of TMGMV in the environment. However, as transmission of TMGMV between species requires mechanical transmission, the likelihood of an environmental reservoir of TMGMV in *Osteospermum* and *Impatiens* species (either weedy or purposefully cultivated) leading to reinfection of *Capsicum* plants is very low.

<u>Long distance spread:</u>
This could occur through the movement of infected seed or plant material, e.g. plants distributed via commercial nurseries/garden centres or by home gardeners.

It may become naturalised (Williams and Randall, undated) through discarded fruit, but this occurs more rarely in comparison to tomato. There is a possibility of spread this way, but the likelihood is considered to be low.

Given that:

- TMGMV in *C. chinense* or *C. annuum* could spread through a crop in a single season, but the plants die at the end of the season;
- There is a negligible likelihood TMGMV will spread beyond the commercial glasshouse to other host species;
- TMGMV could establish in New Zealand via self-sown capsicum seeds from infected fruit, or from deliberate seed harvesting and growing; the likelihood of TMGMV establishing in this way is considered to be low, and is restricted to home garden situations;
- There is a low to moderate likelihood that TMGMV could be spread further afield from infected plant material being distributed by home gardeners, or by garden centres.

The likelihood of TMGMV establishing and spreading within C. chinense *and* C. annuum *crops where the initial infection occurs, and for that first season, is considered to be high.*

The likelihood of TMGMV establishing in the New Zealand environment (beyond one season) is uncertain but considered to range from low to moderate, and is most likely to originate from the occurrence of TMGMV in home garden crops of Capsicum chinense *or* C. annuum*.*

The likelihood of TMGMV establishing in New Zealand via transmission from any commercial capsicum crop is considered to be negligible.

The likelihood of TMGMV spreading in New Zealand beyond an initially infected home garden is considered to be low to moderate.

5.3.2.5 Consequence assessment

Economic consequences

The virus is of economic importance for tobacco crops (Wetter 1989). However tobacco is not grown commercially in New Zealand, the last commercial tobacco crop was planted in 1985 (Te Ara 2011). *Capsicum annuum* is the major *Capsicum* species grown in New Zealand for local consumption and export. There is some production of *Capsicum chinense* in New Zealand but the amounts grown are much smaller. There may be some impact on both of these capsicum crops; symptoms have been described on capsicum plants but no reports of yield loss or reduction in marketability have been found so the impact on capsicums is likely to be low.

The potential economic consequences within New Zealand are considered to be low.

Environmental consequences

There are species native to New Zealand in the Solanaceae, Amaranthaceae, Apocynaceae, Compositae, Gesneriaceae, and Labiatae (Landcare NZ plants database, 2011). There is a high degree of uncertainty about whether these plants would become hosts of TMGMV. It is also unknown whether these species would be symptomatic. However, as some hosts are known to be symptomless, it is moderately likely that if these species were infected, they would be symptomless.

The potential environmental consequences within New Zealand are considered to be low.

Human health consequences

There are no known human health consequences.

Socio-cultural consequences

Poroporo (*Solanum aviculare*) is a native solanaceous plant which has fruits which are sometimes eaten by Maori. There is a very low likelihood that poroporo could be infected by TMGMV as other solanaceous plants are known to be hosts, and a very low likelihood that this infection could cause symptoms in fruit or fruit yield loss.

The potential socio-cultural consequences within New Zealand are considered to be low.

5.3.2.6 Risk estimation

The risk estimation relating specifically to *C. annuum*, as compared to *C. chinense*, is based on an assumption that TMGMV is seed-transmitted in *C. annuum*. The assumption is based on an extrapolation of the observation of seed-to-seedling transmission in *C. chinense*.

The likelihood of entry of TMGMV with *Capsicum* seeds is uncertain but non-negligible, and the likelihood of exposure is high. The likelihood of TMGMV establishing and spreading within *C. chinense* and *C. annuum* crops where the initial infection occurs, and for that first season, is considered to be high. The likelihood of TMGMV establishing in the New Zealand environment (beyond one season) is uncertain but considered to range from low to moderate, and is most likely to originate from the occurrence of TMGMV in home garden crops of *Capsicum chinense* or *C. annuum*. The likelihood of TMGMV establishing in New Zealand via transmission from any commercial capsicum crop is considered to be negligible. The likelihood of TMGMV spreading in New Zealand beyond an initially infected home garden is considered to be low to moderate.

