

Summary of the findings of an EFSA (European Food Safety Authority) Scientific Opinion on solanaceous pospiviroids, and proposal for a future policy approach for *Citrus exocortis viroid*, *Columnea latent viroid*, *Pepper chat fruit viroid* and *Tomato planta macho viroid*.



Leaf symptoms on tomato of *Columnea latent viroid*. Credit: Defra, Crown Copyright. Source: https://gd.eppo.int/



Left: a healthy fruit of sweet pepper cv. Lamborgini. Middle & right: small fruits naturally infected by *Pepper chat fruit viroid*.

Credit: National Plant Protection Organisation of the Netherlands. Source: https://gd.eppo.int/

Background

Viroids are the smallest known pathogen of plants, consisting of a single-strand RNA molecule. Viroids differ from viruses in that they lack a protein shell. *Citrus exocortis viroid* (CEVd), *Columnea latent viroid* (CLVd), *Pepper chat fruit viroid* (PCFVd) and *Tomato planta macho viroid* (TPMVd) are viroids belonging to the genus Pospiviroid. The direct effects of these pospiviroids mainly concern tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) as they cause yield and quality losses. These viroids are not known to occur in Great Britain (all outbreaks have been eradicated) and are currently listed in the legislation as provisional GB quarantine pests (the retained Implementing Regulation 2019/2072, Annex 2A).

A summary of the main findings of an EFSA assessment on the risk posed by solanaceous pospiviroids to the EU is given below, in addition to some comments on the significance of the conclusions to GB. It is proposed that the aforementioned viroids are listed as GB

quarantine pests. Measures for tomato and pepper seeds, and plants intended for planting are also proposed.

The viroids under consideration here will henceforth be referred to by their initialism, whereas those not being considered will be referred to by their species name.

In the EFSA Scientific Opinion on solanaceous pospiviroids (as a whole), the three main pathways for introduction were considered to be: true (botanical) seeds of hosts, host plants for planting, and seed potato (*Solanum tuberosum*) tubers (potato is not a known natural host of the four viroids being considered here; this was considered a main pathway for *Potato spindle tuber viroid*).

N.B. Tomato apical stunt viroid, Tomato chlorotic dwarf viroid, Chrysanthemum stunt viroid and Potato spindle tuber viroid which are also assessed in the EFSA Opinion are already regulated as RNQPs in GB (regulated non-quarantine pests that have a limited presence). The EFSA Opinion also refers to *Mexican papita viroid* which is now thought to be the same species as TPMVd.

The EFSA Scientific Opinion can be found on the following webpage:

https://www.efsa.europa.eu/en/efsajournal/pub/2330

Crop/sector considered most at risk in the UK

All the viroids of concern infect and cause symptoms in tomato. PCFVd infects pepper and is symptomatic in this host. Aubergine (*Solanum melongena*) can become infected with CEVd but without symptoms being expressed. CEVd and CLVd will infect other, mainly ornamental, hosts but these will generally not exhibit any symptoms (Table 1).

Summary and conclusions of the EFSA Scientific Opinion

Risk of entry

True (botanical) seeds of susceptible species: Seed transmission has been shown for several solanaceous pospiviroids in tomato [and pepper]. In addition, a few outbreaks of pospiviroids in tomato crops have been linked to infection transmitted through botanical seeds, where simultaneous outbreaks at unrelated sites were traced to seeds from a common seed lot. The probability of pospiviroids being associated with this pathway was assessed as low, with a high degree of uncertainty (section 3.2.2 in the EFSA Scientific Opinion).

Seed (potato) tubers: not applicable for this proposal as the four viroids under consideration are not known to naturally infect potato.

Plants for planting of susceptible species including cuttings and rooted ornamental plants: pospiviroids can be present in essentially all tissues of their hosts. Therefore, the import of infected plants for planting, including cuttings and rooted plants of both solanaceous and non-solanaceous ornamental species, represents a distinct pathway for entry. Overall, the interception data indicates that the probability of the pospiviroids entering the PRA area via this pathway is moderately likely to likely with a medium uncertainty (section 3.2.4).

