

# SCIENTIFIC OPINION

# Scientific Opinion on the assessment of the risk of solanaceous pospiviroids for the EU territory and the identification and evaluation of risk management options<sup>1</sup>

# **EFSA Panel on Plant Health (PLH)**<sup>2, 3</sup>

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#### ABSTRACT

Following a request from the EU Commission, the EFSA PLH Panel conducted a risk assessment for the EU territory of pospiviroids affecting solanaceous crops, identified and evaluated risk reduction options and evaluated the EU provisional emergency measures targeting Potato spindle tuber viroid (PSTVd). The risk assessment included PSTVd, Citrus exocortis viroid, Columnea latent viroid, Mexican papita viroid, Tomato apical stunt viroid, Tomato chlorotic dwarf viroid, Tomato planta macho viroid, Chrysanthemum stunt viroid and Pepper chat fruit viroid. Four entry pathways were identified, three involving plant propagation material, with moderate probability of entry, and one involving plant products for human consumption, with low probability of entry. The probability of establishment was considered very high. Spread was considered likely within a crop and moderately likely between crop species, with exception of spread to potato, rated as unlikely. The probability of long distance spread within vegetatively propagated crops was estimated as likely/very likely. The direct consequences were expected to be major in potato and tomato, moderate in pepper, minimal/minor in other vegetables and minimal in ornamentals. Main risk assessment uncertainties derive from limited knowledge on pospiviroids other than PSTVd, although all pospiviroids are expected to have similar biological properties. Management options to reduce risk of entry, spread and consequences were identified and evaluated. No management options can prevent establishment. Examples of successful PSTVd eradication are linked to timely and strict implementation of measures. Uncertainty exists on the effectiveness of risk reduction strategies targeting only one pathway. The EU provisional emergency measures appeared to have significantly reduced PSTVd incidence in Solanum jasminoides and Brugmansia sp., even though eradication from the EU is so far incomplete. The low PSTVd incidence in food crops did not permit to conclude whether the reduction in PSTVd prevalence in ornamentals led to a reduction in outbreaks in food crops.

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<sup>&</sup>lt;sup>4</sup> Editorial changes have been made on pages 20 and 21 (references corrected in table 5). These changes do not affect the overall conclusions of the opinion. To avoid confusion the original version has been removed from the website.



**KEY WORDS:** emergency measures, pepper, pospiviroids, potato, PSTVd, solanaceous ornamentals, tomato.



# SUMMARY

Following a request from the European Commission, the Panel on Plant Health was asked to deliver a scientific opinion on the risk of solanaceous pospiviroids for the EU territory. The Panel was requested to provide a pest risk assessment of the solanaceous pospiviroids, to identify risk management options and to evaluate their effectiveness in reducing the risk to plant health posed by this organism. It was also requested to provide an opinion on the effectiveness of the measures listed in Commission Decision  $2007/410/\text{EC}^5$  in reducing the risk to plant health posed by PSTVd.

The Panel conducted the risk assessment following the general principles of the "Guidance on a harmonised framework for pest risk assessment and the identification and evaluation of pest risk management options" (EFSA Panel on Plant Health (PLH), 2010). The risk assessment was conducted without considering the existing plant health legislation. The effectiveness of the current measures in place – specific or not to the pathogen – are evaluated under the Management Options sections.

This risk assessment covers the pospiviroids which are proven in field or in experimental conditions to affect plants of the family Solanaceae, cultivated for both food consumption (e.g. potato, tomato, pepper, aubergine, pepino etc.) and ornamental purpose. The pospiviroid species covered by this document are therefore *Potato spindle tuber viroid* (PSTVd), *Citrus exocortis viroid* (CEVd), *Columnea latent viroid* (CLVd), *Mexican papita viroid* (MPVd), *Tomato apical stunt viroid* (TASVd), *Tomato chlorotic dwarf viroid* (TCDVd), *Tomato planta macho viroid* (TPMVd) *Chrysanthemum stunt viroid* (CSVd) and *Pepper chat fruit viroid* (PCFVd) which are hereafter collectively refered to as "solanaceous pospiviroids". Having never been observed to infect solanaceous hosts, Iresine viroid *I* (IrVd-1) is excluded from the scope of the present risk assessment. Although a detailed assessment of the impacts of pospiviroids species on non-solanaceous hosts (e.g. the impact on flower crops of *Chrysanthemum stunt viroid* and the impact on citrus of the *Citrus exocortis viroid*) is not included in this document, non-solanaceous hosts are examined for their role in entry and spread pathways.

After consideration of the evidence, the Panel reached the following conclusions:

## With regard to the assessment of the risk of solanaceous pospiviroids for the EU territory:

- Four entry pathways have been identified, three of which implicate propagation material [True (botanical) seeds, Seed (potato) tubers and Plants for planting]. The fourth pathway involves plant materials not intended for planting and is considered of minor significance due to the perceived low probability of transfer to a suitable host. The uncertainties associated with this evaluation concern mostly the probability of association of the pathogens with the pathway at origin (due to the limited information available on geographical distribution and prevalence of the pospiviroids) and with the probability of transfer to a suitable host, due to the numerous parameters involved. For the three main pathways, probabilities of survival during transfer and storage, of survival through management procedures and of transfer to a suitable host are considered to be high or very high and there is little uncertainty associated with these ratings. The only limiting factor is the probability of association with the pathway at origin, which as for the pathway involving plant materials not intended for planting, carries a medium uncertainty level. Overall, the probability of entry of solanaceous pospiviroids in the EU territory through the effects of all identified pathways is considered as moderately likely.
- Given previous reports of pospiviroids in many EU Members States, the wide availability of suitable hosts, the suitability of the EU area for these agents and the inability of cultural practices and control measures to decrease the chance of establishment, the probability of establishment of solanaceous pospiviroids upon entry in the 27 EU Member States is considered to be very high

<sup>&</sup>lt;sup>5</sup> Commission Decision 2007/410/EC of 12 June 2007 on measures to prevent the introduction into and the spread within the Community of Potato spindle tuber viroid. OJ L 155, 15.6.2007, p. 71-73.



(certain or close to certain). This evaluation is not associated with any significant level of uncertainty.

- Within a crop species on a short distance, the probability of spread is overall evaluated as likely to very likely, with low uncertainty. The probability of transfer between crop species on a short distance is generally evaluated as being moderately likely, with high uncertainty. However, due to the lower receptivity of the potato crop and to agricultural practices that limits potato crops contacts with other susceptible crops, the probability of spread to potato is rated as very unlikely to unlikely, but with an associated high uncertainty. 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.
- Direct pest effects are expected to be markedly different for the various host plant species. The impact of solanaceous pospiviroids is expected to be major on potato and tomato and moderate on pepper. The uncertainty associated with these evaluations is low in the case of potato and tomato but medium (PSTVd and PCFVd) to high (other pospiviroids) in the case of pepper. The impact on other vegetables is expected to be minimal to minor and that on ornamental species to be minimal. The associated uncertainties are medium and low, respectively. Indirect pest effects are expected to be minimal with low uncertainties, with the exception of the impact on industries producing and commercializing plant propagation materials (seed potato tubers, true botanical seeds, plants for planting) with medium uncertainties.

