



Risk Management Proposal:

Amendment to the testing requirements for *Pepino mosaic virus* in the specific requirements for tomato (*Solanum lycopersicum*) in import health standard *Seeds for Sowing* (155.02.05)

Prepared for public consultation
by Plant Germplasm Imports
Animal and Plant Health Directorate

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Submissions

The Ministry for Primary Industries (MPI) invites comment from interested parties on proposed amendments to the import health standard (IHS) *Seeds for Sowing* (the Standard).

An import health standard “specifies requirements to be met for the effective management of risks associated with arrival of craft or risk goods” (section 22, Biosecurity Act 1993).

MPI would like feedback on the following proposed changes to the Standard:

- Amendment to the testing requirements for *Pepino mosaic virus* in the specific requirements for tomato (*Solanum lycopersicum*)

Include the following in your submission:

- The title of the consultation document in the subject line of your email
- Your name and title
- Your organisation’s name (if applicable)

Send submissions to plantimports@mpi.govt.nz by 22 November 2021.

Feel free to include any technical information you have that supports your submission. While we prefer email, if you would like to send your submission by post, you can mail it to:

Plant Germplasm Imports
Animal and Plant Health Directorate
Biosecurity New Zealand
Ministry for Primary Industries
PO Box 2526
Wellington 6140
New Zealand

Submissions received by the 22nd of November 2021 will be considered during the development of the amended standard. Submissions received after the closing date may be held on file for consideration when the standard is next revised/reviewed.

Official Information Act 1982

Please note that your submission is public information, and it is MPI policy to publish submissions and the review of submissions on the MPI website. Submissions may also be the subject of requests for information under the Official Information Act 1982 (OIA). The OIA specifies that information is to be made available to requesters unless there are sufficient grounds for withholding it, as set out in the OIA. Submitters may wish to indicate grounds for withholding specific information contained in their submission, such as information being commercially sensitive or personal. Any decision to withhold information requested under the OIA is reviewable by the Ombudsman.

1 General information

1.1 PURPOSE

- (1) The purpose of this proposal is to:
 - Provide relevant background information about the import health standard *Seeds for Sowing* (the Standard);
 - Outline the proposed changes to the testing requirements for *Pepino mosaic virus* (PepMV) in the specific requirements for tomato (*Solanum lycopersicum*) in the Standard;
 - Notify the intention to include guidance information in the Standard regarding the use of a bioassay for detection of PepMV in seed samples.
 - Provide the rationale for the proposed changes to the Standard and how they manage biosecurity risk; and to
 - Seek feedback on the proposed Standard.

1.2 BACKGROUND

- (2) The Standard was issued on 20 September 2021 and came into effect on 20 September 2021. The Standard was issued under section 22 of the Biosecurity Act (1993) and applies to seeds for sowing that are imported into New Zealand from foreign countries and territories.

1.3 TIMING AND CONSULTATION

- (3) The proposed amendments to the Standard was released for consultation on **22 October 2021** and will remain open for consultation until **22 November 2021**.

1.4 GENERAL CONTEXT TO CONSULTATION

1.4.1 International regulation of risk goods

- (4) “The World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) sets in place rules that protect each country’s sovereign right to take the measures necessary to protect the life or health of its people, animals, and plants, while at the same time facilitating trade. It embodies and promotes the use of science-based risk assessments to manage the risks associated with the international movement of goods. The SPS Agreement guides how New Zealand sets standards and makes decisions related to biosecurity.” (MAF et al., 2009, p. 1)
- (5) “In particular, [it is] important to maintain the standards of transparency and scientific rigour required by the SPS Agreement and to make decisions as quickly as possible. This will encourage other countries to comply with the rules of the SPS Agreement and also demonstrate that New Zealand’s strict controls are justified to countries that challenge them.” (MAF et al., 2009, p. 13)

1.4.2 Domestic regulation of biosecurity risks associated with craft arrivals

- (6) The New Zealand biosecurity system is regulated through the Biosecurity Act 1993 (the Act). Section 22 of the Act describes an import health standard and requires all risk goods imported into New Zealand to be managed by one. MPI is the New Zealand government ministry responsible for maintaining biosecurity standards for the effective management of risks associated with the importation of risk goods into New Zealand (Part 3, Biosecurity Act 1993).

MPI is committed to the principles of transparency and evidence-based technical justification for all phytosanitary measures, new and amended, imposed on importing pathways. MPI periodically reviews all import health standards, related documents and other standards so that the legal requirements are clear and information is consistently presented and as easy as possible to understand.

