#### National regulatory control systems Systèmes de lutte nationaux réglementaires

### Potato spindle tuber viroid on potato

#### Specific scope

This standard describes a national regulatory control system for *Potato spindle tuber viroid* (PSTVd) that provides guidance on preventing its introduction, surveillance for the pathogen and its containment and eradication if found infecting potato plants or tubers.

#### Introduction

*Potato spindle tuber viroid* (PSTVd) is an A2 pest for EPPO. Details of its biology, distribution and economic importance can be found in EPPO/CABI (1997) and the Plant Quarantine data Retrieval system on the EPPO website.

In potato, PSTVd is spread primarily through the use of infected planting material, produced vegetatively or as botanical seed (Fernow *et al.*, 1970; Singh, 1970). The viroid can also be transmitted mechanically. It survives in dried plant sap and can contaminate machinery and storage facilities. Transmission may occur in the growing crop from plant-to-plant contact and passage of machinery (Merriam & Bonde, 1954; Manzer & Merriam, 1961). The extent of mechanical transmission depends on a number of factors including the host plant species or cultivar which acts as the inoculum source, the viroid concentration, the frequency and severity of damage, and the temperature. The viroid may be present at much lower concentration in plants grown at 25° than at 30°C (Morris & Smith, 1977). Tuber-to-tuber transmission can also occur on cutting knives and seed-piece contact if seed tubers are cut into seed-pieces (Gos, 1926).

Experimentally, aphid transmission has been shown to occur if PSTVd RNA is encapsidated in virions of *Potato leafroll virus* (PLRV) (Salazar *et al.*, 1995), with the efficiency of transmission depending on the potato cultivar used as the inoculum source (Syller & Marczewski, 2001). Although the practical significance of aphid transmission in the spread of PSTVd into and between crops is not known, epidemiological surveys in China have shown a strong correlation between PSTVd infection and the presence of PLRV in potato crops (Querci *et al.*, 1997). The viroid is also known to be transmitted by pollen produced from infected plants under glasshouse conditions (Fernow *et al.*, 1970;

## Specific approval and amendment

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Singh *et al.*, 1992) but the practical significance of pollen transmission under field conditions is not known.

Potato spindle tuber viroid mainly infects species of the Solanaceae, including potato (Solanum tuberosum) and tomato (S. lycopersicum). PSTVd has also been found in avocado (Persea americana; Querci et al., 1995), pepino (S. muricatum; Puchta et al., 1990; Shamloul et al., 1997; Verhoeven & Roenhorst, 1995) and capsicum (Capsicum annuum; Lebas et al., 2005). A wide range of ornamental Solanaceae have also been found to be naturally infected with PSTVd: Brugmansia spp. (Verhoeven et al., 2008a; Mertelik et al., 2009), Cestrum spp. (Luigi et al., 2011), Lycianthes rantonetti (Di Serio, 2007), Petunia spp. (Mertelik et al., 2009), Physalis peruviana (Verhoeven et al., 2009), S. jasminoides (Verhoeven et al., 2008a; Mertelik et al., 2009) and Streptosolen jamesonii (Verhoeven et al., 2008b). In addition, it has been detected in one cultivated plant of Datura sp. (Verhoeven et al., 2010a). Infection has been asymptomatic in all these ornamental species so in the absence of testing, it would go undetected. Ornamental species have been shown to pose a risk to tomato production (Verhoeven et al., 2010a) and may pose a risk to potato production, particularly from mechanical transmission of the viroid if there is a pathway to potato through staff or machinery. Temperature and the plant species acting as the inoculum source may affect the success of transmission since mechanical transmission using finger rubbing from Brugmansia suaveolens and Solanum jasminoides to potato did not occur at a temperature of 15°C but did occur at a higher temperature of 25°C. In addition, S. jasminoides caused infection in potato using lower dilutions of sap  $(10^{-3} \text{ dilution})$  than B. suaveolens  $(10^{-1})$  (Verhoeven et al., 2010b).