With regard to the identification and evaluation of management options, these aim at reducing the risk of entry and spread of pospiviroids within the EU and to limit their impact. A unique feature of pospiviroid infections in solanaceous ornamentals is the lack of visible symptoms hence complicating surveillance and phytosanitary control of production processes and consignments, whereas in potato and tomato pospiviroid infections result in variable symptoms. Natural infections of potato were only reported for PSTVd, however all pospiviroids are infectious to potato and tomato hence present a threat similar to PSTVd for both crops. In general given the very similar biology of pospiviroids, all analyzed management options are expected to have a very similar effectiveness against all solanaceous pospiviroids. In addition, if applied against a particular pospiviroid, most management options are established with the cultivation of an infected plant hence there is no management option that can prevent establishment other than exclusion, avoidance and destruction of the infected plants. Efficient implementation of these management options requires to take into account the difficulties presented by these pathogens and their host plants or products thereof (seeds) for inspection and testing. The following key conclusions were reached:

- Pre-entry measures include import requirements comprising plant materials originating from pest free areas of production and certified free of PSTVd and other solanaceous pospiviroids.
- Implementation of import requirements has a high effectiveness and feasibility only when inspections and viroid tests are conducted with prudence following standardized procedures.
- Testing for pospiviroids at points of entry is highly effective with the possible exception of seed testing due to the potentially low numbers of seeds infected and to the lack of standardized seed testing methods.
- Post-entry quarantine would also be highly effective for vegetative propagation material but only when all plants are tested, such as for nuclear propagation stocks.
- Subsequent surveillance and targeted inspection for pospiviroids would assure efficiency of the measure and freedom from pospiviroids.



- No management options were identified to reduce the likelihood of establishment.
- Management options to reduce probability of spread before cultivation starts and during cultivation most significantly would prescribe the use of planting material (certified) free from pospiviroids.
- Integrated crop management including disease/ pest/ vector control provides a set of measures to reduce disease spreading significantly.
- Official surveillance in nurseries accompanied by targeted testing for pospiviroids assures freedom of these pathogens in plant stocks.
- Hygiene best practice as a suite of prophylactic measures and best practices in crop management, including the use of healthy planting material certified for viroid freedom and best hygiene practices and sanitation before, during and after cultivation to reduce occurrence and spread can provide an effective control of pospiviroids. Although some individual measures can be very effective (e.g. use of certified planting material) most other measures would individually have only a partial effectiveness but their concurrent implementation will reduce the risk of pospiviroid infections to a manageable level and reduce the impact of the disease.
- Options to reduce the probability of spread and to reduce the impact after an outbreak comprise destruction of infected plant materials, sanitation, cleaning, disinfection which when applied as a routine and with prudence effectively eliminate pospiviroids.
- Banning of continuous cropping and intercropping, weed and volunteer control and temporary ban of host plants are rigorous measures to prevent reinfection from alternative hosts and to keep crops free from pospiviroid infections.

As illustrated by examples from Canada to manage outbreaks of PSTVd in potato (Singh and Crowley, 1985), the rigiditiy with which specific measures are applied is crucial for successful risk management in potato. Successful eradication of PSTVd in *Solanum jasminoides* show similar pattern (De Hoop et al., 2008). Following outbreaks, the strict adherence to prescribed measures (destruction of infected source plants, including materials for distribution, sanitation, temporary ban of host plants etc.) and their timely implementation is most decisive for successful outbreak management.

Uncertainties exist over the effectiveness of inspection, sampling and testing measures at points of entry since pathogens in low concentrations in seeds and symptomless crops can escape detection. Since the relative significance of the two main pathways for pospiviroids infection of tomato and potato crops (seed borne infection and transmission from symptomlessly infected ornamentals) is not known, uncertainty exists on the ultimate effectiveness of a strategy targeting only one of these two pathways. In particular uncertainties exist over the effect of disease management in ornamentals to prevent occurrence of PSTVd in tomato and potato. There is low uncertainty on the overall effectiveness of each individual hygiene best practice measure is likely to be low, if applied as a suite of measures, they are considered to be effective, with only low uncertainty.

With regard to the evaluation of the effectiveness of the provisional emergency measures, these were concerned with findings of PSTVd in the ornamental species *S. jasminoides* and *Brugmansia* spp. The available data indicate that although surveys of different intensities have been performed by EU Member States, the emergency measures have resulted in significant increases in inspection activities focused at the reduction of this pathogen in the targeted species. There does not appear, however, to have been a corresponding increase in the numbers of samples tested. Although these activities appear to have significantly reduced the levels of PSTVd inoculum in these species, this reduction is mostly represented by a reduction in findings of infected *S. jasminoides* plants and not necessarily by a similar level of reduction for plants of *Brugmansia* spp. In terms of the overall level



of PSTVd circulating within the EU territory the measures significantly reduced the incidence of this pathogen, eventhough this effect has so far not been complete.

Due to the extremely low incidence of PSTVd in food crops of potato, tomato and pepper in the EU Member States, it is not possible to conclude whether the reduction in prevalence in ornamentals as a consequence of the emergency measures has led to a reduction in outbreaks in these species.

A side effect of the emergency measures was to raise the general level of awareness about other pospiviroids infecting ornamentals. Reported findings of viroids such as TCDVd, TASVd, CSVd and CEVd in ornamental species now far exceed the numbers of PSTVd records. This is probably a result of the increased vigilance combined with the wide use of generic detection methods with broad specificity towards a range of pospiviroids. It would appear that these viroids are now increasing in their prevalence and importance within the EU, eventhough all findings may not be reported as a consequence of the non-quarantine status of non-PSTVd pospiviroids. On the other hand, this increase in reporting may also result from an increased interest on pospiviroids research during the past few years.

The major area of uncertainty regarding the EU emergency measures is the interpretation and application of these measures within individual EU Member States, in particular concerning the intensity of efforts aimed at eradication following detection of PSTVd in ornamentals. It also concerns the voluntary extension by EU Member States of the emergency measures to other host plants or to other viroids. National surveys data compiled by FVO give the number of inspections conducted and number of samples tested but this does not always directly relate to crops or lots being inspected. An area of uncertainty arising from this is that although the number of inspections carried out increased three-fold, this measure alone would not have been adequate to ensure freedom from PSTVd due to the asymptomatic nature of infection in the species targeted by the measures. The number of tests carried out increased slightly, but not proportionally to the increase in number of inspections. Without knowing the number and type of samples taken and how this relates to inspections the effectiveness of surveillance measures cannot be properly evaluated. There are also uncertainties regarding missing data in the FVO reports and missing replies to the EFSA questionnaire for some Countries. Additional uncertainties exist on the side effect of emergency measures on other solanaceous pospiviroids because not all EU Member States report to FVO or Europhyt findings of pospiviroids other than PSTVd. High uncertainty also concerns the protection afforded by the emergency measures to the EU tomato and potato crops. This is because of the very limited number of PSTVd outbreaks in these crops (none in potato), which does not allow to draw meaningful comparisons between pre and postemergency measures periods.



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# BACKGROUND AS PROVIDED BY EUROPEAN COMMISSION

The current common plant health regime is established by Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community (OJ L 169, 10.7.2000, p.1).

The Directive lays down, amongst others, the technical phytosanitary provisions to be met by plants and plant products and the control checks to be carried out at the place of origin on plants and plant products destined for the EU or to be moved within the EU, the list of harmful organisms whose introduction into or spread within the EU is prohibited and the control measures to be carried out at the outer border of the EU on arrival of plants and plant products.

