EPPO Datasheet: *Pospiviroid fusituberis*

Last updated: 2021-03-02

IDENTITY

Preferred name: *Pospiviroid fusituberis*
Taxonomic position: Viruses and viroids: Viroids: Pospiviroidae
Other scientific names: *PSTVd, Potato gothic virus, Potato spindle tuber pospiviroid, Potato spindle tuber viroid, Potato spindle tuber virus, Tomato bunchy top viroid*
Common names: bunchy top of tomato, spindle tuber of potato
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EPPO Categorization: A2 list
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EU Categorization: Emergency measures (formerly), RNQP ((EU) 2019/2072 Annex IV)
EPPO Code: PSTVD0



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Notes on taxonomy and nomenclature

Potato spindle tuber viroid (PSTVd) was the first species to be identified as a viroid, a new type of pathogen different from bacteria and viruses (Diener, 1971). Gross *et al*. (1978) was able to determine the nucleotide sequence and predicted its characteristic secondary structure, a covalently closed circular RNA molecule of 359 nucleotides forming a rod-like structure by internal base pairing. Analysis of the sequence showed that all open reading frames were too small to encode a protein. It was consequently understood that viroids occur as naked RNA molecules. PSTVd is the type species of both the genus Pospiviroid and family Pospiviroidae (Di Serio *et al.*, 2017; 2020).