The distribution of PSTVd within the EPPO region in potato is restricted to some parts of Eastern Europe (EPPO/CABI, 1997). Prevention of further spread of this pest within the region may be achieved mainly by phytosanitary measures that restrict/prevent the movement of infected planting material, and the operation of certification schemes for seed potato. The restrictions for PSTVd, specified in the pest-specific phytosanitary requirements of EPPO Standard PM 8/1 *Commodity-specific phytosanitary measures for potato* (OEPP/EPPO (2004c), are intended to form part of the phytosanitary regulations of EPPO countries.

It is recommended that EPPO countries establish a national regulatory control system for PSTVd based on this standard to include measures to prevent the introduction of PSTVd to the potato production system and to carry out surveillance, containment and eradication of the pest, if present. The national regulatory control system should provide sufficient assurances to allow export of potatoes within the EPPO region, in conformity with the specific phytosanitary requirements for the pest. It is also recommended that EPPO member countries at risk prepare a pest-specific contingency plan (based on EPPO standard PM 9/10 *Generic elements for contingency plans*) to ensure that the necessary management and operational arrangements are in place to deal with an outbreak.

Although the regulatory control system described is for PSTVd, many elements of it could be applied to control of other viroids should they be found in potato. *Citrus exocortis viroid* and *Columnea latent viroid* (Verhoeven *et al.*, 2004), *Tomato chlorotic dwarf viroid* (Singh *et al.*, 1999), *Tomato apical stunt viroid* (Verhoeven & Roenhorst, 2010) and *Tomato planta macho viroid* (Galindo *et al.*, 1982) have been shown to infect potato experimentally, but apart from *Mexican papita viroid* infecting the wild potato species *Solanum cardiophyllum* (Martinez-Soriano *et al.*, 1996) there are no reports of these viroids infecting potato naturally. Nevertheless, these viroids have been detected in tomato and traded ornamental plants of Solanaceae species and so may pose a risk to potato.

#### Outline of the system

Visual inspection of potato plants and tubers is not adequate to prevent the spread of PSTVd because PSTVd infection can often be latent. Control of PSTVd depends primarily on the use of nuclear stock material tested as free of PSTVd and its subsequent propagation within a certification scheme in a pest-free area. If the pest is found, phytosanitary measures are needed to contain, suppress and eradicate it. This includes delimitation of a regulated area (i.e. the 'infested' area and 'probably infested' area) and the restriction of the cultivation of potato and the control of volunteer potatoes, for several years. Other host plants of PSTVd (e.g. tomatoes and other Solanaceae) in the infested area should be assessed for PSTVd infection and, if appropriate, restrictions placed on their cultivation. Hygiene measures are also required since PSTVd may be transmitted mechanically and it is very persistent in dried tuber or leaf sap that can contaminate surfaces, for example, in stores and on graders, potato-harvesting equipment and storage boxes.

Even though PSTVd may be present, countries which apply a national regulatory control system should be able to export potatoes on the same basis as countries which have demonstrated that PSTVd does not occur. Therefore, potatoes can be exported both from areas where PSTVd is known not to occur and from countries where it occurs, provided that the potatoes are produced in areas that lie outside any regulated area for PSTVd and the production system is regulated to prevent its re-introduction. In practice, regulated areas are defined at the level of places of production.

#### **Control system**

This control system for PSTVd has a number of objectives:

- (1) to prevent its introduction into the potato production systems;
- (2) to determine if the pest is present in the potato production systems in the country through surveillance and, if present, to determine its distribution;
- (3) to prevent its spread;
- (4) to eradicate incursions;
- (5) to eradicate the pest from potato production systems in areas where it is present.

#### 1. Prevention of introduction

The holding and handling of PSTVd should be prohibited, except under special permit or licence, as recommended in EPPO Standard PM 3/64 *Intentional import of organisms that are plant pests or potential plant pests* (OEPP/EPPO, 2006c).

To prevent the introduction of PSTVd, breeding material should be tested according to post-entry quarantine requirements (OEPP/EPPO, 2006a).

All seed potatoes intended for marketing in the EPPO region should be free from PSTVd as recommended in EPPO Standard PM 4/28 *Certification scheme for seed potatoes* (OEPP/EPPO, 1999). This should include use of nuclear stock or initial material tested for freedom from PSTVd and where risk is identified, subsequent testing of samples of Propagation Stock<sup>1</sup>. In addition, the requirements in EPPO Standard PM 3/61 *Pest free areas and pest-free production systems for quarantine pests of potato* should be followed (OEPP/EPPO 2004b).