1.4.3 Biosecurity risk associated with seeds for sowing

- (7) Seeds for sowing can be a high-risk pathway for the introduction of regulated pests and diseases into New Zealand. Importation of seeds for sowing may be associated with risks arising from entry, establishment and spread of seed-transmitted and contaminating pests when infested seeds are introduced to an environment favourable to the pest. The transfer of seed-transmitted and contaminating pests to suitable hosts in the wider environment, can lead to them establishing and spreading to cause unwanted economic, environmental and social impacts. To mitigate these risks, MPI has established the Seeds for Sowing import health standard which specifies biosecurity measures that must be met before seeds imported for the purpose of sowing can be imported into New Zealand. These measures ensure that the likelihood of pests and diseases that could cause unwanted impacts entering New Zealand on imported seeds for sowing is reduced to a very low level.

1.5 BACKGROUND TO PROPOSED CHANGES TO THE TESTING REQUIREMENTS FOR PEPINO MOSAIC VIRUS IN THE SPECIFIC REQUIREMENTS FOR TOMATO (*SOLANUM LYCOPERSICUM*)

- (8) *Pepino mosaic virus* (PepMV) (species *Pepino mosaic virus*; genus *Potexvirus*; family *Alphaflexiviridae*) is a regulated¹ quarantine pest and an unwanted² organism in New Zealand. PepMV mainly infects solanaceous species such as tomato, capsicum, eggplant and potato, although it is also known to infect several wild and weedy plants from the families *Amaranthaceae*, *Asteraceae*, *Boraginaceae*, *Brassicaceae*, *Chenopodiaceae*, *Convolvulaceae*, *Malvaceae*, *Plantaginaceae*, *Polygonaceae* and *Solanaceae* (DAWE, 2021).
- (9) Tomato is the primary natural host of PepMV (CABI, 2021). Currently, five strains of PepMV that infect tomato can be distinguished genetically, namely, the Peruvian strain (LP) (Jones et al, 1980), the North American / Chilean strain (US1/Ch1) (Maroon-Lango et al, 2005; Ling, 2007); the European tomato strain (Eu) (van der Vlugt et al. 2000); the Chilean strain (Ch2) (Ling, 2007) and the Peruvian wild tomato strain PES (Moreno-Pérez et al. 2014). All currently

¹ An organism is 'regulated' by MPI if the Chief Technical Officer (CTO) has assessed the organism to be of potential importance to New Zealand and not yet present here, or present but not widely distributed and being officially controlled, or the organism is a vector for another organism that is regulated. The CTO identifies actions required to manage any risk of entry and establishment from specific regulated organisms.

² The Biosecurity Act 1993 defines an unwanted organism as 'any organism that a chief technical officer believes is capable or potentially capable of causing unwanted harm to any natural and physical resources or human health; and (a) includes— (i) any new organism, if the Authority has declined approval to import that organism; and (ii) any organism specified in [Schedule 2](#) of the Hazardous Substances and New Organisms Act 1996; but (b) does not include any organism approved for importation under the [Hazardous Substances and New Organisms Act 1996](#), unless— (i) the organism is an organism which has escaped from a containment facility; or (ii) a chief technical officer, after consulting the Authority and taking into account any comments made by the Authority concerning the organism, believes that the organism is capable or potentially capable of causing unwanted harm to any natural and physical resources or human health.'

identified strains of PepMV, apart from LP, can cause symptomatic, systemic infection in tomato. Symptoms in tomato plants can include marbling, discolouration and splitting of fruit, leaf blistering or bubbling, chlorosis and yellow angular leaf spots, severe leaf mosaics and leaf and stem necrosis. These symptoms range in severity, depending on the PepMV strain and/or variants present, and the age, vigour and variety of tomato plant, and growing conditions in greenhouses. The literature suggests that PepMV does not significantly reduce bulk yields of tomato (although this will depend on tomato variety, strain/variant and growing conditions). However, the quality of the fruit harvested can be reduced significantly (MPI, 2012).

