Code of Practice
for the Management of
Potato Spindle Tuber Viroid (PSTVd)
in Greenhouse Crops

March 2023

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1 Acknowledgements

This Code of Practice for the Management of Potato Spindle Tuber Viroid (PSTVd) in Greenhouse Crops is based on two previous documents:

- New Zealand Code of Practice for the Management of Potato Spindle Tuber Viroid (PSTVd) in Greenhouse Tomato and Capsicum Crops. New Zealand Vegetable & Potato Growers Federation, Wellington (Second Edition, 2003).
- Information on the Management of Potato Spindle Tuber Viroid (PSTVd) in Greenhouse Tomato and Capsicum Crops. Tomatoes New Zealand Inc. and Vegetables New Zealand Inc., Wellington (Draft, 2015).

The code of practice also includes procedures for destruction of infected material and cleaning and disinfection adapted from MPI's Operational Specification for the Potato Spindle Tuber Viroid (PSTVd) response (2022). The publishers of, and contributors to, those three documents are acknowledged.

2 Purpose and scope

This code of practice applies to commercial greenhouse production of tomatoes, capsicums, and eggplants. Its purpose is to enable rapid detection of PSTVd and to minimise the risk of spread of the disease. The code of practice includes measures that apply to two scenarios where PSTVd is detected:

1. The current situation where PSTVd is not present in New Zealand. If PSTVd is detected, MPI and Industry may initiate a response and growers should follow any additional directions issued by MPI.
2. A future scenario where PSTVd is present in New Zealand and there is no incursion response being undertaken. Actions taken following greenhouse detection of PSTVd are implemented by the grower in accordance with this code of practice.

3 Information on PSTVd

3.1 What is PSTVd?

Potato spindle tuber viroid (PSTVd) was first reported in the USA in 1922 and, as its name suggests, causes spindle shaped potato tubers. Since that time it has spread to many potato producing countries. PSTVd also infects other solanaceous crops including tomato, capsicum, aubergine, pepino, and cape gooseberry as well as wild and ornamental solanaceous species (e.g., nightshade, Poroporo, jasmine nightshade).

Viroids are smaller than viruses, but replicate in a similar way. They take over host cells within a plant and uses the plant's metabolic process to produce more viroid particles. The infection progresses to a stage where the metabolism in the whole plant is affected. With PSTVd the time from infection to expression of symptoms can take several weeks. During that time the plant may be infectious and presents a risk to transmission between plants.

3.2 Status of PSTVd in New Zealand

The official status of PSTVd in New Zealand is:

Transient: actionable, under eradication. PSTVd has been detected as an isolated population which may survive into the immediate future and, without phytosanitary measures for eradication, may establish. Appropriate phytosanitary measures have been applied for its eradication.

PSTVd has been detected on a few occasions in New Zealand on greenhouse crops and one instance on cape gooseberry, but has been eradicated each time. PSTVd has never been detected in New Zealand on potato, and there are no records of PSTVd in outdoor tomato or capsicum crops in New Zealand. There are also no records of detection of the viroid from wild host plants in New Zealand.

3.3 PSTVd symptoms

PSTVd is difficult to detect and may be present without any visible signs of infection showing in a plant. Symptoms may take several weeks from infection before symptoms become visible. This presents a risk of transmission to other plants.

Visual signs of PSTVd can be similar to those caused by nutrient deficiency, root disease and pest or spray drift damage.

3.3.1 Tomato symptoms

In tomatoes, there is a long latent period of four to five weeks after infection before the symptoms are expressed. Overseas, young plants (24 true leaf stage) infected with the virulent strain have shown leaf distortion (rugosity), downward bending of leaves caused by increased growth of the upper leaf surface (epinasty) and curling in new leaflets. The internodes become shorter, making the plant appear stunted and malnourished. However, symptoms are not usually evident until after the 5th truss. Later, severe veinal necrosis may develop. So far, no fruit symptoms have been described in tomatoes other than discolouration (yellowing) and blotchiness. Symptoms are exhibited more strongly under high radiation conditions suggesting that spring and summer crops may be more susceptible.