Viroids belonging to the genus Pospiviroid may cause serious damage to solanaceous crops such as tomato, potato and pepper. Presently eight pospiviroids causing damage to solanaceous crops have been presently identified: Citrus exocortis viroid (CEVd), Columnea latent viroid CLVd), Mexican papita viroid (MPVd), Pepper chat fruit viroid (PCFVd), Potato spindle tuber viroid (PSTVd), Tomato apical stunt viroid (TASVd), Tomato chlorotic dwarf viroid (TCDVd) and Tomato planta macho viroid (TPMVd).

Currently potato spindle tuber viroid (PSTVd) is the only solanaceus pospiviroid with an explicit regulated status (listed in Annex I part A section I of Council Directive 2000/29/EC). As a consequence of findings of PSTVd in ornamental plants of *Solanum jasminoides* and *Brugmansia* spp. in some Member States in 2006 the Commission adopted on 12 June 2007 provisional emergency measures to prevent the introduction into and the spread within the EU of PSTVd (Commission Decision 2007/410/EC). These measures target these ornamental plants which were shown to be symptomless hosts of PSTVd and which could therefore act as a source of PSTVd inoculum for infections in solanaceus crop plants. The results of these measures have been assessed yearly, based on the surveys carried out by Member States and their notifications of the suspected occurrence or confirmed presence of PSTVd in their territory.

Provisional emergency measures against a plant harmful organism adopted by the Commission are meant to be, as indicated by their name, temporary measures put in place against an imminent danger of introduction into or spread within the EU of that harmful organism. Based on the experience gained from the application of these measures over a period of time a decision will be taken whether permanent measures are needed (and what type of measures). This decision needs to be based on a recent Pest Risk Analysis covering the whole territory of the EU, which takes into account the latest scientific and technical knowledge for this organism and the experience gained from the implementation of the provisional emergency measures

The finding of other pospiviroids in tomato and pepper, as well as their capacity to infest symptomlessly ornamental plants, warrants for an evaluation of the regulatory status of not only PSTVd but also of the other solanaceous pospiviroids. A simultaneous assessment of the risk posed to plant health by solanaceous pospiviroids, and the identification and evaluation of management measures, seems logical since they cause similar symptoms in tomato, they have similar ways of spread and they have in part common host plants, including symptomless ornamentals. This risk assessment should take into account the latest scientific and technical knowledge about these organisms (such as the results from the EUPRESCO-DET project) as well as their present distribution or absence in the EU. The experience gained from the implementation of the provisional emergency measures against PSTVd should also be taken into account.

## TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

EFSA is requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002, to provide a pest risk assessment of the solanaceous pospiviroids, to identify risk management options and to evaluate their effectiveness in reducing the risk to plant health posed by this organism. The area to be covered by the requested pest risk assessment is the EU territory. EFSA is also requested to provide an opinion on the effectiveness of the measures listed in Commission Decision 2007/410/EC in reducing the risk to plant health posed by PSTVd.



# ASSESSMENT

# 1. Introduction

# 1.1. Purpose

This document presents a pest risk assessment prepared by the Panel on Plant Health for solanaceous pospiviroids, in response to a request from the European Commission. The risk assessment area is the territory of the European Union (hereinafter referred as EU), and the opinion includes identification and evaluation of risk management options in terms of their effectiveness in reducing the risk posed by the organism.

# 1.2. Scope

This risk assessment covers the pospiviroids which are proven in field or in experimental conditions to affect plants of the family Solanaceae, cultivated for both food consumption (e.g. potato, tomato, pepper, aubergine, pepino etc.) and ornamental purpose. Although a detailed assessment of the impacts of pospiviroids species on non-solanaceous hosts (e.g. the impact on flower crops of *Chrysanthemum stunt viroid* and the impact on citrus of the *Citrus exocortis viroid*) is not included in this document, non-solanaceous hosts are examined for their role in entry and spread pathways.

# 2. Data and methodology

# 2.1. Data used in the risk assessment

Literature searches were performed consulting the ISI web of Knowledge database including CABI abstracts 1911-2011. The key word used in the search was: viroid\*. The abstracts retrieved were then screened and considered when one or combination of the following topics was treated: pospiviroid, *Potato spindle tuber viroid* (PSTVd), *Citrus exocortis viroid* (CEVd), *Columnea latent viroid* (CLVd), Iresine viroid 1 (IrVd-1), Mexican papita viroid (MPVd), Tomato apical stunt viroid (TASVd), *Tomato chlorotic dwarf viroid* (TCDVd), *Tomato planta macho viroid* (TPMVd), *Chrysanthemum stunt viroid* (CSVd) and *Pepper chat fruit viroid* (PCFVd). Further references and information were obtained from experts, and from citations within references found.

Personal communications have only been considered when in written form and supported by evidence, and when other sources of information were not publicly available.

In order to collect data and information on the status of pospiviroids and the phytosanitary measures applied in the 27 EU Member States (MS), a questionnaire in Excel format was sent to the National Plant Protection Organisations (NPPO). The questionnaire structure and the summary of the NPPOs replies are presented in Appendix C.

Data from the EU MS surveys on PSTVd, conducted in the years 2007, 2008, 2009 and 2010, were extracted from the respective reports (FVO 2008, 2009, 2010 and 2011) of the Food and Veterinary Office of DG SANCO EC Commission (FVO) and analysed. Details on methods and results are presented in Appendix D.

# 2.2. Methodology

## 2.2.1. The guidance document

The risk assessment has been conducted in line with the principles described in the document "Guidance on a harmonised framework for pest risk assessment and the identification and evaluation of pest risk management options" [EFSA Panel on Plant Health (PLH), 2010].

When expert judgement and/or personal communication were used, justification and evidence are provided to support the statements.

In order to follow the principle of transparency as described under Paragraph 3.1 of the Guidance document on the harmonised framework for risk assessment [EFSA Panel on Plant Health (PLH), 2010] – "...*Transparency requires that the scoring system to be used is described in advance. This includes the number of ratings, the description of each rating.... the Panel recognises the need for further development..."*– the Plant Health Panel has developed specifically for this opinion rating descriptors to provide clear justification when a rating is given, which are presented in Appendix A of this opinion.

# 2.2.2. Conduct and conclusions of the risk assessment

The Panel conducted the risk assessment without considering the existing plant health legislation. The effectiveness of the current measures in place – specific or not to the pathogen – are evaluated under the Management Options sections.

The probability of entry is assessed for entry of pospiviroids from Third Countries, outside EU, towards the EU MS. The probability of spread is assessed for spread of pospiviroids within and between EU MS. When assessing the probabilities of establishment and spread, the main groups of host plants are separately analysed.

The conclusions for entry, establishment, spread and impact are presented separately. The descriptors for qualitative ratings given for the probabilities of entry and establishment and for the assessment of impact are shown in Appendix A.

# **2.2.3.** Evaluation of the management options

The Panel identifies potential risk management options and evaluates them with respect to their effectiveness and technical feasibility, i.e. consideration of technical aspects which influence their practical application. The evaluation of efficiency of management options in terms of the potential cost-effectiveness of measures and their implementation is not within the scope of the Panel evaluation.

The descriptors for qualitative ratings given for the evaluation of the effectiveness and technical feasibility of management options are shown in Appendix A.