Potential risks of ornamental plants, particularly Solanaceae, acting as a pathway to the potato production system should be considered by NPPOs. Specific surveillance such as testing of tomato and other solanaceous crops including ornamentals will help determine the level of risk. The complete separation of potato production from tomatoes and other solanaceous crops including ornamentals will reduce this risk.

Provided that there is no link to seed potato production through transport and handling, the risk associated with ware potatoes is much lower as they are not intended to be planted. EPPO standard PM 8/1 specifies that ware potatoes should come from pest-free areas for PSTVd and originate from pest-free potato production and distribution system for PSTVd according to EPPO Standard PM 3/61.

<sup>&</sup>lt;sup>1</sup>Propagation Stock = basic and certified seed potatoes from UNECE terminology (UN/ECE, 2008).

#### 2. Surveillance

*Potato spindle tuber viroid* should be considered as a notifiable pest. All persons suspecting or confirming the presence of PSTVd should notify the NPPO. Surveillance may be general surveillance based on historical data and/or specific surveys (see ISPM No. 6 Guidelines for Surveillance). Specific surveys of growing potato crops and tubers in store should be conducted if justified by pest risk assessment.

There is no need for specific surveys in potato if:

- there is no recent data on presence of PSTVd in the country in the potato production system, and
- all potato material imported or marketed and planted in the country is derived from PSTVd-tested material.

In certification schemes, the growing crop and tubers are inspected. Although an unreliable indicator of infection, plant symptoms (reduction in plant size, changes in plant growth habit ranging from severe symptoms of uprightness with dark green rugose leaves to mild mosaic) may be present depending on the PSTVd strain, potato cultivar and environmental conditions. Infected tubers may be reduced in size and may be misshapen with spindling and conspicuous eyes. Plant symptoms may be more obvious after flowering. All suspect leaf or tuber material should be tested in the laboratory.

#### Specific surveys

Where specific surveys are considered justified (e.g. after a recent PSTVd outbreak, or when the status of PSTVd is not certain in the country, or to specifically target potato material not known to be derived from PSTVd-tested material) laboratory testing using EPPO Diagnostic Protocol PM 7/33<sup>2</sup> (OEPP/EPPO, 2004a) should form the basis of the survey. ISPM No 31 *Methodologies for sampling of consignments* may be used as a basis for establishing sampling rates. However, as a minimum for the growing crop, one fully expanded leaflet should be taken from the top of each of 200 plants. Alternatively or additionally, after harvest a minimum of 200 tubers should be sampled randomly from each crop. Increasing the number of plants or tubers tested for each crop will increase the probability of detection.

#### 3. Determination of the presence and containment

If an outbreak is suspected the NPPO should initially prohibit movement of all suspect material. This may involve the prohibition of movement of all potatoes or other host plants from the place of production<sup>3</sup> concerned and restrictions on the movement of staff and farm machinery. Suspect host material should be subject to confirmatory testing according to EPPO Diagnostic protocol PM  $7/33^2$ .

If the initial suspicions are not confirmed then any prohibitions should be lifted. If infection by PSTVd is confirmed the prohibitions should be continued.

The NPPO should designate as 'infested':

- the potatoes (plants and tubers) from which the sample was taken.
- contaminated equipment and other articles (machinery, packing material, store, etc.) which have been in contact with the potatoes.
- the place of production, where the potatoes were grown.

The extent of 'probable' infestation<sup>4</sup>, should be determined by considering all other potatoes and other hosts that may be linked with the designated infestation by place of production, storage or usage of machinery. Clonally related stocks also need to be considered. A regulated area should be demarcated composed of places of production designated as 'infested' and places of production designated as 'probably infested'.

The NPPO should investigate the extent and the primary source of the PSTVd outbreak. All potatoes grown at the 'infested' place of production should be sampled and tested, in particular, any clonally related potato stocks (i.e. sister stocks and earlier propagation material). If the seed potato production system is confirmed as the primary source of the outbreak, the testing should be extended to all potato material which is clonally related of other relevant places of production. If still available this should include testing of the nuclear stock used to produce the infested potatoes. Also other lots of potatoes may be tested at relevant places of production, that may have been in contact with the infected potatoes, e.g. through machinery and storage, potato cleaning and grading systems.