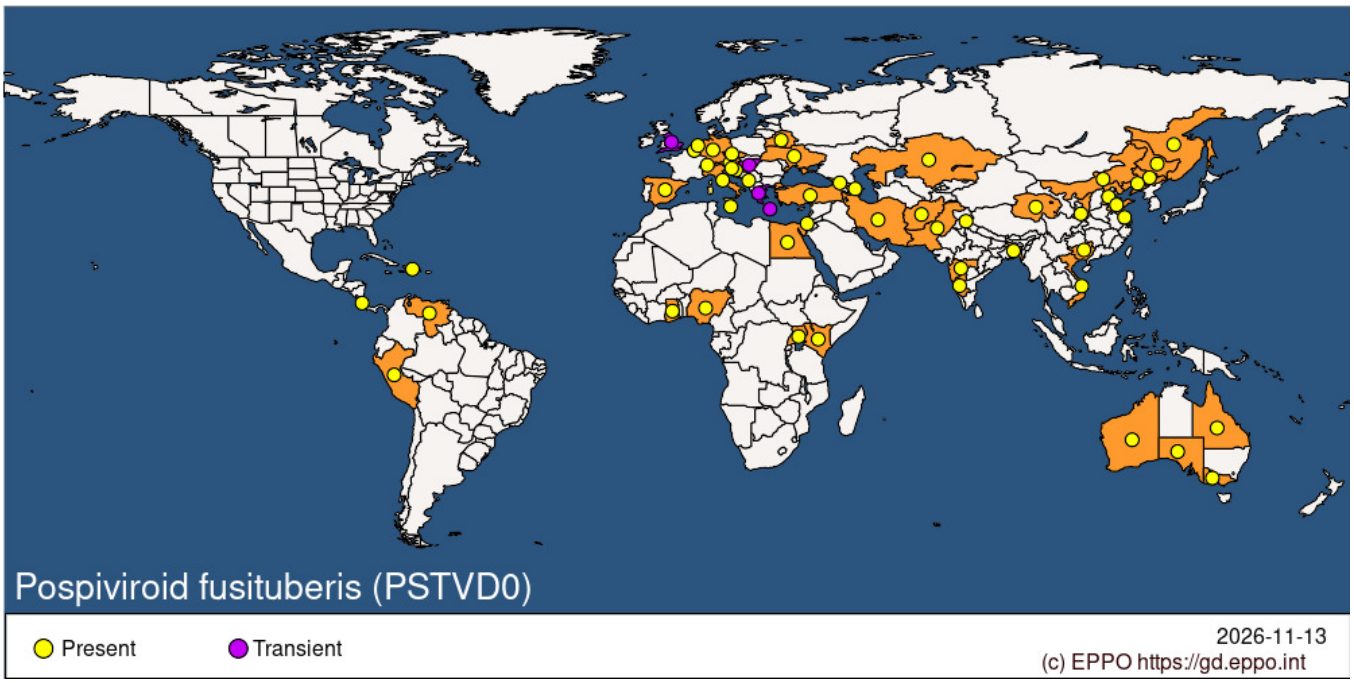
HOSTS

The main host is potatoes (*Solanum tuberosum* and other tuber-bearing *Solanum* spp.). Many other Solanaceous species have been reported as hosts, including important vegetable crops such as tomato (*Solanum lycopersicum*), pepper (*Capsicum* spp.), and a wide range of other fruit and ornamental crops and weeds (see Host list). Infections in ornamental crops and weeds often remain symptomless. A wide range of other members of the Solanaceae have been experimentally infected, as well as a few species in other families (Singh, 1973). In the EPPO region, potatoes and tomatoes are the main hosts of concern.

Host list: *Atriplex semilunaris, Brugmansia hybrids, Brugmansia sanguinea, Brugmansia suaveolens, Calibrachoa sp., Capsicum annuum, Cestrum aurantiacum, Cestrum elegans, Cestrum endlicheri, Cestrum nocturnum, Chenopodium eremaeum, Dahlia sp., Datura leichhardtii, Datura sp., Erigeron bonariensis, Hevea brasiliensis, Ipomoea batatas, Lycianthes rantonnetii, Lycium sp., Nicandra physalodes, Persea americana, Petunia sp., Physalis angulata, Physalis peruviana, Solanum anguivi, Solanum coagulans, Solanum dasyphyllum, Solanum laxum, Solanum lycopersicum, Solanum muricatum, Solanum nigrum, Solanum pseudocapsicum, Solanum sisymbriifolium, Solanum tuberosum, Streptoglossa sp., Streptosolen jamesonii*

GEOGRAPHICAL DISTRIBUTION

Potato spindle tuber disease caused by PSTVd was first described in the United States of America (EFSA, 2011 citing Martin, 1922). Since then, the viroid has been reported from the Americas, Asia, Africa, Europe and Oceania. PSTVd has been reported to be successfully eradicated from various countries throughout the world (Singh & Crowley, 1985; Verhoeven *et al.*, 2018). However, since PSTVd infections may remain symptomless in many hosts, there is some uncertainty on the current distribution (EFSA, 2011).



EPPO Region: Austria, Azerbaijan, Belarus, Belgium, Croatia, Czechia, Georgia, Germany, Greece (mainland, Kriti), Hungary, Israel, Italy (mainland), Kazakhstan, Malta, Montenegro, Netherlands, Russian Federation (Far East), Slovenia, Spain (mainland), Switzerland, Türkiye, Ukraine, United Kingdom (England)
Africa: Egypt, Ghana, Kenya, Nigeria, Uganda
Asia: Afghanistan, Bangladesh, China (Guangxi, Hebei, Heilongjiang, Jiangsu, Jilin, Liaoning, Neimenggu, Qinghai, Shaanxi, Shandong), India (Himachal Pradesh, Karnataka, Maharashtra), Iran, Islamic Republic of, Israel, Kazakhstan, Pakistan, Vietnam
Central America and Caribbean: Costa Rica, Dominican Republic
South America: Peru, Venezuela
Oceania: Australia (Queensland, South Australia, Victoria, Western Australia)

BIOLOGY

In tomato plants, PSTVd has been shown to spread systematically via the phloem, from an inoculated leaf to actively growing tissues such as young leaves and fruits (Palukaitis, 1987). It is likely to be the same for other hosts as this is a common route used by plant viruses. In potato, the viroid could be detected throughout the whole plant, including tubers (Weideman, 1987; Roenhorst *et al*., 2005). In leaves from different parts of the plant, only marginal differences in concentrations were found. The same was found for different parts of tubers. For pepper no details are available, but PSTVd has been detected in both leaves and fruit (Verhoeven *et al.*, 2020). In early infections the highest concentrations are generally found in the top leaves. The incubation period is difficult to indicate, because it depends on the viroid strain, the amount of inoculum, host species and variety, as well as environmental conditions, in particular temperature.