The economic consequences are low, the environmental consequences are very low, the human health consequences are negligible, and the socio-cultural consequences are very low.

See the table below for a visual summary of the risk estimation.

Risk estimation table: *Tobacco mild green mosaic virus* on *Capsicum* seeds from all countries				
	Considered to be:			
Likelihood of:	Negligible	Low	Moderate	High
Entry		?	?	?
Exposure				?
Establishment		In environment ?	In environment ?	Within crops
Spread		In environment ?	In environment ?	Within crops
Consequences of establishment				
Economic				
Environmental				
Socio-cultural				
Human Health				

? denotes uncertainty

In consideration of these assessments, TMGMV *is considered to be a non-negligible risk on imported capsicum seeds. The risk is worth considering and further analysis may be undertaken to decide if additional measures are warranted.*

5.3.3 Risk management – efficacy of risk management options

Risk Management for all the viruses and viroids assessed is considered in section 6.

5.3.4 References for TMGMV

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6 Overview of potential risk management options

6.1 Introduction

Risk management in the context of risk analysis is the process of identifying measures to effectively manage the risks associated with the commodity under consideration. Since zero-risk is not a reasonable option, the guiding principle for risk management should be to manage risk to achieve the required level of protection that can be justified and is feasible within the limits of available options and resources.

Recommendations for the appropriate phytosanitary measures to achieve the effective management of risks are not made in this document. These will be determined when the Risk Management Proposal (RMP) and Import Health Standard (IHS) are drafted.

This Chapter presents a brief assessment of options that have been considered for managing the risk of seed-borne viruses and viroids.

6.2 Pre-border - Options that could manage risk for all growing situations (i.e. commercial, outdoor crops, backyard growers):

6.2.1 Efficacy of seed treatments

Treatment of seeds with trisodium phosphate and hypochlorite is likely to reduce the seed-to-seedling transmission rate only of viruses and viroids *that occur on the surface of the seed* (Matsuura et al., 2010; Ling, 2010; DEFRA, 2010). At least one major seed company already treats tomato seeds with both trisodium phosphate and hypochlorite (Monsanto, 2011, personal communication). However, only one of the pathogens under consideration is known to occur as a surface contaminant. Therefore, this management option will not reduce the risk of all the pathogens assessed. At least one pathogen (PSTVd) occurs within tomato seeds (Matsushita et al 2011).

Pathogens on the seed surface:

PepMV - Efficacy of seed treatments for PepMV: Ling (2010) showed that treatment of seeds with 0.5% or 1% sodium hypochlorite (commercial bleach) for 30 minutes reduced or even eliminated infectious virus particles on PepMV-contaminated tomato seed. Treatment with 20% trisodium phosphate was also an effective treatment. None of these treatments significantly affected the rate of seed germination (Ling, 2010). According to DEFRA (2010), the industry standard apparently involves soaking seeds in hypochlorite, using concentration of 0.46% available chlorine for 15 minutes. This reduces PepMV contamination on tomato seeds by 70%. If the soaking in increased from 15 to 60 min, detectable levels of PepMV can be eliminated.

Pathogens within the seed:

PSTVd;

possibly TASVd (based on finding that trisodium phosphate treatment was ineffective at preventing seed-to-seedling transmission; Antignus et al. (2007))

possibly PZSV (based on finding that trisodium phosphate and hydrochloric acid treatment was ineffective at preventing seed-to-seedling transmission; Lapidot et al. (2010))
Efficacy of seed treatments for pathogens within the seed: Pathogens located inside a seed will not be exposed to chemical treatments. Therefore, such treaments will not eliminate or inactivate PSTVd or other viruses/viroids that occur inside the seed.

<u>Unknown seed location of pathogen</u>:
PCFVd
TMGMV
TCDVd - Efficacy of seed treatments for TCDVd: incubation of TCDVd-infected tomato seed in sodium hypochlorite did not eliminate the viroid from the seed (Singh & Dilworth, 2009). There is conflicting evidence from Matsushita et al., (2011); TCDVd could not be detected in tomato ovules.