- (10) PepMV is highly contagious in tomato and is capable of being efficiently transmitted mechanically i.e. through natural or artificial injury of the plant cuticle. It spreads easily by standard crop handling procedures in a glasshouse through contaminated tools, hands and clothing and direct plant-to-plant contact. Invertebrates are also capable of mechanically transmitting PepMV. In glasshouse experiments PepMV has been shown to be transmitted mechanically by bumblebees (*Bombus terrestris*). Bumblebees have been shown to carry PepMV when exposed to infected plants (Crop Protection Compendium, 2021; Shipp et al. 2008) and have a widespread distribution in New Zealand (NZOR, 2021). PepMV can also be transmitted by the root-infecting parasitic fungus vector *Olpidium virulentus* through soil and water. *Olpidium virulentus* is considered present in New Zealand under the name *Olpidium brassicae* (MPI, 2012). PepMV has also been reported to be transmitted between tomato plants by the greenhouse whitefly *Trialeurodes vaporariorum* which is also present in New Zealand (Noël et al, 2014).
- (11) Infected seed can be a pathway for PepMV because it is seedborne and seed-transmitted in tomato. Seeds for sowing are considered a high-risk pathway for the introduction of PepMV. PepMV has been detected many times in consignments of traded tomato seeds since 2001. Although transmission from contaminated seeds to seedlings occurs infrequently, at an incidence reported to range between 2% and 0.005%, it is believed that transmission via contaminated seeds is responsible for initiating outbreaks and for transport of the virus to new regions (DAWE 2021, Hanssen and Thomma 2010). Although PepMV is transmitted at a low rate, the sheer volume of traded tomato seed for commercial sowing results in a high likelihood of spread on this pathway. The potential economic impacts caused by PepMV entering and becoming established in New Zealand have been assessed as low to high. The potential impact depends on factors such as the PepMV strain, tomato cultivar, environmental factors, and co-infection with *O. virulentus*. (MPI, 2012).
- (12) MPI introduced requirements in the Standard to manage the risk from PepMV entering New Zealand on tomato seeds for sowing imported from all countries in 2012. The requirements are:
 - The seeds are produced in a ‘pest-free area’, free from PepMV; or
 - The seeds are produced in a ‘pest-free place of production’, free from PepMV; or
 - The seeds are officially tested, on a representative sample, and using appropriate methods, and found to be free from PepMV.
- (13) If testing is the chosen phytosanitary measure, it can be completed offshore prior to export, or on arrival in New Zealand. Pre-export testing for each seed lot must be endorsed by the NPPO on the phytosanitary certificate, or if tested on arrival in New Zealand, must be completed by an MPI-approved testing laboratory.
- (14) In April and May 2021, PepMV was detected in tomato plants in four commercial tomato growing greenhouse facilities in the Auckland region of New Zealand (MPI, 2021). Infected plants tested positive for the mild variant of the Ch2 PepMV strain. MPI and industry partners

under the Government Industry Agreement (GIA) initiated a joint biosecurity response to manage the incursion, which is ongoing. Strict hygiene measures and movement controls are in place at the affected properties. Additional surveillance and testing activities are being undertaken, including tracing and testing inputs to affected properties. Investigation is ongoing to determine the source of the virus in New Zealand. While imported tomato seeds for sowing have not been determined as the source of the outbreak, they are a possible source.

- (15) Tomato seeds for sowing can be imported into New Zealand from all countries. Between January 2018 and December 2020, 899 lines of tomato seeds for sowing were imported into New Zealand in 382 consignments. Seed was imported from 25 countries, including from countries where PepMV is known to be present. Almost all New Zealand-grown fresh tomato produce is grown from imported seed.
- (16) The post-border detections of PepMV in New Zealand in 2021 indicate an increased likelihood of the virus entering New Zealand on tomato seeds imported for sowing. It is important that the measures in the Standard are sufficiently robust to ensure that the risk of PepMV entering New Zealand on imported tomato seed is reduced to a very low level. One way to gain a higher level of assurance that risk is reduced to a low level is by specifying testing parameters that provide high confidence that low levels of virus on the seeds will be detected by the test.
- (17) MPI proposes strengthening the current testing requirements for PepMV (currently “official testing on a representative sample using appropriate methods”). We propose a specified seed sample size, sampling methodology and diagnostic testing method that an NPPO may use to demonstrate that seeds exported to New Zealand are free from PepMV. This declaration on phytosanitary certificates will provide increased assurance that risk is managed appropriately on the pathway and will match the seed testing requirements for PepMV performed onshore.
- (18) At the time of drafting this risk management proposal, PepMV is a regulated quarantine pest for New Zealand. Although there has been an incursion from the Ch2 strain of PepMV in New Zealand, it is currently being officially controlled. If the regulatory status of PepMV or the specific Ch2 variant of PepMV changes during the course of this consultation, this may impinge on MPI’s ability to require measures to manage the pest. In this case, MPI will reconsult affected parties on the proposed strengthening of testing requirements for PepMV in the Standard.

2 Proposed changes to the Standard

- (19) MPI proposes a change to the testing requirement for PepMV in the specific requirements for tomato (*Solanum lycopersicum*) in the Standard to require that seeds are “officially tested, on a representative sample of a minimum of 3,000 seeds officially drawn according to the ISTA or AOSA sampling methodology, using an NPPO-approved ELISA or NPPO-approved PCR testing method and found free from *Pepino mosaic virus*”. The change as it will appear on the Standard can be found in Appendix 1 of this risk management proposal.