# 2.2.4. Level of uncertainty

For the risk assessment conclusions on entry, establishment, spread and impact and for the evaluation of the effectiveness of the management options, the levels of uncertainty have been rated separately

The descriptors for qualitative ratings given for the level of uncertainty are shown in Appendix A.

# 3. Risk assessment

# **3.1.** Pest characterisation

## **3.1.1.** Identity of the pest

The genus *Pospiviroid* in the family *Pospiviroidae* comprises 10 species: *Potato spindle tuber viroid* (PSTVd), *Citrus exocortis viroid* (CEVd), *Columnea latent viroid* (CLVd), Iresine viroid 1 (IrVd-1), *Mexican papita viroid* (MPVd), *Tomato apical stunt viroid* (TASVd), *Tomato chlorotic dwarf viroid* (TCDVd), *Tomato planta macho viroid* (TPMVd) and *Chrysanthemum stunt viroid* (CSVd) (Flores et al., 2005a, 2005b). *Pepper chat fruit viroid* (PCFVd) (Verhoeven et al., 2009a) has recently been accepted as the 10<sup>th</sup> species of the genus<sup>6</sup>.

<sup>&</sup>lt;sup>6</sup> <u>http://talk.ictvonline.org/files/proposals/taxonomy\_proposals\_plant1/m/plant01/2211.aspx</u>

All but one species can infect plants of the family Solanaceae. IrVd-1 has never been found on solanaceous hosts and was not infectious to tomato or potato under experimental conditions (Spieker, 1996a; Verhoeven et al., 2010a). Since it does not seem to pose a threat to solanaceous crops and therefore does not qualify as a solanaceous pospiviroid as defined in this opinion, IrVd-1 was excluded from this assessment. Henceforth, for the purpose of this opinion, the term pospiviroid or solanaceous pospiviroid will be used throughout without reference to IrVd-1 unless it is otherwise stated.

Reported diseases (Hadidi et al., 2003) are: potato spindle tuber disease (PSTVd), tomato bunchy top disease (PSTVd), gothic disease (PSTVd), Indian tomato bunchy top disease (CEVd), tomato chlorotic dwarf disease (TCDVd), tomato planta macho disease (TPMVd), tomato apical stunt disease (TASVd) and pepper chat fruit disease (PCFVd) (Verhoeven et al., 2009a).

The discovery of viroids as pathogens distinct from viruses is relatively recent. Prior to the late 1960s, the spindle tuber disease of potato was recognized as a disease similar to those caused by many viruses (Raymer and Diener, 1969). It was only in 1971 that a characterization of PSTVd to emphasize similarities to and differences from conventional viruses was presented by T.O. Diener and the term viroid proposed to designate the potato spindle tuber RNA and other RNAs with similar properties (Diener, 1971). Following this initial discovery, the etiologies of several diseases were assigned to viroids (Diener 1979) now included in the family *Pospiviroidae*.

PSTVd has never been isolated from any of the wild potato species from the Andes, the center of origin for the cultivated potato (*Solanum tuberosum* L.). This observation, and the absence of any known source of genetic resistance to PSTVd in cultivated potatoes, has been taken as an indication that this viroid and its potato host did not co-evolve (Diener, 1987). Viroid infections are assumed to have been introduced by chance in crops from wild plants, with further spread by propagation and breeding activities (Diener, 1995). How and when PSTVd was introduced into cultivated potatoes is unclear, but it appears to have been a comparatively recent event. Several wild *Solanum cardiophyllum* plants growing in Mexico were latently infected with MPVd, a viroid closely related to TPMVd (Martinez-Soriano et al., 1996), suggesting that wild solanaceous species introduced into the United States from Mexico in the late 19<sup>th</sup> century may have acted as the initial sources of pospiviroids for infection of potatoes or other solanaceous crops. However, PSTVd has been recently identified in ornamentals originating from South America (e.g. *Brugmansia* Pers. spp., *Solanum jasminoides* Paxton, *Physalis peruviana* L.) selected from wild plants that may already have been infected (see Table 16 in Appendix B).

3.1.1.1. Taxonomic position, detection and identification methods

Viroids are subviral agents with small genomes (245-401nt) composed of covalently-closed circular single-stranded RNA molecules replicating autonomously in infected plants.

The taxon is unique among plant pathogens and consists of two families, the *Pospiviroidae* and the *Avsunviroidae*. The genus *Pospiviroid* is one of five genera of the *Pospiviroidae* (Flores et al., 2005b).

Species discrimination is based on sequence similarity level (less than 90% sequence identity of the total viroid genome) and on distinctive biological properties (Flores et al., 2005b). Some pospiviroids represent clusters of very similar genome sequences (e.g. TPMVd/MPVd and PSTVd/TCDVd) but differ in host range and symptom expression (Martinez-Soriano et al., 1996; Singh et al., 1999; Matsushita et al., 2009) and are therefore accepted as distinct species. Recently, however, Verhoeven et al. (2011b) failed to reproduce the biological differences between TPMVd and MPVd and therefore, reclassification of both pospiviroid species to a single species has been proposed.

Pospiviroid detection is done by using RT-PCR with generic primers for broad but specific amplification of pospiviroids (Bostan et al., 2004; Verhoeven et al., 2004). In addition other molecular methods, including (i) Northern blot hybridisation assays using species-specific probes that to a lower extent also show cross-hybridization with other members of the genus (Owens and Diener, 1981; Singh, 1999), (ii) RT-PCR (Shamloul et al., 1997) and (iii) real-time RT-PCR (Boonham et al., 2004;



Monger et al., 2010), allow reliable detection of pospiviroids. Sequence analysis of RT-PCR products permits identification of pospiviroid species. Overall, methods for reliable detection and identification/discrimination of pospiviroids are available, although their high sensitivity implies the risk of false-positive reactions because of cross-contamination (Borst et al., 2004). These techniques are already widely used by EU MS as indicated by the answers received to the questionnaire sent by EFSA (see Appendix C).

Recently, simultaneous infections of *S. jasminoides* and *Cestrum auranticum* by PSTVd and TASVd, and by PSTVd and CEVd, respectively, have been reported (Luigi et al., 2011). Similarly, mixed infections of CSVd and IrVd or of CEVd and TCDVd have been reported in ornamental *Verbena* species from Canada or from India (Singh et al., 2006). Since viroid titers in mixed infected hosts have been shown to be host-dependent (Pallás and Flores, 1989), we cannot exclude that in ornamental solanaceous plants infected by more than one pospiviroid, identification of one of them by RT-PCR with generic primers could be impaired by the concurrent presence of the other accumulating at higher levels.

Pospiviroids are mainly found in solanaceous species, but also in other hosts including citrus (CEVd), avocado (PSTVd), and non-solanaceous ornamentals (CSVd). The range of plant species where pospiviroids have been found in nature is rather narrow (Appendix B, Table 16), but their experimental host range is wider (Appendix B, Table 17) and pospiviroids are infectious to plant species from diverse families. Natural resistance against pospiviroids of practical significance has so far not been reported in cultivated host species.

Under experimental conditions, all pospiviroids could be transmitted to potato (Table 1), except for IrVd-1 (Verhoeven et al., 2010a), and caused spindle tuber symptoms similar to those induced by PSTVd (Verhoeven et al., 2004; Verhoeven et al., 2009a).