PSTVd can be spread by vegetative propagation and transmission via contact, insects, pollen, and seeds (Owens & Verhoeven, 2009).

Vegetative propagation

The most efficient way of spreading of PSTVd is through vegetative propagation. Once an infection is established in ‘mother plants’, the viroid will be present in all progeny derived from these infected plants, including bulbs, tubers, cuttings, and microplants. Vegetative propagation has been the major cause of the spread of PSTVd in potatoes and vegetatively-propagated ornamentals (Singh *et al.*, 1993; Owens *et al.*, 2009; Verhoeven *et al.*, 2010a).

Contact (mechanical transmission)

PSTVd-infected plants (including dried sap) form a source of inoculum for local spread by contact between diseased and healthy plants during cultivation. Mechanical transmission may be enhanced by crop handling, contaminated tools and machinery. The efficiency of transmission was found to increase at temperatures above 20°C (Verhoeven *et al.*, 2010b). This might explain that PSTVd infections did not spread in potatoes under temperate maritime climates (Verhoeven *et al.*, 2018), whereas extensive outbreaks in potatoes have been reported under continental climates in Canada, China, Ukraine and the USA in the past (Singh *et al.*, 1993, 2014; Qiu *et al.*, 2016). In protected tomato crops, with relatively high temperatures, PSTVd infections have been found to spread rapidly within a row (Verhoeven *et al.*, 2004). The ease of mechanical transmission has been found to be related to the plant species and presence of leaf hairs (Navarro *et al.*, 2009; Verhoeven *et al.*, 2010b).

Pollen and seeds

Transmission of PSTVd via pollen and true seeds of potato has been demonstrated by Singh *et al.* (1992). In addition to potato, PSTVd has been reported to be seed borne in tomato and pepper (Singh, 1970; Matsushita & Tsuda, 2016). Seed-transmission rates have been reported from 0 to 100% for different plant species and circumstances (Hunter *et al.*, 1969; Faggioli *et al.*, 2015). Reports on high seed-transmission rates were mainly obtained under experimental conditions (Simmons *et al.*, 2015; Verhoeven *et al.*, 2021). However, given that PSTVd is highly contagious and easily transmitted via contact and aerosols, it cannot be excluded that some reports of seed transmission are based on false positive results due to cross contamination during growing of plants, sample collection, and/or testing. Therefore, the role of pollen and seed transmission in the epidemiology of PSTVd is still a matter of debate (see Pathways for movement).

Insects

The role of insects in spreading of PSTVd is not clear (EFSA, 2011). In potato, transmission by aphids has been reported in the past (De Bokx & Piron, 1981), but was not confirmed in other studies (Schumann *et al.*, 1980; Van Bogaert *et al.*, 2016). However, aphid transmission of PSTVd in potato has been reported from plants co-infected by potato leaf roll virus (Querci *et al.*, 1997; Singh & Kurz, 1997). In tomato, aphid transmission of pospiviroids has been reported, but could not be confirmed for PSTVd (EFSA, 2011). Pollination by bumblebees has been associated with the spread of tomato apical stunt viroid in protected tomato crops (Antignus *et al.*, 2007; Matsuura *et al.*, 2010), but was not observed for PSTVd (Nielsen *et al.*, 2012). Given the non-specific interaction between the bumblebee and the viroid, transmission by pollinating insects cannot be excluded.