2.1 NON-REGULATORY CHANGES TO THE STANDARD

- (20) MPI has included guidance in the specific requirements for *Solanum lycopersicum* in the Standard to state that:
 - (i) MPI does not accept bioassays to detect the presence of PepMV on seed samples.

- (ii) Additional declarations on phytosanitary certificates to meet the offshore testing requirements for PepMV in the import health standard *Seeds for Sowing* should be based only on a negative result obtained in an NPPO-approved ELISA or NPPO-approved PCR and not on results from a bioassay.
- (21) None of these non-regulatory changes alter the intent of or the requirements within the Standard. The inclusion of guidance is considered a minor amendment in accordance with section 24B(2) of the Act and, as such, do not require consultation under section 23(2) of the Act. Please note that these minor amendments have only been included for clarity and transparency purposes, and they do not form part of the consultation. The inclusions in guidance as they will appear in the Standard can be found in Appendix 1 of this risk management proposal.

3 Rationale for the proposed change to the Standard

3.1 PROPOSED CHANGE TO THE TESTING REQUIREMENTS FOR PEPINO MOSAIC VIRUS

- (22) For tomato seeds for sowing imported into New Zealand that are tested before export, MPI gains assurance that seeds are free from PepMV by the exporting country NPPO endorsing the measure in an additional declaration on the phytosanitary certificate. MPI proposes an amendment to the current additional declaration for PepMV to prescribe a seed sample size for testing (3,000 seeds), seed sampling methodology (ISTA or AOSA methodology) and a diagnostic testing method (NPPO-approved ELISA or PCR).
- (23) PepMV was the first quarantine pest on tomato seed for which specific measures were introduced in the Standard in 2012. Prior to this, tomato seeds for sowing were eligible to import into New Zealand under basic requirements. The additional declaration for seed testing for PepMV in the Standard was modelled on the declaration on phytosanitary certificates that was required by the European Union for tomato seeds originating in non-EU countries.
- (24) Since introducing measures for PepMV, MPI has regulated other quarantine viruses on tomato seeds for sowing in the Standard, with more prescriptive requirements. For example, for *Tomato mottle mosaic virus*, testing is to be performed on a representative sample of a minimum of 3,000 seeds drawn according to ISTA or AOSA methodology using an NPPO-approved ELISA or NPPO-approved PCR testing method. MPI has concluded that the testing requirements for PepMV should be aligned in their strength and specificity with viruses of a similar risk profile.
- (25) PepMV is a seedborne pathogen, and it can be detected by testing a representative sample from a seed lot. Sampling of the seed lot performed according to ISTA or AOSA methodology will ensure that the sample drawn for testing accurately reflects the characteristics of the whole seed lot (International Rules for Seed Testing, 2021). A 3,000-seed sample is a suitable sample size for testing for the presence of PepMV. Testing a representative sample of 3,000 seeds, drawn from a large, well-mixed seed lot, will detect pest infestation at levels as low as 0.1% with 95% confidence (FAO, 2008). The prevalence of virus pests in seed lots is assumed to be at 0.1%. Testing a 3,000-seed sample is in line with the sample size requirements for testing for other quarantine viruses such as tomato brown rugose fruit virus and tomato mottle mosaic virus in the specific requirements for tomato (*Solanum lycopersicum*) in the Standard.

- (26) Molecular (PCR) and serological (ELISA) diagnostic methods are routinely used to detect seedborne pests. Both methods are sensitive for detecting specific viruses, including PepMV. Both ELISA and PCR tests are developed for testing for PepMV. The European Plant Protection Organization (EPPO) recommends both ELISA and PCR tests for detecting and identifying PepMV on tomato seeds (EPPO, 2013). For qualitative detection and identification of PepMV, double antibody sandwich ELISA (DAS-ELISA) is recommended as the initial test of choice. Real-time reverse transcription PCR (real-time RT-PCR) is recommended as the molecular test of choice. The ELISA and PCR diagnostic protocols for PepMV are both based on a recommended minimum sample size of 3,000 seeds with a maximum sub-sample size of 250 seeds and are both able to detect one infected seed in a sub-sample of 250 seeds (EPPO, 2013).
- (27) Amending the testing requirements for PepMV to specify a sample size (3,000 seeds), sampling methodology (ISTA or AOSA sampling) and testing method (PCR or ELISA) will align the offshore testing requirements with the onshore testing requirements for PepMV. Currently, onshore testing is performed by the MPI Plant Health and Environment Laboratory on a tomato seed sample of 3,000 seeds, officially drawn using ISTA methodology, using a real-time RT-PCR assay. PHEL's accredited PepMV assay is based on primers designed by Ling et al. (2017) and is in accordance with the recommended PCR diagnostic protocol in the EPPO diagnostic standard for PepMV.