Viroid	Tomato	Potato*	References
PSTVd	YES	YES	Fernow, 1967; Fernow et al., 1970; Diener, 1979
CEVd	YES	YES	Mishra et al., 1991; Verhoeven et al., 2004
CLVd	YES	YES	Owens et al., 1978; Hammond et al., 1989; Singh et al., 1992a; Spieker, 1996b; Verhoeven et al., 2004; Nixon et al., 2010; Steyer et al., 2010
MPVd	YES	YES	Martinez Soriano et al., 1996; Ling and Bledsoe, 2009; Ling and Zhang, 2009; J.Th.J. Verhoeven, Plant protection Service of The Netherlands, personal communication, March 2011
PCFVd	YES	YES	Verhoeven et al., 2009b
TASVd	YES	YES	Walter, 1987; Verhoeven and Roenhorst, 2010
TCDVd	YES	YES	Singh et al., 1999; Verhoeven and Roenhorst, 2010
TPMVd	YES	YES	Galindo et al., 1982; J.Th.J. Verhoeven, Plant protection Service of The Netherlands, personal communication, March 2011
CSVd	YES	YES	Niblett et al., 1978; J.Th.J. Verhoeven, Plant protection Service of The Netherlands, personal communication, March 2011

 Table 1:
 Susceptibility of tomato and potato to infection by solanaceous pospiviroids.

\* Except for PSTVd, results for potato are from experimental transmissions.

# **3.1.2.** Occurrence of the pest

## 3.1.2.1. Global occurrence (outside the risk assessment area)

Pospiviroids have been reported from many countries. A synthesis of those reports is presented in Table 2 which is based on the available CAB International datasheets on pospiviroids (CABI online a, b, c, d, e and f) and, for pospiviroids for which CABI datasheets are not available, on recent literature. It should be stressed however, that a significant amount of uncertainty is associated with the data presented in this table because: (i) interceptions are not necessarily separated from outbreaks when reported, (ii) the information may no longer be valid due to successful eradication efforts.

Viroid	Country	References
PSTVd	ASIA: Afghanistan, present, no further details; China, restricted distribution; India, present, few occurrences; Turkey, present, no further details. AFRICA: Egypt and Nigeria, present, no further details. NORTH AMERICA: USA, restricted distribution. CENTRAL AMERICA: Costa Rica, present, no further details. SOUTH AMERICA: Chile, Peru and Venezuela, present no further details. EUROPE: Belarus, widespread; Russian Federation and Ukraine, present, no further details. OCEANIA: New Zealand, present, few occurrences.	CABI, online d.
CEVd	ASIA: China, present, no further details India, restricted distribution; Indonesia, Iran, Iraq, Israel, Japan, Jordan, Republic of Korea, Lebanon, Malaysia, Oman, Pakistan, Philippines, Saudi Arabia, Syria, Taiwan, Thailand, Turkey, United Arab Emirates, Vietnam and Yemen, present, no further details. AFRICA: Algeria, Cameroon, Cote d'Ivoire, Egypt, Ethiopia, Ghana, Libya, Madagascar, Mauritius, Morocco, Mozambique, Nigeria, Reunion, Sierra Leone, Somalia, South Africa, Sudan and Tunisia, present, no further details. NORTH AMERICA: Mexico, present, no further details; USA, restricted distribution. CENTRAL AMERICA: Cuba, Guadeloupe, Jamaica, Trinidad and Tobago, present, no further details; Brazil, restricted distribution; Chile, Colombia, Peru, Suriname and Uruguay, present, no further details. EUROPE: Montenegro, Russian Federation and Serbia, present, no further details; OCEANIA: Australia, restricted distribution; Cook Islands, Fiji, French Polynesia, New Zealand, Papua New Guinea and Samoa, present, no further details.	CABI, online b.
CLVd	CENTRAL AMERICA: Costa Rica, present, no further details Canada USA.	CABI, online c; Hammond et al., 1989; Singh et al., 1992.
MPVd	NORTH AMERICA: Canada. CENTRAL AMERICA: Mexico.	Ling and Bledsoe, 2009; Ling and Zhang, 2009.
PCFVd	NORTH AMERICA: Canada.	Verhoeven et al., 2011a.
TASVd	ASIA: Indonesia and Israel, present, no further details. AFRICA: Ivory Coast and Senegal, present, no further details; Tunisia, present, few occurrences.	CABI, online e.
TCDVd	ASIA: India and Japan, present, no further details. NORTH AMERICA: USA, present, few occurrences; Canada, eradicated according to CABI; CENTRAL AMERICA: Mexico.	CABI, online f; Ling and Zhang, 2009; Singh and Dilworth, 2009.
TPMVd	CENTRAL AMERICA: Mexico.	Galindo et al., 1982.
CSVd	ASIA: China, restricted distribution; India, Japan, Republic of Korea and Turkey, present, no further details. AFRICA: South Africa, present, few occurrences. NORTH AMERICA: Canada and USA, restricted distributions. SOUTH AMERICA: Brazil, present, no further details.	CABI, online a.

<b>Table 2.</b> Current reports of pospivitorus outside EC	Table 2:	Current reports	of pospiviroids	outside EU
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	OCEANIA: Australia, restricted distribution; New Zealand, present, few
	occurrences.

The pospiviroids have mainly been recorded in symptomatic crops e.g. tomato, potato, chrysanthemum and citrus. However, they may be more widely distributed because of the existence of many asymptomatic host plants (Verhoeven et al., 2008a) and of the lack of systematic surveys including such hosts. Therefore, uncertainty exists on geographical distribution and prevalence of pospiviroids. For example, after initial reports and application of stringent regulations, PSTVd was eradicated from potato stocks in several countries (Singh and Crowley, 1985; Singh et al., 1988; De Boer et al., 2002; Sun et al., 2004; De Boer and Dehaan, 2005), nevertheless the state of PSTVd in other asymptomatic host plants in those countries is not known.

# 3.1.2.2. Occurrence in the risk assessment area (EU)

Pospiviroid occurrences have mainly been reported in EU MS in nurseries (solanaceous ornamentals) and protected crops (tomatoes). The official surveys conducted in EU MS starting in 2007, following the Commission Decision 2007/410/EC<sup>7</sup>, focused initially on assessing the presence of PSTVd in the solanaceous ornamentals, *S. jasminoides* and *Brugmansia* spp. Although the Commission Decision 2007/410/EC refers only to *Brugmansia* and *S. jasminoides*, surveys in some EU MS included also other solanaceous ornamentals, as *Lycianthes rantonneti* (syn. *Solanum rantonneti*), *Calibrachoa* sp., *Datura* sp., *Cestrum* sp. and *Petunia* as well as tomato and potato (FVO 2008, 2009, 2010 and 2011). At a later stage in 2009, some EU MS also extended laboratory testing to identification of other pospiviroid species (see summaries of replies to the EFSA Questionnaire, Appendix C).

For each year the survey results were presented in EU MS reports and summarised by the FVO in EUwide survey reports. These reports cover surveys of domestic production, monitoring of internal market and import control. The EU MS reports generally do not provide data on lot size and/or area of host plants sufficient for a quantitative evaluation. In addition, due to a bias towards targeted sampling of plants for planting rather than random sampling at plant production sites, individual reports do not necessarily imply a widespread occurrence.