Yield of tomatoes from affected plants will be reduced, however losses depend on the age of the plant and the severity and strain of the infection. Lost production may be from 10-20%, and as high as 60% with severe strains.

Further information can be found in the Tomatoes NZ factsheet or the Horticulture NZ factsheet.

3.3.2 Capsicum symptoms

In capsicums, PSTVd can show itself in very subtle ways. Some common indicators are:

- Ruffled or wavy edges to the leaves. (Note: some varieties; e.g. the orange variety Boogie, have naturally ruffled or wavy leaves).
- Leaves may be smaller than normal.
- The calyx may curl up at the edges.
- Fruit may be smaller than normal.
- The fruit stem may be thin and skinny looking.
- Plant heads tend to be smaller and thinner than normal.

The severity of PSTVd in capsicums may vary from crop to crop and from variety to variety, as some varieties may be more susceptible to PSTVd infection than others. The production of capsicums from affected plants may not be significantly reduced, however the fruit is normally smaller, and the calyx may curl up at the edges.

3.3.3 Aubergine / eggplant symptoms

In eggplant, PSTVd can cause stunting and epinasty (downward pointing of leaves) in infected plants.

3.4 PSTVd transmission

PSTVd is transmitted in four different ways:

- Mechanical transmission. The main way that PSTVd is transmitted within crops is through routine plant handling operations and transfer of PSTVd on knives, equipment and clothing. Leaf hairs contain high concentrations of viroid and are easily transferred to other surfaces by normal cultivation activities. This occurs with potatoes and tomatoes, where viroid spread is mainly along the row.
- Infected seed and pollen. PSTVd is transmitted on seed and also on pollen. The rate of seed transmission is variable and can depend on variety. All solanaceous seed is imported to New Zealand under import conditions with strict requirements for PSTVd freedom.
- Aphid transmission. PSTVd can be transmitted by *Myzus persicae* (peach potato aphid) when infected with both *Potato leafroll virus* (PLRV) and PSTVd.
- Vegetative propagation. PSTVd can be transmitted from infected material during grafting and propagation by cuttings.

3.5 Survival of PSTVd on surfaces

PSTVd can survive in dried tomato sap for more than eight weeks and in infected leaf debris for over six months (DPI-NSW, 2023[1]).

PSTVd survives on many contaminated materials, remaining infective on contaminated metal after 1 hour; contaminated cotton after 6 hours; and contaminated wood, plastic and leather remained infective after 24 hours (Barbetti, 2012)[2]. However PSTVd survived for only 30 minutes on human skin. PSTVD survived for at least 4 weeks on aluminium but survived poorly on plastic (Spence and O'Neill, 2004[3]).

3.6 Disease prevention

Prevention includes all measures to prevent the introduction of PSTVd into a crop. It is very important to start a new production cycle with viroid-free planting material (seeds or plants).

[1] https://www.dpi.nsw.gov.au/biosecurity/plant/insect-pests-and-plant-diseases/pstvd
[2] https://ausveg.com.au/app/data/technical-insights/docs/130014_VG09110.pdf
[3] Protected tomato: Sources, survival, spread and disinfection of Potato spindle tuber viroid (PSTVd). Final report to Horticultural Development Council, UK. 2004

In addition to the use of healthy planting material, it is also important to prevent viroid introduction via human activities. Because PSTVd is easily transmitted mechanically it can be introduced into a greenhouse from other infected sources via the hands, clothes, or equipment used by people working in or visiting the greenhouse. The use of disposable gloves and specific clothing and equipment that stays inside a greenhouse unit may help prevent PSTVd spread to other greenhouse units.

Eradication is based on destruction of PSTVd-infected plants and thorough cleaning of equipment and greenhouses where infected plants have been grown.

4 Requirements of the Code of Practice

Key requirements of the code of practice are:

- Hygiene measures to prevent spread of PSTVd into and within greenhouses.
- Surveying of greenhouse crops for PSTVd.
- Isolation of suspect PSTVd infected plants and sampling for testing.
- Management of growing operations in greenhouses confirmed PSTVd.
- Cleaning and disinfection between growing cycles.