If infestation cannot be explained by clonal relationships or contact relationships with other infested propagation material, other host plants in the 'infested' place of production should be tested. If the source of infestation cannot be found, the NPPO should consider whether aphid-borne transmission may have occurred (i.e. whether aphids and PLRV were present in the growing crop).

If PSTVd is detected (and confirmed by testing) in the seedpotato production system, earlier propagations should be tested for the pest. If the source of infestation could not be identified, sampling and testing for PSTVd in the seed-potato production system should be intensified in accordance with sound scientific and statistical principles. It also is recommended to sample and test each seed potato crop within the seed potato certification scheme.

If the 'infested' potatoes present a risk to another country, the NPPO of that country should be informed immediately of a suspect finding so that it can take appropriate measures. Information for the potato lots exported should at least consist of:

- (1) the variety name of the potato lot;
- (2) the type (ware, seed, etc.) and where applicable the seed category of potatoes;
- (3) the name and address of the consignor and the consignee;
- (4) the date of delivery of the potato lot;
- (5) the size of the potato lot delivered.

 $<sup>^2\</sup>mathrm{Under}$  revision to include testing of growing crops, tubers and other Solana-ceae.

<sup>&</sup>lt;sup>3</sup>A place of production is defined as 'Any premises or collection of fields operated as a single production or farming unit. This may include production sites which are separately managed for phytosanitary purposes' (ISPM No. 5, *Glossary of phytosanitary terms*).

<sup>&</sup>lt;sup>4</sup>No positive test result, but a strong presumption that infection is possible.

Additionally, the registration number of the grower or merchant and a copy of the delivery notice should be provided if available.

If a confirmed outbreak is associated with material received from another country, evidence such as appropriate specimens or material and documentation should be kept for up to 1 year according to the requirements in ISPM No. 13 *Guidelines for the notification of non-compliance and emergency action*.

#### 4. Eradication from the potato production system

In order to ensure that PSTVd is eradicated from the potato production system, the following measures should be implemented as appropriate.

Successful application of the disposal methods recommended below for all material designated as 'infested' or 'probably infested' will require careful implementation to ensure pathogen containment during treatment and thorough pathogen elimination prior to release from containment of treated material. Regular monitoring and auditing of disposal procedures should ensure their efficient and effective application at all times. In addition, if material is transported for processing a system of cleansing and disinfection (see below) should be in place for at least the vehicles that have been used for transportation.

#### 4.1 Measures applied at 'infested' places of production

#### 4.1.1 Measures for infested tubers

Tubers designated as 'infested' should be disposed of using, for example:

- incineration.
- heat sterilization.
- industrial processing at a processing plant with appropriate waste facilities.
- fermentation and composting at an officially approved composting site following EPPO standard PM 3/66 Guidelines for the management of plant health risks of biowaste of plant origin (OEPP/EPPO, 2008).
- · steaming and feeding to animals.
- deep burial.
- Alternatively:
- Tubers can be fed directly to cattle on a contained hard-standing area provided all the manure and tuber debris is collected and composted in a contained area for a period of at least 2 months and is not subsequently returned to arable land.
- Fermentation of contaminated potatoes during silage production may also be a convenient pre-treatment prior to direct feeding to animals under the same controlled conditions as for direct tuber feeding.
- 4.1.2 Measures for infested fields
- No potatoes or other host crops of PSTVd should be grown in the 'infested' fields until no volunteer potato plants have been found for two consecutive years.

- Solanum weeds should be controlled.
- Where there is a risk of volunteer plants from true potato seeds, longer intervals may be necessary unless results of testing these volunteer plants show that they pose no risk.
- When potatoes are grown for the first time after the infestation, only ware potatoes should be produced. Harvested tubers should be tested according to EPPO Standard PM 7/33. Then after the normal rotation cycle, either seed or ware potatoes may be produced.
- Alternatively:
- 'Infested' fields may be maintained, either in bare fallow or in permanent pasture with frequent close cutting or intensive grazing for 3 years.
- After this period either seed or ware potatoes may be produced.
- Harvested tubers should be tested according to EPPO Standard PM 7/33.