The 2011 questionnaire on pospiviroids sent by EFSA (see Section 2.1 and Appendix C) to the NPPO of the EU MS was answered by 17 NPPO. From those EU MS providing replies, PSTVd was never detected in 3 EU MS and occurred in the past in at least one crop group in 8 EU MS. Six EU MS reported PSTVd as currently present in at least one crop group but generally with limited distribution, eradicated or under eradication (one EU MS reports it as eradicated, four as under eradication and one with uncertain eradication status). Current PSTVd presence is generally linked to the solanaceous ornamentals, with only one EU MS reporting PSTVd also in other solanaceous vegetables. There is no report of current presence in potato, seed potatoes or tomato. With regard to past occurrence, PSTVd was detected in the past in seed potato in one EU MS; in potato in three EU MS, although generally in gene banks or germplasm collections (Appendix C; FVO 2008, 2009, 2010 and 2011); and in greenhouse-grown tomato in five EU MS.

**Table 3:** Summary of the EU MS replies to the EFSA questionnaire<sup>8</sup> with regard to PSTVd occurrence and eradication (details in Appendix C).

Crop / crop group	Report of occurrence of PSTVd in the crop /crop group	If currently present, report of eradication of PSTVd in the crop /crop group
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<sup>&</sup>lt;sup>7</sup> Commission Decision 2007/410/EC of 12 June 2007 on measures to prevent the introduction into and the spread within the Community of Potato spindle tuber viroid. OJ L 155, 15.6.2007, p. 71-73.

<sup>&</sup>lt;sup>8</sup> Data reported in this table refer only to the MS replying the EFSA questionnaire: Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finnland, Germany, Ireland, Italy, Latvia, Malta, Poland, Slovakia, Slovenia, The Netherlands, United Kingdom.



	N° of EU MS where PSTVd has been never detected	N° of EU MS where PSTVd was present in the past but no longer present	N° of EU MS where PSTVd is currently present	N° of EU MS not reporting	N° of EU MS where PSTVd is under eradication	N° of EU MS where PSTVd is eradicated	N° of EU MS where PSTVd eradication is unknown or not reported
Potato	13	4	-		-	-	-
Potato seeds	15	1	-	1	-	-	-
Tomato	12	5	-	-	-	-	-
Other solanaceous vegetables	13	2	1	1	-	-	1
Solanaceous ornamentals	4	7	6	-	4	1	1
Other hosts	14	-	-	3	-	-	-

**Table 4:** Summary of past and current PSTVd situation in EU MS, based on the yearly PSTVd surverys (FVO 2008, 2009, 2010 and 2011) and on the EU MS replies to EFSA questionnaire  $(Appendix C)^9$ .

EU MS	MS Total number of crops /lots with PSTVd outbreaks per year in the period 2007-2010 (FVO 2008, 200 2010, 2011)				Current status according to EU M replies to EFSA questionnaire		
	Year 2007	Year 2008	Year 2009	Year 2010	Currenly present in at least one crop / crop group	Eradicated or under eradication in the crop / crop group	Eradica tion unkno wn or not reporte d
Austria	N.A. <sup>1</sup>	4	2	1	Yes	-	Yes
Belgium	N.A <sup>1</sup> .	4	0	0	Yes	Yes	-
Bulgaria	0	0	0	0	No	-	-
Cyprus	N.A. <sup>1</sup>	N.A. <sup>1</sup>	0	0	N.A. <sup>1</sup>	N.A <sup>1</sup> .	N.A. <sup>1</sup>
Czech Republic	25	27	11	8	Yes	Yes	-

<sup>&</sup>lt;sup>9</sup> Data reported in this table for the EFSA questionnaire replies refer only to the replying MS: Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finnland, Germany, Ireland, Italy, Latvia, Malta, Poland, Slovakia, Slovenia, The Netherlands, United Kingdom.



Denmark	1	3	1	0	No	-	-
Estonia	1	0	0	0	No	-	-
Finland	N.A. <sup>1</sup>	2	0	0	No	-	-
France	N.A. <sup>1</sup>	30	25	19	N.A. <sup>1</sup>	N.A. <sup>1</sup>	N.A. <sup>1</sup>
Germany	65	16	18	4	Yes	Yes	-
Greece	N.A. <sup>1</sup>	N.A. <sup>1</sup>	3	0	N.A. <sup>1</sup>	N.A. <sup>1</sup>	N.A. <sup>1</sup>
Hungary	0	0	0	N.A. <sup>1</sup>	N.A. <sup>1</sup>	N.A. <sup>1</sup>	N.A. <sup>1</sup>
Ireland	0	0	0	0	No	-	-
Italy	40	1 <sup>10</sup>	21	8	Yes	Yes	-
Latvia	N.A. <sup>1</sup>	0	0	0	No	-	-
Lithuania	0	0	0	0	N.A. <sup>1</sup>	N.A. <sup>1</sup>	N.A. <sup>1</sup>
Luxemburg	N.A. <sup>1</sup>	0	0	0	N.A. <sup>1</sup>	N.A. <sup>1</sup>	N.A. <sup>1</sup>
Malta	N.A. <sup>1</sup>	1	0	0	No	-	-
Netherlands	3	5	3	0	Yes	Yes	-
Poland	1	0	1	0	No	-	-
Portugal	N.A. <sup>1</sup>	5	3	0	N.A. <sup>1</sup>	N.A. <sup>1</sup>	N.A. <sup>1</sup>
Romania	0	0	0	0	N.A. <sup>1</sup>	N.A. <sup>1</sup>	N.A. <sup>1</sup>
Slovakia	0	2	1	0	No	-	-
Slovenia	37	28	11	1	No	-	-
Spain	13	16	22	27	N.A. <sup>1</sup>	N.A. <sup>1</sup>	N.A. <sup>1</sup>
Sweden	N.A. <sup>1</sup>						
United Kingdom	1	0	0	0	No	-	-

1: no information provided by the EU MS.

The FVO yearly reports of the official PSTVd surveys conducted following the Commission Decision 2007/410/EC and the responses of EU MS NPPOs to the EFSA questionnaire provide a compelling picture of the past and current PSTVd status in EU MS (Table 3 and Table 4).

PSTVd is currently absent from many EU MS, while in several EU MS it is reported on solanaceous ornamentals and considered of limited distribution and under eradication. Nevertheless a significant uncertainty remains on the overall presence of PSTVd in EU MS due to the low average number of samples tested per EU MS and to the lack of details on the sampling strategy applied. In some cases there are discrepancies between FVO report and questionnaire replies, however this seems rather a matter of differences in interpretation of the term eradication, e.g. eradication of single outbreaks versus total eradication of the pathogen from the country.

<sup>&</sup>lt;sup>10</sup> PSTVd outbreak in 2008 reported with note n. 13619/17 June 2010 from Ministero delle Politiche agricole alimentari e forestali.



The information on the presence in the EU MS of pospiviroids other than PSTVd is not as detailed, because only few EU MS included detection and identification of the other pospiviroids in their surveys (pospiviroids other than PSTVd are not currently listed in the Council Directive 2000/29/EC<sup>11</sup>). As a consequence, information about pospiviroids other than PSTVd is mainly derived from data on interceptions (as reported in Europhyt database<sup>12</sup>), from scientific references and from the EU MS replies to the EFSA questionnaire (Table 5).