These requirements are presented below, and growers should consider how they will meet the requirements. PSTVd is highly transmissible through handling and working with plants. Ideally, the requirements will fit in with existing hygiene practices, but growers should review their hygiene and isolation practices against these requirements and modify practices if necessary.

Growers should document how they will meet the requirements, so that all staff are aware of their roles in PSTVd management. The procedures need to identify what actions are taken, who is responsible for the action, when it will be done, and how the outcome will be recorded. Alternatively, growers can adopt or modify the generic procedure provided in Appendix 1.

4.1 Responsibility for implementing the PSTVd code of practice

Greenhouse operators should nominate a person responsible for implementing the code of practice including monitoring of hygiene procedures, surveying of crops, sampling and isolation suspected PSTVd infected plants, removal and destruction of confirmed PSTVd infected plants, management of harvest operations, supervision of disinfection of production sites, and record keeping. This person is referred to as the PSTVd manager.

4.2 Source of planting material

Greenhouse operators should ensure that planting material is free from PSTVd. This can be achieved by ensuring that seed or plant suppliers have had the seed tested and found free from PSTVd, and are part of the Plant Pass scheme.

4.3 Hygiene measures to prevent the spread of PSTVd

The activities and movement of people and equipment is one of the main ways that PSTVd is spread within and between greenhouses. Greenhouse operators or PSTVd managers should document the procedures that they will follow to minimise the likelihood of PSTVd entering greenhouses and spreading between plants and

between units or other production sites. These procedures should address the following:

- Ensuring the greenhouse environs are free from non-crop PSTVd host plants.
- Control over movement of staff and equipment (including pollinators) into and between greenhouse units.
- How hands are kept clean.
- How knives and tools are kept clean or sterilised.
- Cleaning of equipment such as trolleys.
- Control over visitors to the greenhouse.
- Removal and destruction of volunteer plants.

4.4 Surveying crops for PSTVd

Greenhouse operators or PSTVd managers should document procedures for monitoring greenhouses for PSTVd. The monitoring procedures must:

- Identify who will undertake surveying activities.
- Describe how surveying will be undertaken.
- Ensure that the surveying will be carried out, at least
    - weekly in infected greenhouses.
    - monthly in all other greenhouses.
- Describe how proficiency of persons surveying for PSTVd will be ensured.
- Describe how samples of plant material from suspected PSTVd infected plants will be taken and submitted for identification.
- Describe how records will be kept.

4.5 Isolation of suspect PSTVd infected plants

Greenhouse operators or PSTVd managers will document procedures for dealing with suspect PSTVd infections. The procedures must:

- Describe how the suspect plant and the 5 plants on either side will be marked to allow for future identification.
- Describe how the suspect plant will be isolated from the rest of the production unit (to prevent possible spread of PSTVd).
- Describe how the marked plants will be isolated and additional hygiene measures applied to prevent disease spread until test results are received.

4.6 Collection and submission of samples

Where it is suspected that a plant is infected with PSTVd, procedures should describe how to take leaf samples and submit them to the MPI Approved Laboratory in accordance with Appendix 1 (5.6 Collection and submission of samples).

4.7 Management of greenhouses with confirmed PSTVd

Where PSTVd has been detected in a greenhouse, MPI may issue directions to take actions and those directions must be followed.

Growers should develop procedures that describe how greenhouses with confirmed PSTVd will be managed in circumstances where there is no direction from MPI (e.g., no incursion response has been initiated).

Describe the procedure for removal of a confirmed PSTVd infected plant and the 5 plants on either side ensuring:

- Staff wear disposable gloves.
- Plants are bagged for disposal.
- Equipment used with the infected plants is sterilised or disposed of.

It is recommended that additional measures are taken to reduce the risk of spread to other units. Procedures should describe:

- Increased monitoring frequency from monthly to weekly.
- Further isolation of the affected unit.

4.8 Cleaning and disinfection between crop cycles

PSTVd can be difficult to detect and may be present in a greenhouse without the grower's knowledge. Thorough cleaning and disinfection between crop cycles is an opportunity to break a disease cycle by removing potentially infected plants and cleaning surfaces where PSTVd may survive and be transferred to new plants. Even if there have been no confirmed detections of PSTVd, there may be latent or undetected PSTVd infection present. To break any potential disease cycle growers should follow the procedure described in Appendix One (5.8 Cleaning and disinfection between crop cycles).