DETECTION AND IDENTIFICATION

Symptoms

The type and severity of symptoms of PSTVd depend on the viroid strain, host species and variety, as well as environmental conditions. PSTVd infections may be symptomless or produce symptoms ranging from mild to severe. PSTVd accumulates faster at higher temperatures, which might result in more severe symptoms (Harris & Browning, 1980). For the major crops, i.e. potato, tomato and pepper, symptoms have been described by Owens & Verhoeven (2009). A concise description of the most prominent symptoms in these crops is given below. Infections of PSTVd in other Solanaceous fruit crops, such as *Solanum melongena* (eggplant) and *Solanum muricatum* (pepino), and most ornamental species, generally remain symptomless.

On potatoes

Infected plants may be stunted, leaves may be smaller, more upright, darker green and slightly rugose in comparison to healthy plants. Infected tubers may be small, elongated (spindle-shaped), misshaped and cracked. Eyes may be more pronounced and knob-like protuberances may develop into small tubers.

On tomatoes

At an early stage of infection, infected plants show reduced growth and chlorosis in the top part of the plant. In time growth reduction may become more severe, and chlorotic leaves may turn red and/or purple and become brittle. At

this stage, flowering and fruit onset stops. Plants may eventually die or partially recover.

On peppers

Infected plants generally show either no symptoms or mild symptoms, i.e. distortion of the margins of apical leaves (Lebas *et al.,* 2005; Verhoeven *et al.,* 2016). Under experimental conditions, flower onset was delayed and fruits irregularly coloured and malformed (Verhoeven *et al.,* 2020).

Morphology

PSTVd consists of a covalently closed circular, single-stranded RNA molecule of approximately 359 nucleotides. The naked RNA molecule forms a rod-like structure by internal base pairing (Gross *et al.,* 1978).

Detection and inspection methods

PSTVd infections may be detected by visual inspection in the field or greenhouse, dependent on the circumstances, i.e. viroid variant, host species and cultivar, and environmental conditions. Crop inspection procedures for potato as well as for tomato and pepper are available respectively in EPPO Standards PM 3/71 (EPPO, 2007) and PM 3/77 (EPPO, 2015). Spindle-shaped potato tubers might be recognised during transit inspections. Laboratory tests are essential for detection in symptomless hosts and for identification of the viroid, particularly since other pospiviroids might induce similar symptoms (Verhoeven *et al.,* 2004). The preferred testing methods for detection of PSTVd are molecular methods, i.e. RT-PCR and real-time RT-PCR. However, none of these tests is able to discriminate PSTVd from other pospiviroids, in particular tomato chlorotic dwarf viroid and some isolates of tomato planta macho viroid. For identification, amplicons obtained by RT-PCR, preferably comprising the whole genome, should be sequenced and analysed. Further details and other tests for detection and identification of PSTVd are described in the EPPO and IPPC diagnostic standards (IPPC, 2016; EPPO, 2021).

PATHWAYS FOR MOVEMENT

PSTVd can be spread by vegetative propagation and transmission via contact, insects, pollen, and seeds (Owens & Verhoeven, 2009). Infected propagation material, including bulbs, tubers, cuttings, microplants and seeds, may serve as vehicles for introduction of the viroid into newly established crops and regions worldwide. Transmission via contact (mechanical transmission), pollen and insects, can account for further spread within a crop and/or region.