3.2 INCLUSION OF GUIDANCE REGARDING THE USE OF A BIOASSAY IN SEED TESTING FOR THE PRESENCE OF PEPINO MOSAIC VIRUS

- (28) MPI does not accept the use of a bioassay to detect PepMV in tomato seed samples exported to New Zealand. This guidance has been included in the Standard to clarify the phytosanitary requirements concerning offshore testing of tomato seeds for sowing for the presence of PepMV and to facilitate compliance with these requirements by importers, exporters and exporting countries' NPPOs.
- (29) Bioassay results do not provide New Zealand with an appropriate level of protection against PepMV on imported tomato seeds for sowing. This guidance is included in the Standard because there are certain diagnostic protocols that rely on a bioassay on susceptible herbaceous indicator plants to draw conclusions on whether a seed lot is infected with PepMV. For example, the International Seed Health Initiative for Vegetable Crops (ISHI-Veg) has published a diagnostic protocol for detecting PepMV on tomato seeds using ELISA that relies on a bioassay (ISHI-Veg, 2021). The ISHI-Veg protocol approves two bioassay methods for detecting infectious PepMV in tomato seed extracts. MPI does not approve of either of these methods.
- i. In the first method, *Nicotiana benthamiana* plants are inoculated and then ELISA is conducted on the new non-inoculated leaves of the plant to confirm the presence of systemic infection by PepMV.
 - ii. In the second method, ELISA is used on seed extracts as a pre-screen.
 - If the pre-screen by ELISA does not detect PepMV, the protocol concludes that the seeds are free from the virus.
 - If the pre-screen by ELISA detects PepMV, the protocol involves using a bioassay to confirm the presence of infectious PepMV. If no symptoms are observed in the bioassay, the protocol concludes that seeds are free from PepMV.
 - If symptoms are observed in the bioassay, the protocol involves confirmation by ELISA. If the results of this confirmatory ELISA detects PepMV, then the seed lot is

considered infected with PepMV.

- If the confirmatory ELISA does not detect PepMV, the protocol concludes that the seed lot is free from the virus, despite the positive result obtained in the initial pre-screen ELISA. The protocol concludes that the pre-screen ELISA identified biologically inactive PepMV.

- (30) The EPPO diagnostic standard recommends DAS-ELISA as the initial test of choice for qualitative detection and identification of PepMV. It does not recommend a bioassay as a test for detecting PepMV in seeds (EPPO, 2013). Bioassays are less sensitive than ELISAs and are more prone to variability and false negatives. The two methods, as described above, are prone to false negative results at low incidence of infected seed. This means tomato seeds that are infected with PepMV and tested with either of these methods could be passed as healthy and be approved for export.
- (31) MPI has concluded that the advice of the EPPO, that a bioassay is not recommended for detecting PepMV in seeds, is suitable for the New Zealand context.

4 Feasibility of the proposed change to the Standard

- (32) Procedures for detecting and identifying PepMV in a representative sample from a tomato seed lot comprising 3,000 seeds using ELISA and PCR methods are available for exporting NPPOs to use for pre-export testing. The NPPOs of exporting countries routinely certify tomato seeds for export to New Zealand as free from PepMV based on seed testing for PepMV by ELISA or PCR. This amendment brings the wording of the additional declaration in line with the declarations required for other quarantine pests of tomato in the Standard.
- (33) ELISA and PCR diagnostic protocols for detecting and identifying PepMV on tomato seed have been available for more than 10 years. These protocols are published, validated and have been used internationally for phytosanitary testing of seed. The EPPO Standard (PM 7/113 (1) *Pepino mosaic virus*), first published in April 2013 describes a diagnostic protocol for detection of PepMV in tomato seeds using DAS-ELISA and real-time RT-PCR. MPI recognises the ISHI-Veg diagnostic protocol “Detection of *Pepino mosaic virus* in tomato seeds” as effective for detecting and identifying PepMV on tomato seeds, as long as the method is not used in conjunction with a bioassay.
- (34) DAS-ELISA kits containing all necessary components needed for the test are widely available commercially and can be used in the diagnostic protocol described in the EPPO Standard PM 7/113 (1). Commercial antisera against PepMV that detect all known strains of PepMV are included in these kits that are available worldwide, for example from Prime Diagnostics and Bioreba (Prime Diagnostics 2021; Bioreba 2021).
- (35) PCR primers and probes have been published for a highly sensitive and specific real-time RT-PCR assay in Ling et al 2007. PCR testing of tomato seeds for phytosanitary certification using this assay has been adopted by exporting NPPOs such as Australia, as well as by PHEL for onshore testing of tomato seeds for PepMV for import and export certification.
- (36) Allowing both ELISA and PCR will be trade-enabling. The EPPO standard for seed health testing for PepMV recommends both DAS-ELISA and real-time RT-PCR-based assays. Several tomato seed-producing countries exporting to the EPPO region are likely to be using either of these methods to test their tomato seeds for export. Re-export is integral to the international