6.2.2 Assurance of pest-free place of seed-production or pest free area

If the original source of seeds can be verified, a 'pest-free place of seed-production' (International Standards for Phytosanitary Measures number10, ISPM No 10) and/or a 'pest free area' (International Standards for Phytosanitary Measures number 4, ISPM No 4) would reduce the risk in all three categories of tomato crop grown in New Zealand (greenhouse, outdoor commercial crops, backyard crops). The efficacy of a pest free place of production would be limited for pathogens that can occur symptomlessly.

The following are examples of requirements for the risk management of one of the pathogens, PSTVd, in other jurisdictions, namely the EU and in Australia:

EU (as specified in Directive 2000/29/EC Annex IV part A Section I, for origin outside of EU)	**Australia** (as specified in AQIS Condition C18144 seed for sowing conditions for tomato seed)
	Condition 8: Seed must be accompanied by an official government Phytosanitary certificate from the country of origin where the seed was grown and be endorsed with the following additional declarations (see rows below). Condition 9: Where seed has been grown in a country other than the country of export each consignment must be accompanied by: a) A Phytosanitary certificate from the country where the seed was grown endorsed with one of the mandatory additional declarations listed in condition 8; **AND** b) A re-export Phytosanitary certificate from the country of export. Condition 12: Consignments not accompanied by an acceptable Phytosanitary certificate are subject to re-export or destruction at the importer's expense. Alternatively, some consignments may be eligible for seed testing for potato spindle tuber viroid.
Either the seeds originate in areas where PSTVd is <u>not known</u> to occur; or	Condition 8 cont: The tomato seed in lot(s) (insert lot numbers) in the consignment was grown in (insert name of 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,
No symptoms of diseases caused by PSTVd has been observed on the plants at the place of production during their complete cycle of vegetation; or	Condition 8 cont: 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. Or,
The seeds have been subjected to official testing PSTVd on a representative sample and using appropriate methods, and have been found, in these tests, free from PSTVd	Condition 8 cont: The tomato seed in lot(s) (insert lot numbers) in the consignment was derived from seed and pollen parent plants grown by (insert name of producer) in (insert name of country) that were tested during the growing period and found free of potato spindle tuber viroid.

6.2.3 Seed testing pre-border or at border

Destructive seed-testing

It is possible to carry out diagnostic tests on seeds to detect the presence of the seven viruses and viroids analysed in this risk analysis. However, the testing would involve destruction of the seeds. As the tests involve seed destruction, the seeds approved for entry into New Zealand would not be those that have been tested. No analysis has been done in this risk analysis on the efficacy of destructive seed-testing, nor of the commercial availability of tests, for the viroids or viruses assessed in this risk analysis.

Non-destructive seed-testing

The UK-based Food and Environmental Research Agency (FERA) has developed non-destructive seed testing methods for PepMV and PSTVd which are capable of detecting a single seed infected with PepMV or PSTVd in a batch of 600 healthy seeds. However, the method has not been published and, as at January 2012, is not commercially available in New Zealand (MAF, 2012). The non-destructive seed testing method has no adverse effects on seed viability (germination) or plants grown from those seeds. However, the test does impact the long-term viability of seeds. Storage needs to occur at 4°C and it is recommended that seeds which have been exposed to the non-destructive test should be left no longer than 2 weeks before planting (MAF, 2012).

To determine whether the non-destructive seed-testing methods would be effective as a risk management measure, further statistical analysis is required to determine what is the acceptable threshold of prevalence for PepMV-infected or PSTVd-infected seed within an imported consignment. Specifically, the question needs to be asked whether a batch with a negative test result, indicating a prevalence of less than 1 in 600 (0.17%), is likely to result in an exposure event[39].

[39] Note: for PepMV, which has a very low seed-to-seedling transmission frequency, if the prevalence of PepMV-infected seeds is >0.14% (~ 1 in 700 seeds infected), seed-to-seedling transmission, (i.e. an exposure event), is likely to occur at least once a year because New Zealand imports more than 2.5 million seeds annually (refer to the PepMV RA for further details).