Whereas movement of infected vegetatively-propagated plants and plant material is the most efficient way of spreading PSTVd over long distances, the role of seed transmission is less clear. For potato, most publications on seed transmission date back to 1980-1990 and were related to germplasm collections. No recent reports on seed transmission are available, despite the fact that trading true potato seed for production is increasing. More insight in the role of true potato seed transmission as a pathway for movement of PSTVd is desirable. For tomato and pepper, PSTVd has been reported in many seed lots (Constable *et al.,* 2019). Nevertheless, only limited numbers of outbreaks have been reported (Verhoeven *et al.,* 2021). Grow-outs of commercially produced seed lots of tomato and pepper, testing positive for PSTVd or other pospiviroids, did not result in any infection in over 100 000 seedlings (Verhoeven *et al.,* 2021). For PSTVd, Van Brunschot *et al.* (2014) reported one out of 370 seedlings from an infested tomato seed lot to be infected. Similarly, for tomato chlorotic dwarf viroid, Candresse *et al.* (2010) reported 2-20 out of 2,500 tomato seedlings to be infected. The fact that in 2016 tomato apical stunt viroid was detected in a pepper seed lot produced in 1992, at least 10 years before the testing of pospiviroids became mandatory (Verhoeven *et al.,* 2017), indicates that pospiviroids were present in seed lots, whereas outbreaks were rarely recorded at that time. These results indicate that in practice seed transmission in tomato seems exceptional, whereas it has not been reported for pepper so far. The role of seed transmission in the spread of PSTVd, therefore, might have been overestimated in the past.

Regarding local spreading of PSTVd, mechanical transmission is the most important pathway, including human activities e.g. via equipment and machinery used during cultivation. For example, several outbreaks in tomato crops could be related to infections in vegetatively propagated ornamentals, based on a phylogenetic analysis of the viroid isolates in these crops (Navarro *et al.,* 2009; Verhoeven *et al.,* 2010a). Where nucleotide sequences of different PSTVd isolates from vegetatively propagated crops grouped per plant species, the isolates from tomato grouped with

isolates from potato, *Solanum laxum* (synonyme *Solanum jasminoides*) and *Physalis peruviana*. The absence of a specific group of tomato isolates indicates that the infections in tomato are more likely to originate in these ornamentals than in tomato (seeds). In addition, an outbreak of PSTVd in vegetatively propagated peppers was thought to probably originate from infected plants of *S. laxum* that had been handled in the same facilities (Verhoeven *et al.*, 2016). Pollen and insects may also contribute to local spread, but this pathway can be considered of minor importance.

PEST SIGNIFICANCE

Economic impact

The economic impact of PSTVd consists of direct and indirect impacts. Direct impacts include yield and quality losses, whereas indirect impacts are related to changes in prices and effects on international trade. Soliman *et al.* (2012) made an assessment of the total economic impact based on modelling, taking into account prevalence, yield losses, climatic data (temperature), as well as price and changes in trade. This impact assessment showed large differences dependent on the estimated incidence of the viroid, and were difficult to quantify, in particular due to uncertainties about the possibility of introduction and further spread in a crop and region. Nevertheless, it was concluded that direct impacts combined with export losses for potatoes and tomatoes, justify the costs of current phytosanitary measures in the European Union.

Over the last century, significant yield losses due to PSTVd infections in potatoes have been reported (EFSA, 2011). It should be noted, however, that reported losses were recorded under different conditions than the current agricultural practices that include extensive phytosanitary measures. Similarly, PSTVd may occasionally cause serious infections in tomatoes (Verhoeven *et al.*, 2004), but the overall significance in this crop seems limited seeing the low frequency and restricted extent of the outbreaks reported. For pepper crops no symptoms or only mild symptoms have been reported (Lebas *et al.*, 2005; Verhoeven *et al.*, 2016). Under experimental conditions fruit size was reduced (Verhoeven *et al.*, 2020). Further data on outbreaks and impact in pepper are not available. For other crops that do not show symptoms, no direct impact is expected but export of these and other crops might be affected.

Control

There are no chemical or biological methods available to control PSTVd within infected plants. Therefore, prevention is necessary to avoid infection with PSTVd. Eradication measures are necessary in the case of outbreaks (Owens & Verhoeven, 2009).