movement of seeds, and Europe is a key destination from which tomato seeds are re-exported to New Zealand. Given the acceptance of ELISA and PCR testing of tomato seeds exported to the European Union for PepMV, it could become trade-restrictive if New Zealand only specifies PCR testing as a requirement, which would necessitate retesting seed lots already tested by ELISA and certified as free from PepMV. Tomato seeds are often imported into New Zealand in shipments comprising low volumes of seed. Requiring PCR for PepMV of seed shipments to New Zealand that may have already been tested by ELISA could lead to destruction of a significant proportion of seeds in the shipment, which could become prohibitive to importers.

- (37) The proposed testing requirements for PepMV align with the sample sizes for testing for other quarantine virus and viroid pests of tomato seed in the Standard, namely, *Columnnea latent viroid*, *Potato spindle tuber viroid*, *Tomato chlorotic dwarf viroid*, *Tomato brown rugose fruit virus*, *Tomato apical stunt viroid*, *Tomato planta macho viroid* and *Tomato mottle mosaic virus*. PCR is a common option for all these pests, and it is likely that testing for all these pests can be done on a single sample of 3,000 seeds with a maximum subsample size of 250 seeds (or 400 seeds if the seeds do not need to be tested for tobamoviruses). Therefore, at a minimum a total of 3,000 seeds needs to be sacrificed per tomato seed lot for testing for all the quarantine pests in the Standard.
- (38) Onshore testing for PepMV at PHEL is routinely performed using PCR. The indicative cost of RT-PCR testing of a sample of 3,000 untreated seeds for PepMV onshore at PHEL is NZ\$1,020 (excl. GST). This testing would be done on 12 subsamples of 250 seeds each. However, seeds normally received by PHEL for testing for PepMV also require testing for the other quarantine pests of tomato seed in the Standard. PHEL can combine testing efficiencies for PCR testing for PepMV and the pospiviroids (*Columnnea latent viroid*, *potato spindle tuber viroid*, *tomato chlorotic dwarf viroid*, *tomato apical stunt viroid* and *tomato planta macho viroid*) by testing 3,000 seeds as subsamples of up to 400 seeds, i.e. eight subsamples at the cost of \$720 (excl. GST). However, if the seeds also need to be tested for *Tomato brown rugose fruit virus* and *Tomato mottle mosaic virus*, the testing is done on 12 subsamples of 250 seeds each. On the other hand, the indicative cost of an ELISA assay is NZ \$650 (excl. GST). Note that PHEL does not currently offer onshore seed testing for PepMV by ELISA.