Plant products not intended for planting (food consumption, cut flowers, pollen, animal feed): As produce is intended for human or animal use, generally there will be destruction of the material. However, there are three potential routes by which pospiviroid inocula could be transferred to suitable hosts:

- unintended use of foodstuffs for propagation likelihood was assessed as being low with a high degree of uncertainty;
- mechanical-assisted transfer to susceptible plants:
 - packing houses at production sites, where fruit for marketing is brought on site and packed during the growing season to enable year-round working of the packhouse;
 - contamination from pospiviroids picked up on workers' hands from consuming contaminated foodstuffs.
 - likelihood was assessed as being very unlikely with a high degree of uncertainty;
- or vector-assisted transfer to susceptible plants likelihood was assessed as being very unlikely with a high degree of uncertainty

Overall, this pathway was considered of minor significance due to the low probability of transfer to a suitable host being the limiting factor (section 3.2.5 & 3.2.6).

Significance to GB: True (botanical) seeds of susceptible species: The EFSA opinion assessed that the possibility of pospiviroids being associated with true seed is low. However, transmission rates can be high; experimentally seed transmission rates of 80% have been demonstrated for *Tomato apical stunt viroid* in tomatoes and transmission rates of 19% for PCFVd in peppers. Additionally, very low levels of infection associated with seed could cause a significant impact on the crop due to the potential for mechanical spread of viroids resulting from working on the crop. This is particularly true for tomatoes.

Plants for planting of susceptible species including cuttings and rooted ornamental plants: The survey which was carried out under the EU emergency measures for *Potato spindle tuber viroid* has shown that it is present in ornamentals within GB. There is evidence that *Potato spindle tuber viroid* has been transferred from infected ornamentals to tomatoes and the possibility that other viroids have been transferred from ornamentals to tomatoes cannot be ruled out. However, there is no evidence to suggest this has occurred in GB. Additionally, plants for planting of tomato, pepper and aubergine can be a pathway for the introduction of pospiviroids.

Plant products not intended for planting (food consumption, cut flowers, pollen, animal feed): A significant volume of ware potatoes, tomatoes and other produce is imported into GB which has the potential to carry pospiviroids. The probability of pospiviroids being associated with this pathway was estimated by EFSA as being unlikely to moderately likely (section 3.2.5.1). However, a particular scenario which may require special consideration is the import of infected produce (in particular tomatoes) for packing on a production site. There is some evidence to suggest that viroids can be spread within a crop via pollen. However, the risk associated with pollen as a commodity was not addressed within the EFSA Opinion.

Risk of establishment and spread

Establishment: With the exception of TPMVd, all other solanaceous pospiviroids have been reported from one or more EU countries. Overall, host plants suitable for solanaceous pospiviroids are widely present in GB, although mostly under protected cultivation. Given that many host plants of solanaceous pospiviroids can be grown in GB, the environment is considered suitable for these pathogens. Overall, the probability of establishment of solanaceous pospiviroids upon entry to EU Member States (MS) (GB was an EU MS at the time of publication) was considered to be very high. This evaluation was not associated with any significant level of uncertainty (sections 3.3 & 3.6.2).

Spread: Transmission of pospiviroids by aphids or bumblebees, within and between crops, has an unlikely to moderately likely probability rating. The presence of other viruses in a plant can facilitate aphid transmission of viroids. High uncertainties on the assessment derive from the limited number of virus-viroid-host-vector combinations for which experimental data are available. The potential for pospiviroids to spread through contaminated seeds and pollen of their hosts was considered very high for those hostviroid combinations for which experimental evidence is available. For those combinations for which experimental evidence is lacking, a high probability rating was suggested by analogy, but this rating was associated with high uncertainty. Viroid infection in mother plants results in a high rate of infection in their vegetatively propagated progeny plants. Consequently, vegetatively propagated plants are permanent sources of infections for other lots and crops. Uncertainties regarding spread through vegetative propagation are negligible, while those related to mechanical transmission were higher due to variations in data. The potential for mechanical transmission of *Potato spindle tuber viroid* from ornamentals to tomato and potato has been experimentally demonstrated, although with a much higher efficiency in tomato. By analogy, similar conclusions were extended to the other pospiviroids but with medium uncertainty in the absence of specific studies.

Within a crop species, all four mechanisms (seed and pollen transmission, insect transmission, vegetative propagation and mechanical transmission) contribute to short-distance pospiviroid spread, and overall the probability of spread was evaluated as likely to very likely, with low uncertainty. The probability of spread from symptomless ornamentals to tomato was rated moderately likely, with high uncertainty; as was spread from ornamentals to 'other vegetable' crops. These ratings were based on evidence of *Potato*

spindle tuber viroid transmission. The probability of long-distance spread, to give widespread epidemics (as opposed to localized outbreaks) is evaluated as likely to very likely for vegetatively propagated species and as moderately likely for non-vegetatively propagated ones, with overall medium uncertainty (sections 3.4 & 3.6.3).