4.9 Monitoring and record keeping

The PSTVd manager will monitor CoP activities and keep records of:

- Surveying, including the location and marking of suspect infections.
- Locations and dates of samples submitted for diagnosis, and the results
- Actions taken on receipt of PSTVd positive results.
- disinfection operations between crop cycles.

5 Appendix 1. Procedure for compliance with the code of practice

5.1 Responsibility for compliance with this procedure

The PSTVd manager is responsible for this procedure. The PSTVd manager for [Business name and address] is [Name……]

5.2 Source of planting material

- Record details of planting material, such as:
    - Source of seeds (including batch numbers, supplier, dates) used for rootstocks and grafting.
    - Confirmation by suppliers that seeds
        - were imported in compliance with MPI's Import Health Standard, or
        - if New Zealand grown a representative sample of at least 3000 seeds, drawn according to the ISTA or AOSA sampling methodology, was tested and found free from PSTVd.
    - Confirmation that plants were grown in accordance with PlantPass.
    - Dates of receipt and planting (including units / rows planted).

5.3 Hygiene measures to prevent the introduction and spread of PSTVd

- Ensure greenhouse environs are free from wild and ornamental hosts of PSTVd
    - Inspect the area around the greenhouse and remove any wild host plants such as nightshade.
- Ensure staff are familiar with requirements for cleaning and disinfection, including use of disinfectant products:
    - General equipment to be cleaned and disinfected with virkon at 2% with contact time of at least 1 hour, rinse with water afterwards.
    - Sensitive equipment cleaned with virkon 2% should then be wiped with 70% ethanol to remove any residual virkon.
    - Scissor trolleys – make watertight and steam clean. Wipe external parts of the trolley with virkon 2% where it is safe and practical to do so. Rinse with water after.
    - Equipment able to be treated with virkon 2% must follow the label directions for use, with an increased contact time to 1 hour. Rinse with water afterwards.
- Develop and follow a containment plan for the greenhouse complex (separating the greenhouse complex into isolation units where movement of people and equipment between units is minimised may reduce the spread of PSTVd across the site)
    - Identify all greenhouses on a diagram.
    - Identify which greenhouses (or sections of greenhouses) are considered separate isolation units because -
        - They have doors between sections.
        - Are managed by separate teams or on separate days.
        - Have equipment (e.g., trolleys, knives) that stay within the unit and are not shared between units.
        - Have independent hydroponic systems.

    - Instruct all staff on the operation of the containment plan and monitor implementation.
- Instruct all staff on hygiene requirements and monitor implementation:
    - No animals are allowed inside the greenhouse at any time (other than pollinators).
    - Staff must wear clean clothes or overalls each day or when moving between units.
    - Staff must wash and sanitise hands when entering or leaving the unit and before and after breaks.
    - Staff working the crop must
        - wear latex (or other impermeable) gloves that are disposed of at the end of each day or when moving to a new unit.
        - Dip gloved fingers and knives into disinfectant solution before moving into the crop or before commencing work in a new unit.
        - Dip gloved fingers or pruning tools into disinfectant solution inside a container and/or sponge when twisting, layering or leafing.
        - Replace disinfectant frequently (as these products break down in contact with light and organic matter) and wash sponges daily.
        - Perform all plant handling tasks such as twisting, leaf removal and picking in the same direction along the row. (This is very important, as this will ensure that if PSTVd does spread in the greenhouse, it will spread mainly in one direction).
- Instruct all visitors on hygiene requirements and monitor implementation
    - Contractors entering the premises to work on plants wear new gloves and clean protective clothing (or disposable suits) that remain in the greenhouse or are disposed of when finished.
    - Advisors, seed and other company representatives wear clean protective clothing and also gloves if handling plants.
- Supervise visitors not working with the crop to ensure that they keep to the pathways and do not handle the crop.
- Place mats at all doorways and keep them moist with disinfectant throughout the day.
- Ensure staff comply with equipment cleaning instructions:
    - Clean and spray trolleys with disinfectant plus any other equipment being moved from one unit to another.
    - Sweep and clean trolleys and bins before moving to a new row.
    - Keep concrete paths free of plant material at all times by ensuring bins are not overfilled and any spillage is promptly cleaned up.
    - Place leaves and prunings into designated containers.
    - Don't get into bins to tramp down leaf matter.
    - Keep trolleys free of plant material and sweep and clean at the end of each day.
    - Sanitise reused equipment; e.g. bobbins, clips.
- Ensure that pollinator colonies are not moved between units.
- Ensure staff removing rogue plants:

- Remove any tomato or capsicum seedlings emerging from the drains or holes.
- Do this as a separate job and ensure other plant material is not touched.
- Wear gloves and dispose of these afterwards.

5.4 Surveying of crops for PSTVd

- Ensure that all staff working plants in the designated production units exhibit competency in the recognition and reporting of symptoms of PSTVd.
  - Instruct staff on PSTVd symptoms and reporting of suspect PSTVd to the PSTVd surveyor.
  - Ensure that PSTVd identification resources (e.g., booklets distributed by TNZ and VNZ) are readily available to all staff working the crop during the surveying period.
- Ensure that greenhouses are surveyed for PSTVd at least weekly in infected greenhouses and at least monthly in all other greenhouses.
  - Survey activities may be part of normal plant management activities.
  - Ensure that survey personnel are competent in recognition of PSTVd symptoms and recording and reporting requirements:
    - Train survey personnel in symptom recognition.
    - Assess competency of survey personnel in symptom recognition, recording and reporting.
    - Maintain records of competency assessments.
- During the surveying programme, ensure that survey personnel inspect each plant for symptoms of PSTVd.
- Monitor survey personnel during PSTVd surveying to ensure that where symptoms resembling those of PSTVd are detected, these are reported to the PSTVd manager.
- Inspect suspect plants and determine whether the plants are likely to be infected with PSTVd, or if there is another cause of infection.
- Maintain records of surveying activities and actions taken.

5.5 Isolation of suspect PSTVd infected plants

- When a plant has been sampled for suspect PSTVd, attach a label to the plant so that it can be identified as a suspect plant (in case resampling is necessary or if positive results are obtained).
- Attach a label to the 5 plants on either side of the suspect plant, indicating that they are adjacent to a suspect plant.
- Cordon off the row (both sides) and ensure that staff working in the area are aware of suspect PSTVd plants in that row and of the following requirements.
- Ensure staff working plants in the rows with suspected infected plants follow additional hygiene measures:
  - Work the isolated row from each end if possible, working towards the isolated plants, working the isolated plants last.
  - Wear latex (or other impermeable) gloves that are disposed of at the end of each day or when moving to a new row.
  - Dip gloved fingers and knives into disinfectant solution before moving into the crop or before commencing work in a new row.

- Dip gloved fingers or pruning tools into disinfectant solution inside a container and/or sponge when twisting, layering or leafing.
- Replace disinfectant frequently (as these products break down in contact with light and organic matter) and wash sponges daily.
- Clean and spray trolleys with disinfectant plus any other equipment being moved from one row to another.
- Place leaves and prunings into designated containers.
- Don't get into bins to tramp down leaf matter.
- Sweep and clean trolleys and bins before moving to a new row.
- Keep concrete paths free of plant material at all times by ensuring bins are not overfilled and any spillage is promptly cleaned up.
- Keep trolleys free of plant material and sweep and clean at the end of working the affected row.
- Sanitise reused equipment; e.g. bobbins, clips.