5 References

- Bioreba AG (2021) Startseite. <http://www.bioreba.com/> Accessed 29 August 2021.
- Blystad, D-R; van der Vlugt, R; Alfaro-Fernández, A; del Carmen Córdoba, M; Bese, G; Hristova, D; Pospieszny, H; Mehle, N; Ravnikar, M; Tomassoli, L; Varveri, C; Nielsen, S L (2015) Host range and symptomatology of *Pepino mosaic virus* strains occurring in Europe. *European Journal of Plant Pathology* 143(1): 43–56.
- CABI (2021) Pepino mosaic virus. *Crop Protection Compendium*. <https://www.cabi.org/isc/datasheet/43661> Accessed 27 August 2021.
- DAWE (2021) Final pest risk analysis for Pepino mosaic virus and pospiviroids associated with tomato seed. Australian Government, Department of Agriculture, Water and the Environment. Feb 2021.
- EPPO (2013). EPPO Standard (PM 7/113 (1) Pepino mosaic virus). <https://doi.org/10.1111/epp.12023> Accessed 29 August 2021.
- FAO (2008) International Standards for Phytosanitary Measures (ISPM) No. 31. Methodologies for sampling of consignments. Secretariat of the International Plant Protection Convention, Food and Agricultural Organization of the United Nations; Rome, Italy.
- Hanssen, I M; Paeleman, A; Wittemans, L; Goen, K; Lievens, B; Bragard, C; Vanachter, A C R C; Thomma, B P H J (2008) Genetic characterization of *Pepino mosaic virus* isolates from Belgian greenhouse tomatoes reveals genetic recombination. *European Journal of Plant Pathology* 121(2): 131–146.
- Hanssen, I M; Thomma, B P H J (2010) Pepino mosaic virus: a successful pathogen that rapidly evolved from emerging to endemic in tomato crops. *Molecular Plant Pathology* 11(2): 179–189. <https://www.agriculture.gov.au/biosecurity/risk-analysis/plant/pepino-mosaic-virus-pospiviroids-tomato-seed>
- ISHI-Veg (2021) *Detection of Pepino mosaic virus in Tomato Seed*. International Seed Health Initiative for Vegetable Crops (ISHI-Veg). International Seed Federation; Nyon, Switzerland. https://www.worldseed.org/wp-content/uploads/2021/03/Protocol_Tomato_PepMV_v5_March-2021-1.pdf Accessed 29 August 2021.
- ISTA (2021) Chapter 2: Sampling. *International Rules for Seed Testing 2021*. International Seed Testing Association; Bassersdorf, Switzerland. https://www.seedtest.org/upload/cms/user/s4_ISTA_Rules_2021_02_sampling.pdf Accessed 27 August 2021.
- Jones, R A C; Koenig, R; Lesemann, D E (1980) *Pepino mosaic virus*, a new potexvirus from pepino (*Solanum muricatum*). *Annals of Applied Biology* 94(1): 61–68.
- Ling KS, Wechter WP, Jordan R. (2007). Development of a one-step immunocapture real-time TaqMan RT-PCR assay for the broad-spectrum detection of *Pepino mosaic virus*. *Journal of Virological Methods* 144: 65–72.
- Ling, K-S (2007) Molecular characterization of two *Pepino mosaic virus* variants from imported tomato seed reveals high levels of sequence identity between Chilean and US isolates. *Virus Genes* 34(1): 1–8.
- MAF; MFAT; FSA (2009) *Balance in Trade*. Ministry of Agriculture and Forestry, Ministry of Foreign Affairs and Trade, New Zealand, Food Safety Authority; Wellington, NZ. <https://www.mpi.govt.nz/dmsdocument/12576-balance-in-trade>
- Maroon-Lango, C J; Guaragna, M A; Jordan, R L; Hammond, J; Bandla, M; Marquardt, S K (2005) Two unique US isolates of *Pepino mosaic virus* from a limited source of pooled tomato tissue are distinct from a third (European-like) US isolate. *Archives of Virology* 150(6): 1187–1201.
- Moreno-Pérez, M G; Pagán, I; Aragón-Caballero, L; Cáceres, F; Fraile, A; García-Arenal, F (2014) Ecological and genetic determinants of *Pepino mosaic virus* emergence. *Journal of Virology* 88(6): 3359–3368.

- MPI (2012) Import Risk Analysis: Tomato and capsicum seed for sowing from all countries. <https://www.mpi.govt.nz/dmsdocument/2887> Accessed 27 August 2021.
- MPI (2021) Pepino mosaic virus (PepMV) in Auckland. Ministry for Primary Industries; Wellington, NZ. <https://www.mpi.govt.nz/biosecurity/major-pest-and-disease-threats/pepino-mosaic-virus-pepmv-in-auckland/> Accessed 27 August 2021.
- Noël, P; Hance, T; Bragard, C (2014) Transmission of the *Pepino mosaic virus* by whitefly. *European Journal of Plant Pathology* 138(1): 23–27.
- NZOR (2021) The New Zealand Organisms Register. <http://nzor.org.nz> Accessed 29 August 2021.
- Prime Diagnostics (2021) <https://www.wur.nl/en/show/Prime-Diagnostics-2.htm> Accessed 29/08/2021
- Shipp, J L; Buitenhuis, R; Stobbs, L; Wang, K; Kim, W S; Ferguson, G (2008) Vectoring of *Pepino mosaic virus* by bumble-bees in tomato greenhouses. *Annals of Applied Biology* 153(2): 149–155.
- Spence, N J; Basham, J; Mumford, R A; Hayman, G; Edmondson, R; Jones, D R (2006) Effect of *Pepino mosaic virus* on the yield and quality of glasshouse-grown tomatoes in the UK. *Plant Pathology* 55(5): 595–606.
- van der Vlugt, R A A; Stijger, C C M M; Verhoeven, J T J; Lesemann, D-E (2000) First report of *Pepino mosaic virus* on tomato. *Plant Disease* 84(1): 103–103.

6 Appendix 1

2.74 *Solanum lycopersicum*

The following requirements only apply to species in the Plant Biosecurity Index listed under Import Specifications for Seed as "see 155.02.05 under *Solanum lycopersicum*."