FVO survey reports for PSTVd (FVO 2008, 2009, 2010 and 2011) provide records of: TASVd which was found in *Solanum jasminoides* and *Brugmansia* in one EU MS in 2009 and 2010, CEVd found in *Solanum jasminoides* in two EU MS in 2009, TCDVd infecting tomato in one EU MS in 2009, TCDVd infected *Petunia* in two EU MS in 2010 (Table 5).

Information on interceptions of other pospiviroids within the EU MS from Europhyt database is summarised in Table 20 in Appendix B.

EU MS responses to the EFSA questionnaire (out of the 17 EU MS responses, only 16 addressed questions on the other solanaceous pospiviroids) (Appendix C) also confirmed the presence of other solanaceous pospiviroids in EU MS. Only TPMVd and MPVd were never detected in EU MS, while the other solanaceous pospiviroids were found in some EU MS mostly in solanaceous ornamentals in few locations or localised to some regions. Occurrences in vegetable crops are rare while none of these pospiviroids was ever found on potato.

In details, CEVd was never detected in eight EU MS, present in the past in one EU MS and currently present in six EU MS; no information was provided by two EU MS. CSVd was never detected in seven EU MS, present in the past in four EU MS and currently present in five EU MS; no information were provided by one EU MS. CLVd was never detected by 11 EU MS, present in the past in three EU MS and currently present in one EU MS; no information was provided by two EU MS. MPVd was never detected in 15 EU MS; no information were provided by 2 EU MS. PCFVd was never detected in 14 EU MS and present in the past in one EU MS; no information was provided by 2 EU MS. TASVd was never detected in 9 EU MS, present in the past in 2 EU MS and currently present generally in few locations in 4 EU MS; no information was provided by 2 EU MS. TCDVd was never detected in 8 EU MS, present in the past in 3 EU MS and currently present in 4 EU MS; no information was provided by 2 EU MS. TCDVd was never detected in 8 EU MS, present in the past in 3 EU MS and currently present in 4 EU MS; no information was provided by 2 EU MS; no information was provided by 2 EU MS. TCDVd was never detected in 8 EU MS, present in the past in 3 EU MS and currently present in 4 EU MS; no information was provided by 2 EU MS. TCDVd was never detected in 2 EU MS.

**Table 5:** Reports of pospiviroids other than PSTVd in the risk assessment area (EU) according to FVO reports and MS notifications, published references and the EU MS replies to EFSA questionnaire.

Pospiviroid	Reports in EU MS and published references
CEVd*	Austria (FVO, 2010), Belgium (FVO, 2010), Czech Republic (EFSA questionnaire, 2011, current), Germany (Verhoeven et al., 2008c), Italy (EFSA questionnaire, 2011, current), Netherlands (Verhoeven et al., 2008a and c), Slovenia (EFSA questionnaire, 2011, present in the past).
CLVd	Denmark (Nielsen and Nicolaisen, 2010; EFSA questionnaire, 2011, present in the past), Belgium (Verhoeven et al., 2004; EFSA questionnaire 2011, present in the past), France (Steyer et al., 2010), Italy (Parrella et al., 2010), The Netherlands (Verhoeven et al., 2004;

<sup>&</sup>lt;sup>11</sup> Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ L 169, 10.7.2000, p. 1-148.

<sup>&</sup>lt;sup>12</sup> Europhyt is a web-based network launched by DG Health and Consumers Protection, and is a sub-project of PHYSAN (Phyto-SanitaryControls) specifically concerned with plant health information. Europhyt database manages notifications of interceptions of plants or plant products that do not comply with EU legislation notifications.



	EFSA questionnaire 2011, current), UK (EFSA questionnaire, 2011, present in the past; Nixon et al., 2010),.
MPVd	
PCFVd	Netherlands (Verhoeven et al., 2009a; EFSA questionnaire, 2011, present in the past).
TASVd	Austria (EFSA questionnaire, 2011, present in the past), Belgium (FVO, 2010 and 2011; Verhoeven et al., 2008c; EFSA questionnaire, 2011, current), Czech Republich (EFSA questionnaire, 2011, current), Finland (EFSA questionnaire, 2011, present in the past; Lemmetty et al., 2011), Germany (Verhoeven et al., 2008c; EFSA questionnaire, 2011, current), Italy (Luigi et al., 2011), Netherlands (Verhoeven et al., 2008a; 2010b; EFSA questionnaire, 2011, current).
TCDVd	Belgium (FVO, 2011; EFSA questionnaire, 2011, current), Czech Republic (EFSA questionnaire, 2011, present in past), Finland (EFSA questionnaire, 2011, present in the past; Lemmetty et al., 2011), (France (FVO, 2010; Candresse et al., 2010), Germany (EFSA questionnaire, 2011, current), Netherlands (Verhoeven et al., 2010d; EFSA questionnaire, 2011, current), Portugal, (James et al., 2008), Slovenia (Marn and Plesko, 2010; EFSA questionnaire, 2011, current), UK (FVO, 2011; EFSA questionnaire, 2011, present in the past).
TPMVd	
CSVd	Austria (FVO, 2010), Czech Republic (Czech Republic State Phytosanitary Administration, 2010), Finland (EFSA questionnaire, 2011, present in the past; Lemmetty et al., 2011), Germany (EFSA questionnaire, 2011, current), Italy (La Rosa et al., 1983,), Netherlands (Verhoeven et al., 1998), Slovenia (Mehle et al., 2010; EFSA questionnaire, 2011, present in the past), Spain (Duran-Vila et al., 1996), UK (Hadidi et al., 2003).

\* CEVd is likely to be present wherever citrus species are grown.

In summary, PSTVd appears to be present in solanaceous ornamentals in a number of EU MS and may still have been under-reported in the EU MS surveys, beause of limitations in sampling (number of samples per country, sampled species...). There appears to have been only rare reports in other crops. As for other pospiviroids, the situation is more uncertain but all of them, with the exception of TPMVd and MPVd, have been reported from one or more EU MS in ornamentals or in tomato. None of them has ever been reported in potato.

## 3.1.2.3. Regulatory status outside the risk assessment area

The official regulatory status for solanaceous pospiviroids, for some EU trade partners and for Third Countries ranked according to FAOSTAT<sup>13</sup> statistics as top 10 producers of potato, seed potatoes and tomato, is shown in Table 6. This overview is restricted to countries for which the relevant information is published on the IPPC (International Plant Protection Convention) web site<sup>14</sup>.