To prevent infection, it is essential to produce healthy planting material (Morris & Smith, 1977). This implies that the absence of the viroid in mother plants or nuclear stock has to be confirmed by testing, as recommended in the certification scheme for seed potatoes (EPPO, 1999), Petunia (EPPO, 2002), and schemes for production of herbaceous ornamentals (EPPO, 2008a). During the whole production process hygiene procedures should be in place to prevent infection by cross contamination. Measures may concern spatial separation of (host) plants as well as the use of clean (disinfected) tools and protective clothing by staff during crop handling. Post-entry quarantine programs as implemented for potato (EPPO, 2019b), have been shown to be effective in preventing the introduction of PSTVd in potato breeding and subsequent production systems (Verhoeven *et al.*, 2018). In addition, cold treatment can be used to eliminate PSTVd from infected germplasm or potato clones, before release from quarantine (Paduch-Cichal & Kryczynski, 1987).

For eradication in the case of outbreaks, all PSTVd-infected plants should be traced and destroyed. A regulated area should be demarcated, composed of places of production designated as ‘infested’ and places of production designated as ‘probably infested’ (EPPO, 2011). To prevent re-infection, strict hygiene procedures should apply and equipment and machinery, if applicable, should be cleaned thoroughly. Plants to be destroyed as well as disposables should be e.g. discarded in closed containers to an incinerator, or plants should be industrially processed, fermented and composted (EPPO, 2008b), steamed and fed to animals, or deposited at a waste disposal site and covered with soil. After completion of protected cultivations, all surfaces, substrates and equipment should be cleaned or destroyed. In the case of field-grown potatoes, crop rotation with non-PSTVd hosts is recommended, combined with elimination of volunteer plants (EPPO, 2020). Upon resuming the cultivation of PSTVd susceptible crops,

monitoring for PSTVd symptoms and/or testing is advised. More detailed measures are recommended in Standard PM 9/13 *National regulatory control system for PSTVd* (EPPO, 2011).

Phytosanitary risk

The viroid is regulated as a quarantine pest or regulated non-quarantine pest (RNQP) by many countries worldwide. Given that PSTVd can be present in asymptomatic hosts, the current geographical distribution is unclear. This implies that PSTVd can be introduced without being noticed by plants not known to be infected and from countries where the viroid has not been reported. Once established PSTVd is expected to cause most direct damage under warm and dry climatic conditions.

PHYTOSANITARY MEASURES

To prevent the introduction and spread of PSTVd, import requirements for different host species apply worldwide. These requirements can vary with regards to crop, type of consignment, and prevalence at the place of origin. When deregulated as a quarantine pest, PSTVd was recommended for regulation as a RNQP for seed potatoes and propagation material (including seeds) of pepper and tomato during the EU Quality pest project (Picard *et al*., 2018).

For potato, EPPO recommends that countries where PSTVd is not known to occur, or which have implemented eradication measures, should require measures for import of seed potatoes (except microplants and minitubers) and ware potatoes. According to EPPO Standard PM 8/1 (EPPO, 2017) seed and ware potatoes imported from a country where the pest occurs should be subject to transitional arrangements. Imported potatoes should come from a pest-free area and originate from a pest-free potato production and distribution system, according to EPPO Standard PM 3/61 (EPPO, 2019a), or the exporting country should have implemented an official regulatory control system according to EPPO Standard PM 9/13 (EPPO, 2011). If potatoes are imported from a country where PSTVd is not known to occur, the absence should be confirmed by survey following ISPM 6 *Surveillance* (IPPC, 2019). In addition, post-entry quarantine programs are established to allow safe movement of potato germplasm for research and breeding purposes (EPPO, 2019b).

For tomato and pepper seeds, quarantine testing requirements may apply to allow seed lots to be traded and/or imported (EPPO, 2016).

As well as preventing introduction, it is essential to start cultivation with non-infected plants or seeds. Therefore, the absence of the viroid in germplasm, mother plants and nuclear stock should be assured before the start of breeding, propagation and/or production of plants or seeds. During the EU Quality pest project, recommended measures included a zero tolerance for all categories of tomato and pepper plants, based on testing and absence of symptoms.

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