5.6 Collection and submission of samples

Where it is suspected that a plant is infected with PSTVd, take leaf samples and submit them to the MPI Approved Laboratory in accordance with the following procedure:

- Do not touch the suspect plants with bare hands but wear disposable gloves for sampling.
- Use a new pair of disposable gloves for each plant sampled.
- Take a sample of 5 leaves from each suspect plant. Take leaves at equal distances from between 5cm from the top down to 1m from the top.
- Collect each sample of 5 leaves using gloved hand and while still holding sample turn glove inside out leaving sample well inside (so the leaves do not come out of the glove).
- Place samples in a ziplock plastic bag with the air expressed prior to sealing.
- This bag should be placed inside another ziplock bag and sealed.
- Label the bag with full details of the grower's name, address, and contact phone numbers.
- Keep bags containing leaf samples cool at all times and ensure they are not frozen. Do not leave samples in the sun or inside hot vehicles. Place samples in a small chilly bin with a cold pack wrapped in several paper towels or newspaper to prevent freezing of samples.
- Wash hands thoroughly with soap and water and decontaminate any equipment.
- Label the chilly bin with laboratory address and full grower return address and contact phone numbers.
- Contact the laboratory in advance of sending off samples to advise that a suspect sample is being submitted. If samples are collected on Sunday through Thursday, dispatch samples immediately on overnight courier. For samples collected on Friday or Saturday, leave a message on the answer phone and keep samples cool, and courier to the laboratory on Sunday (for Monday delivery).

The laboratory address is:

Attention: Lisa Ward

URGENT- Virology – Plant sample for testing
MPI PHEL
231 Morrin Road
St Johns
AUCKLAND
Ph: 09 909 3015
Fax: 09 909 5739
Email: lisa.ward@mpi.govt.nz

5.7 Management of greenhouses with confirmed PSTVd

- If laboratory tests confirm PSTVd follow any directions issued by MPI.
- If no directions are issued, supervise staff in the removal of suspect plants and the 10 marked neighbouring plants as follows:
    - Wear gloves and protective clothing when removing infected plants;
    - Remove plants and place in bags for disposal.
    - Dispose of, or sanitise, other equipment (such as bobbins, support clips and truss clips) used in conjunction with infected plants.
- Implement measures to reduce the risk of spread to other units:
    - Increase monitoring frequency to weekly monitoring.
    - Review isolation plans and determine whether isolation measures can be strengthened:
        - If the isolation unit comprises more than one greenhouse, can the affected greenhouse be separated from the others in terms of movement of people and equipment.
        - If teams manage the affected greenhouse as well as others in the current isolation unit, can the movement of teams be restricted by splitting the team or timing of their work so they aren't moving between the affected greenhouse and others on the same day.

5.8 Cleaning and disinfection between crop cycles

- At the end of production, supervise staff in the removal of all plants, their disposal, and cleaning and disinfection of surfaces in the production unit.
- Confirm all persons involved in this process wear disposable suits that are left in the greenhouse during breaks and discarded at the end of the cleanup.
- Ensure that plant material and growing slabs/bags are removed as quickly as possible and the floor is swept clean of all debris.
- Confirm that the plant material is transferred to vehicles and taken for disposal.
- After the crop has been removed confirm that the following is undertaken:
    - Waterblast gutters and toughs and floors.
    - Spray and treat concrete floors and paths with virkon 2% with contact time of 1 hour. Rinse with water afterwards.
    - Clean and disinfect plastic sheeting or alternatively remove it.
    - Treated glass house walls with virkon 2%, then rinse with water. Only clean the glasshouse walls as high as the second windowpane taking care to avoid splashing any virkon onto the ceiling and screens. Then clean the remainder with GS4 before washing with water.

    - Clean the underside of the roof glass and ceiling screens using the underside roof washer with pressurised fresh water.
    - Flush and sterilize all irrigation lines with nitric acid (following the label directions for concentration) to remove build-up of organic material, and then flush with water. The system should then be filled with hyperstat (following the label directions for concentration) which will sit there for a week before it is then flushed with water again.
    - Replace CO2 lines.
    - Replace or sanitise all bobbins, support clips and truss clips.
    - Disposal of pollinator colonies.
    - Water tank systems are decontaminated and cleaned out with sodium hypochlorite following the label directions for concentration, with a contact time of 1 hour. Flush thoroughly with water. Check with supplier/technician for compatibility.
    - If biofilters are used, consult the supplier for decontamination procedures.

- Maintain record of disinfection operations, including the following information:

    - Date harvesting finished.
    - Date(s) disinfection commenced and finished;
    - Method and location of disposal of plant material;
    - Method of treatment/disposal of growing substrate and other substrate;
    - Chemicals used to disinfect/sanitise.