Significance to GB: Tomato apical stunt viroid, Tomato chlorotic dwarf viroid, Chrysanthemum stunt viroid and Potato spindle tuber viroid (not under consideration here) are viroids that have established in GB albeit with a limited presence.

Table 1: Natural hosts of pospiviroids							
Viroid	Potato	Tomato	Pepper	Aubergine	Ornamentals		
CEVd		~		~	~		
CLVd		~			~		
PCFVd		~	~				
TPMVd		~					

Tomatoes are grown throughout GB mostly under protection; as are peppers, though commercial growers are not as well distributed. Tomato is host to the four pospiviroids considered here (Table 1). EFSA considered that the probability of these viroids establishing was very high. There are no reasons to contradict this rating nor the ratings for spread.

Impact

Overall, there was ample evidence that significant yield losses may result from pospiviroid infections in tomato and the impact on the EU was therefore expected to be major, with low uncertainty. The potential impact of PCFVd (and *Potato spindle tuber viroid*) on the pepper industry was rated as moderate, with medium uncertainty. The impact on 'other vegetables' was expected to be minimal to minor and that on ornamental species to be minimal. The associated uncertainties were medium and low, respectively (sections 3.5 & 3.6.4).

Significance to GB: British growers supply approximately 20% of the UK's tomato market (~100,000 tonnes). During the summer, this goes up to around 50%. In total, approximately £190 M is spent on British-grown tomatoes (British Tomato Growers' Association 2021). The potential impact of these viroids could therefore be significant.

Identification of management options

The EFSA Opinion assesses many management options in sections 4 and 5. The effectiveness of these are summarised in tables 11 to 15. Given the very similar biology of the pospiviroids, all analysed management options were expected to be as effective on each viroid. Key conclusions are given in section 5.4.1.

Proposed measures for GB and justifications:

There are no chemical or biological controls available for viroids. Therefore, the exclusion of infected seed, planting material and destruction of infected plants coupled with hygiene measures to prevent infection of subsequent crops are the most effective means of control.

SCHEDULE 1 New Annex 2 to the Phytosanitary Conditions Regulation

"ANNEX 2
List of GB quarantine pests
Plant pests not known to occur in any part of Great Britain

Add:

F). Viruses, viroids and phytoplasmas

Citrus exocortis viroid (CEVD00) Columnea latent viroid (CLVD00) Pepper chat fruit viroid (PCFVD0) Tomato planta macho viroid (TPMVD0)

Justification: Citrus exocortis viroid, Columnea latent viroid, Pepper chat fruit viroid and Tomato planta macho viroid are not known to occur in the UK. They will be regulated on all hosts and pathways.

Schedule 7, Part A

Plants, plant products and other objects originating in third countries which may only be introduced into Great Britain if special requirements are met

(1)	(2)	(3)
Description of plants, plant products or other objects	Origin	Requirements
Seeds of Solanum lycopersicum L. and its	Any third country	The seeds must be accompanied by an official statement that they:
hybrids		(a) originate in an area* established by the national plant protection organisation in accordance with ISPM4 as an area that is free from Citrus exocortis viroid, Columnea latent viroid, Pepper chat fruit viroid and Tomato planta macho viroid
		(b) are derived from plants grown throughout their life in a place of production** established by the national plant protection organisation in accordance with ISPM10 as a place of production that is free from Citrus exocortis viroid, Columnea latent viroid, Pepper chat fruit viroid and Tomato planta macho viroid and verified through official inspections and, where appropriate, testing, or
		(c) have been subjected to official testing for Citrus exocortis viroid, Columnea latent viroid, Pepper chat fruit viroid and Tomato planta macho viroid on a statistically based sample in accordance with ISPM 31 and using an appropriate method and have been found, in these tests, free from these harmful organisms

- * The name of the area(s) must be included in the phytosanitary certificate under the heading "Additional declaration
- ** The name of the place of production(s) must be included in the phytosanitary certificate under the heading "Additional declaration

Seeds of *Capsicum* spp.