Approved countries: All countries

Quarantine pests: *Columnnea latent viroid*, *Pepino mosaic virus*, *Potato spindle tuber viroid*, *Tomato chlorotic dwarf viroid*, *Tomato brown rugose fruit virus*, *Tomato apical stunt viroid*, *Tomato planta macho viroid*, *Tomato mottle mosaic virus*

Import permit: Not required

PEQ: Not required

Approved treatment: Not required

Phytosanitary certificate: Required

2.74.1 Phytosanitary certificate - Additional declarations

(1) If satisfied that the pre-shipment activities have been undertaken, the exporting country NPPO must confirm this by providing the certifying statement as per Part 1.5.2 of this import health standard and also the following additional declaration (s) to the phytosanitary certificate:

- a) "The *Solanum lycopersicum* seeds have been prepared to industry standards with thorough cleaning to remove all traces of flesh from the seeds".

AND

- b) "The *Solanum lycopersicum* seeds have been:

- i) produced in a 'pest free area' free from *Pepino mosaic virus*.

OR

- ii) produced in a 'pest free place of production' free from *Pepino mosaic virus*.

OR

- iii) officially tested, on a representative sample of a minimum of 3000 seeds officially drawn according to the ISTA or AOSA sampling methodology, using an NPPO-approved ELISA or NPPO-approved PCR testing method, and using appropriate methods, and found to be free from *Pepino mosaic virus*".

AND

- c) "The *Solanum lycopersicum* seeds have been:

- i) produced in a 'pest free area' free from *Columnnea latent viroid*, *Potato spindle tuber viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid*, and *Tomato planta macho viroid*.

OR

- ii) produced in a 'pest free place of production' free from *Columnnea latent viroid*, *Potato spindle tuber viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid*, and *Tomato planta macho viroid*.

OR

- iii) produced in a 'pest free place of production' where parent plants have been tested according to a NPPO approved methodology and found free from *Columnnea latent viroid*,

Potato spindle tuber viroid, Tomato apical stunt viroid, Tomato chlorotic dwarf viroid, and Tomato planta macho viroid.

OR

- iv) officially tested, on a representative sample of a minimum of 3000 seeds officially drawn according to the ISTA or AOSA sampling methodology using a NPPO approved PCR testing method, and found to be free from *Columnea latent viroid, Potato spindle tuber viroid, Tomato apical stunt viroid, Tomato chlorotic dwarf viroid, and Tomato planta macho viroid*².

AND

- d) ² The *Solanum lycopersicum* seeds have been:

- i) produced in a 'pest free area', free from *Tomato brown rugose fruit virus*

OR

- ii) produced in a 'pest free place of production' free from *Tomato brown rugose fruit virus*.

OR

- iii) officially tested, on a representative sample of a minimum of 3000 seeds officially drawn according to the ISTA or AOSA sampling methodology, using an NPPO-approved PCR testing method and found free from *Tomato brown rugose fruit virus*².

AND

- e) ² The *Solanum lycopersicum* seeds have been:

- i) produced in a 'pest free area', free from *Tomato mottle mosaic virus*

OR

- ii) produced in a 'pest free place of production' free from *Tomato mottle mosaic virus*.

OR

- iii) officially tested, on a representative sample of a minimum of 3000 seeds officially drawn according to the ISTA or AOSA sampling methodology, using an NPPO-approved ELISA or NPPO-approved PCR testing method and found free from *Tomato mottle mosaic virus*².

2.74.2 Testing requirements

- (1) Testing is required to be completed offshore prior to export, or on arrival in New Zealand.
- (2) Pre-export testing for each seed lot must be endorsed by the NPPO on the phytosanitary certificate, or if tested on arrival in New Zealand, must be completed by an MPI-approved testing laboratory.
- (3) Testing on-shore will be performed using an MPI-approved testing method.

Guidance

- The ISHI-Veg local lesion bioassay for *Tomato brown rugose fruit virus* and *Tomato mottle mosaic virus* is not accepted as a valid test by MPI.
- The use of a bioassay to detect the presence of *Pepino mosaic virus* on seed samples is not accepted as a valid test by MPI.
- Additional declarations on phytosanitary certificates to meet the offshore testing requirements for *Tomato brown rugose fruit virus* in Import Health Standard 155.02.05: *Seeds for sowing* should be based only on a negative result obtained in an NPPO-approved PCR test and not on results from a bioassay.
- Additional declarations on phytosanitary certificates to meet the offshore testing requirements for *Tomato mottle mosaic virus* and *Pepino mosaic virus* in Import Health Standard 155.02.05: *Seeds for*

sowing should be based only on a negative result obtained in an NPPO-approved ELISA or NPPO-approved PCR test and not on results from a bioassay.

- For tomato seed lots tested for quarantine pests onshore in New Zealand at an MPI-approved testing laboratory, additional declarations by the exporting NPPO are not required to be endorsed on the phytosanitary certificate.
- Removal of ELISA as a testing option for *Tomato brown rugose fruit virus* will come into force on 19 October 2021.