# 4.2 Measures applied at 'probably infested' places of production

#### 4.2.1 Measures for 'probably infested' tubers

All potato tubers designated as 'probably infested' should also be disposed of, as above (see 4.1.1). Alternatively:

- Tubers can be used as ware potatoes under the control of the NPPO for direct consumption, provided that they are packed, ready for direct delivery and use without repacking, on a site with appropriate waste disposal facilities.
- On-farm composting at the place of production is also suitable provided it is conducted under official supervision to ensure that the entire quantity of material is adequately composted by exposure to a temperature of at least 55°C during an uninterrupted period of at least 2 weeks.
- During winter tubers may also be returned to the field of origin and left on the surface to be killed by exposure to frost but there should be no significant risk of any movement of the material away from the field.
- Anaerobic digestion for production of biogas at an officially approved site is also a suitable method for disposal provided the entire volume of material is subjected to a minimum temperature of 55 °C maintained over a period of 24 h without interruption with an hydraulic dwell time in the reactor of at least 20 days.

#### 4.2.2 Measures for 'probably infested' fields

When potatoes are grown for the first time after the infestation in fields designated as 'probably infested', only ware potatoes should be produced. Then after the normal rotation cycle, either seed or ware potatoes may be produced.

#### 4.3 Measures applied in the regulated area

#### 4.3.1 Potato plants

Potato plants designated as 'infested' and 'probably infested' should be destroyed promptly with a herbicide (preferably systemic to reduce tuber viability) if the crop is still actively

			Active ag	gainst			Application	rates			Efficacy in the	
Active substance	Commercial product	Country	Bacteria	Fungi	Viruses	Viroids	Spray	Fog	Dip	Corrosive	presence of organic matter	PSTVd
Benzoic acid	Menno florades;	AT, FR, GB, LT, LV, NL, DE, BE	2	7	7	2			1:50-1:100	ć	1	7*
Chlorine (hypochlorite etc.)†	Menno clean	GB, FR, NL, PL, SK	7	2	7	7	1% available		1% available	Metals (except stainless steel)		7
Potassium monopersulphate, potassium hydrogen sulphate, potassium sulphate	Virkon S	GB, FI, FR,	7	2	2	7	chlorine 1:100	1:25	chlorine 1:100	I	7	7
*Timmermann et al. (2001).												

Table 1 Examples of disinfectants used in potato production against PSTVd (List gathered in 2008) (Products should be used following the instructions on the label)

and solution a 5% generally S oleach household hypochlorite, of solution stock the п chlorine available the ĥ determined required is therefore the dilution four parts wate bleach to tA solution of 1% available chlorine is required, part one p herefore requires a dilution of growing) or by burning, and the tubers harvested where appropriate.

#### 4.3.2 Other objects

All objects designated as 'infested' or 'probably infested', such as equipment, machinery, storage facilities including those used for local distribution should be thoroughly cleaned and disinfected following EPPO Standard PM 10/1 *Disinfection in potato production* (OEPP/EPPO, 2006b), but using the disinfectants listed in Table 1, or destroyed if this is appropriate.

#### 4.3.3 Long-term measures

The following measures should be implemented for 2 years (or 3 years if fields in the 'infested' places of production are subject to 3 years of measures described above):

- Potato production, handling and storage should be done under official supervision.
- Harvested seed- and ware-potato stocks should be kept separate or cleansing and disinfection should be carried out between the handling of seed and ware stocks.
- Only certified seed potatoes, or potatoes grown under official control, should be planted.
- Official monitoring should be conducted using laboratory testing at a sampling rate to be established by the NPPO.

If nuclear stock microplant material is found to be infested, the material should be destroyed and a thorough investigation carried out to determine the potential source of PSTVd infection and why it was not detected. The risk of transmission of PSTVd to other nuclear stock material held should be assessed and this material tested if appropriate. Decontamination of the facility should be carried out. Plants and tubers derived from infested nuclear stock should be designated as infested and appropriate measures described above should be taken.

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