Any third country

The seeds must be accompanied by an official statement that they:

- (a) originate in an area* established by the national plant protection organisation in accordance with ISPM4 as an area that is free from Pepper chat fruit viroid
- (b) are derived from plants grown throughout their life in a place of production** established by the national plant protection organisation in accordance with ISPM10 as a place of production that is free from Pepper chat fruit viroid and verified through official inspections and, where appropriate, testing, or
- (c) have been subjected to official testing for Pepper chat fruit viroid on a statistically based sample in accordance with ISPM 31 and using an appropriate method and have been found, in this test, free from this harmful organism.
- * The name of the area(s) must be included in the phytosanitary certificate under the heading "Additional declaration
- ** The name of the place of production(s) must be included in the phytosanitary certificate under the heading "Additional declaration

Justification: Seed transmission of *Potato spindle tuber viroid*, *Tomato apical stunt viroid* and *Tomato chlorotic dwarf viroid* in tomatoes has been demonstrated. There is good circumstantial evidence that CLVd can be associated with seed. This is based on the outbreaks in the UK in 2007, where the only link between the four outbreak sites was that the seed used was from the same lot. Seed transmission of CEVd and TPMVd has not

been demonstrated but the EFSA opinion does not rule out the possibility that these could be seed transmitted, stating that in tomatoes 'a similar behaviour is assumed with those viroids that are seed transmissible'. PCFVd has also been included here, the EFSA opinion does not include tomato as a host of PCFVd. However, there is a more recent New Disease Report from Thailand which reports an outbreak of PCFVd in tomatoes (Reanwarakorn *et al.* 2011: https://www.ndrs.org.uk/article.php?id=024006).

The EFSA opinion does not comment on the possibility of seed transmission in peppers. However, given the assumption for tomatoes that all pospiviroids will behave similarly, it would seem appropriate to include requirements for pepper seeds for PCFVd.

Measures being proposed here are not as stringent as for *Tomato brown rugose fruit virus* as there does not seem to be the same level of risk of these viroids being present in seed moving in trade.

(1)	(2)	(3)
Description of plants, plant products or other objects	Origin	Requirements
Plants intended for planting of <i>Solanum</i>	Any third country	The plants must be accompanied by an official statement that they:
lycopersicum L. and its hybrids		(a) have been derived from seed comply with the requirements set out in entry **,
		and
		(b)(i) originate in an area* established by national plant protection organisation in accordance with ISPM4 as an area free to Citrus exocortis viroid, Columnea latent viroid, Pepper chat fruit viroid and Tomat planta macho viroid
		Or
		(ii) have been produced in a site of production where, since the beginning of last cycle of vegetation, no symptoms of disease caused by Citrus exocortis viroic Columnea latent viroid, Pepper chat fruit viroid and Tomato planta macho viroid habeen observed and where the following actions have been taken:

(aa) staff and items, such as tools, machinery, vehicles, vessels and packaging material, from other sites producing solanaceous plants and other host plants of Citrus exocortis viroid, Columnea latent viroid, Pepper chat fruit viroid and Tomato planta macho viroid have been prevented from coming into contact with the site or other appropriate hygiene measures have been taken to prevent infection by staff working, or items used, at other sites producing solanaceous plants and other host plants of Citrus exocortis viroid, Columnea latent viroid, Pepper chat fruit viroid and Tomato planta macho viroid

Plants intended for planting of *Capsicum* spp.

Any third country

The plants must be accompanied by an official statement that they:

(a) have been derived from seed complying with the requirements set out in entry **,

and

(b) (i) originate in an area* established by the national plant protection organisation in accordance with ISPM4 as an area free from Pepper chat fruit viroid.

Or

- (ii) have been produced in a site of production where, since the beginning of the last cycle of vegetation, no symptoms of disease caused by Pepper chat fruit viroid have been observed and where the following actions have been taken:
- (aa) staff and items, such as tools, machinery, vehicles, vessels and packaging material, from other sites producing solanaceous plants and other host plants of Pepper chat fruit viroid have been prevented from coming into contact with the site or other appropriate hygiene measures have been taken to prevent infection by staff working, or items used, at other sites

producing solanaceous plants and other host plants of Pepper chat fruit viroid

Justification: These measures are needed to ensure that all plants for planting of tomato and pepper entering GB are free from the relevant viroids. It is proposed that all plants should be derived from seed which had been subject to the same requirements as seeds of tomato and pepper entering GB. Also, plants need to either be produced in a pest free area or have been produced on a site of production which implements appropriate hygiene measures to prevent infection.

The measures being proposed here are not as stringent as for *Tomato brown rugose fruit virus* as there does not seem to be the same level of risk of these viroids being present in seed moving in trade and therefore in young plants.