6.3 Post-border

6.3.1 Risk management in greenhouse crops: Disease and crop management practices

Post-border management of viroids can be effective at reducing the risk of viroids and viruses in greenhouse crops. In New Zealand there are already disease and crop management practices used in export tomato crops. Known as the "New Zealand code of practice for the management of potato spindle tuber viroid (PSTVd) in greenhouse tomato and capsicum crops", these crop management practices are effective at early identification and eradication of the potato spindle tuber viroid when symptoms are apparent (PPIN, 2008).

Wherever the code of practice is implemented, if the pathogens show symptoms, the pathogens are highly likely to be identified and eradicated early, and spread of the pathogen to other plants prevented. However, PSTVd and other pathogens can occur asymptomatically and so there will be instances that the code of practice would not result in early detection of infected plants.

It may be that the industry already has plant hygiene practices in place that can prevent spread of pathogens that are initially asymptomatic and can become symptomatic later on in the life of the plant. But they are not documented in the Code of Practice. This could be ascertained through discussion with the sector. In the meantime and in the current absence of such documentation, it cannot be concluded that the Code of Practice is effective at managing the risk of the pathogens that can initially occur asymptomatically and produce symptoms later.

6.3.2 Risk management in commercial outdoor tomato crops

Weed control and increased crop monitoring

Elimination of weedy solanaceous plants and of weedy perennials that can act as hosts of the pathogens, and provide a mechanism for establishment of the pathogens in New Zealand, is likely to reduce the opportunities for the pathogens to be spread to or from outdoor crops by bumblebees and/or other insects.

Increased crop monitoring

Consideration could be given to whether elements of the 'code of practice for greenhouse growers' could be adopted for outdoor crops where practical. This risk management option would be aimed at identifying infected plants and destroying them before bumblebees or routine plant maintenance practices could spread the pathogens. This would only be effective against pathogens that are causing plant symptoms.

6.3.3 Risk management in backyard tomato and capsicum crops

The use of imported tomato and capsicum seed in the home garden is likely to be the main source of environmental reservoirs of the pathogens. It is considered unlikely that the risk posed by the use of tomato and capsicum seeds in the home-garden can be achieved in the post-border space.

A decision will be required as to whether it is necessary to manage the risk posed by seeds used in home gardens.

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Appendix 6 Glossary of definitions and abbreviations

BORIC	Biosecurity Organisms Register for Imported Commodities
CPC	Crop Protection Compendium. Internet Database
Endemic	an animal, plant, pest, or disease that is native to and is not naturally found outside a defined geographical area
Establishment	perpetuation, for the foreseeable future, of an organism or disease within an area after entry.
Exposure	the process of the hazard organism moving from the commodity it arrived on to another host
Exotic	this word has different meanings in different fields, but in this document is defined as an animal, plant, pest or disease that is not indigenous to New Zealand.
Hitch-hiker organism	an organism that has an opportunistic association with a commodity or item with which it has no biological host relationship.
Indigenous	native; organism originating or occurring naturally in a specified area.
Introduced	not indigenous, not native to the area in which it now occurs, having been brought into this area directly or indirectly by human activity.
IHS	Import Health Standard
IRA	Import risk analysis
MAF	Ministry of Agriculture and Forestry, New Zealand
MAFBNZ	MAF Biosecurity New Zealand
QuanCargo	Database of commercial consignments, and interceptions of pests made by quarantine inspection.
PCFVd	*Pepper chat fruit viroid*
PepMV	*Pepino mosaic virus*
PSTVd	*Potato spindle tuber viroid*
PPIN	Plant Pest Information Network database, MAF.

PZSV	*Pelargonium zonate spot virus*
Regulated Pest	a pest of potential economic importance to New Zealand and not yet present here, or present but either not widely distributed and being officially controlled, having the potential to vector another organism, or a regulated non-quarantine pest.
Risk	in the context of this document risk is defined as the likelihood of the occurrence and the likely magnitude of the consequences of an adverse event.
TASVd	*Tomato apical stunt viroid*
TCDVd	*Tomato chlorotic dwarf viroid*
TMGMV	*Tobacco mild green mosaic virus*
USDA	United States Department of Agriculture.
Viable	capable of living; able to maintain a separate existence (on its own accord).
Vector	an organism or object that transfers a pest, parasite, pathogen or disease from one area or host to another.