**Table 6:** Examples of Third Countries where pospiviroids are regulated

Viroid	Country where the pospiviroid is regulated								
PSTVd	ASIA: India (PQOI, online); Turkey (Plant Quarantine regulation of Turkey, 2003, on line).								
	NORTH AMERICA: Canada (Pests regulated by Canada, on line: Canada-seed potatoes directiv								
	2009, on line). CENTRAL AMERICA: Mexico (IPPC, on line). SOUTH AMERICA: Brazi								
	(Ministério da Agricultura, Pecuária e Abastecimento, on line). EUROPE: Ukraine (List of								
	regulated harmful organisms Ukraine, on line). OCEANIA: Australia (Quarantine Proclamation								
	1998, on line).								
CEVd	OCEANIA: Australia (Quarantine Proclamation 1998, on line); CENTRAL AMERICA: Mexico								
	(IPPC, on line).								
CLVd	OCEANIA: New Zealand (Biosecurity Organisms register, on line); Australia (Quarantine								
	Proclamation 1998, on line);								
MPVd	OCEANIA: Australia (Quarantine Proclamation 1998, on line);								
PCFVd	OCEANIA: Australia (Quarantine Proclamation 1998, on line);								
TASVd	OCEANIA: Australia (Quarantine Proclamation 1998, on line);								

 <sup>&</sup>lt;sup>13</sup> FAOSTAT, FAO (Food and Agriculture Organisation)Statistics Division 2011 (<u>http://faostat.fao.org/site/339/default.aspx</u>).
 <sup>14</sup> International Plant Protection Convention (IPPC), https://www.ippc.int /



TCDVd	OCEANIA: Australia (Quarantine Proclamation 1998, on line);
TPMVd	OCEANIA: New Zealand (Biosecurity Organisms register, on line); Australia (Quarantine
	Proclamation 1998, on line);
CSVd	ASIA: India (PQOI, online); Turkey (Plant Quarantine regulation of Turkey, 2003, on line);
	CENTRAL AMERICA: Mexico (IPPC, on line); Peru (SENASA, on line); EUROPE: Ukraine
	(List of regulated harmful organisms Ukraine, on line). OCEANIA: Australia (Quarantine
	Proclamation 1998, on line).

Few Third Countries list all pospiviroids as quarantine pests and regulate them on all commodities (e.g. Australia). Some countries regulate only few pospiviroids on all commodities (e.g. New Zealand, Ukraine), others regulate only PSTVd either in general (e.g. Brazil) or specifically on potato seed tubers (e.g. Canada). The Turkey Regulation on Agricultural Quarantine regulates PSTVd in general (in Annex I, Harmful organisms that constitute a barrier for importation) and CSVd when found in some plants and plant products (Annex II).

The Plant Quarantine Order of India requires that true seed/micro-tubers (*in vitro*) of potato are obtained from plants tested and certified free from viroids of potato and of other tuber bearing solanaceous plant species.

Tomato seed for sowing imported to India from any country has to be free from PSTVd. In case of imports of *Chrysanthemum* spp from Columbia, Denmark, France, Finland, Germany, Japan, UK, USA to India, a certificate is needed declaring that the tissue cultured plants were obtained from mother stock tested and maintained free from CSVd.

The official regulations for import of vegetative propagation material of Mexico (IPPC, on line) require freedom of PSTVd, CSVd and CEVd for tomato, chrysanthemum and citrus originating respectively from the Netherlands and the USA.

Some Third Countries do not include any solanaceous pospiviroids in their lists of regulated/quarantine plant pests (e.g. Belarus, USA). However, although the regulated plant pest list (USDA APHIS, on line a) of USA does not include any pospiviroids, viroids are listed among the taxa against which USDA APHIS (Animal and Plant Health Inspection Service) may take quarantine action (USDA APHIS, on line b).

3.1.2.4. Regulatory status in the risk assessment area (EU)

PSTVd is a harmful organism listed under Council Directive 2000/29/EC, Annex I Part A, Section I and hence its introduction into and spread within all EU MS has to be prohibited.

Other pospiviroids are not listed in this Directive. As these pathogens can infect potato under experimental conditions but have never been observed naturally infecting this host, it is unclear but doubtful whether they could be considered as being included in Annex I Part A, Section I as "Potato viruses and virus-like organisms".

In addition, in June 2007 the EU Commission adopted emergency measures to further prevent the introduction into and the spread within the EU territory of PSTVd (Commission Decision 2007/410/EC). These measures apply to plants of the genus *Brugmansia* and of the species *S. jasminoides*, intended for planting, including seeds. The Commission Decision defines additional measures for import and movement of the specified plants within the EU territory and requires the EU MS to conduct official surveys and to notify the results to the Commission.

CSVd is listed in the Directive 2000/29/EC Annex II Part A Section II as a harmful organism occurring in the EU territory, whose introduction into and spread within EU MS shall be banned in plants of the genus *Dendranthema* (DC.) Des Moul intended for planting, other than seeds.



Specific legislation exists for solanaceous plants. The phytosanitary legislation of the EU (Directive 2000/29/EC Annex III Part A (10-13) prohibits the introduction of:

- i. tubers of *Solanum tuberosum* L., seed potatoes, from Third Countries other than Switzerland;
- ii. plants of stolon- or tuber-forming species of *Solanum* L. or their hybrids, intended for planting, other than seed potatoes, from Third Countries;
- iii. tubers of species of *Solanum* L., and their hybrids (different from seed potatoes and plants from planting), from Third Countries other than Algeria, Egypt, Israel, Libya, Morocco, Syria, Switzerland, Tunisia and Turkey, and other than European Third Countries which are either recognised as being free from *Clavibacter michiganensis* ssp. *sepedonicus* (Spieckermann and Kotthoff) Davis et al., or in which provisions recognised as equivalent to the Community provisions on combating *C. michiganensis* ssp. *sepedonicus* have been complied with.
- iv. plants of Solanaceae intended for planting (other than seeds, tubers of *Solanum tuberosum* both for propagation and consumption purposes and plants for planting of other stolon or tuber forming *Solanum* sp.) from Third Countries other than European and Mediterranean countries).

Moreover, according to the provisions laid down in Directive 2000/29/EC Annex IV part A Section I (origine outside EU), the following requirements apply for the introduction and movement within all EU MS of:

- i. tubers of *S. tuberosum* for consumption, other than early potatoes, originating from countries where PSTVd is known to occur: suppression of the faculty of germination (point 25.3);
- ii. plants for planting of Solanaceae (other than tubers of *S. tuberosum* and tomato seeds), originating in countries where PSTVd is known to occur: official statement that no symptoms of PSTVd have been observed on plants at the place of production since the beginning of the last complete cycle of vegetation (point 25.6);
- iii. plants for planting of *Dendranthema* (DC.) Des Moul (other than seeds) should be no more than third generation stock derived from material which has been found free from CSVd during virological tests, or directly derived from material of which a representative sample of at least 10 % has been found to be free from CSVd during an official inspection carried out at the time of flowering (point 28);
- iv. tomato seeds: official statement that the seeds have been obtained by means of an appropriate acid extraction method or an equivalent method approved and (a) either the seeds originate in areas where PSTVd is not known to occur; or (b) no symptoms of diseases caused by PSTVd has been observed on the plants at the place of production during their complete cycle of vegetation; or (c) the seeds have been subjected to official testing PSTVd on a representative sample and using appropriate methods, and have been found, in these tests, free from PSTVd (point 48);

With regard to the plants and plant products originating in the EU, the following requirements are laid down in Directive 2000/29/EC Annex IV part A Section II for the introduction and movement within all EU MS of:



- i. Tubers of potato, intended for planting (other than tubers of those varieties officially accepted in one or more EU MS pursuant to Council Directive 70/457/EEC<sup>15</sup>: official statement that the tubers belong to advanced selections, have been produced within the EU terrirory and have been derived in direct line from material which has been maintained under appropriate conditions and has been subjected within the Community to official quarantine testing in accordance with appropriate methods and has been found, in these tests, free from harmful organisms (point 18.2);
- ii. Plants for planting of stolon or tuber-forming species of Solanum L., or their hybrids (other than potato tubers as specified in Annex IV-A-II-18.1 or 18.2, and other than culture maintenance material being stored in gene banks or genetic stock collections): (a) the plants shall have been held under quarantine conditions and shall have been found free of any harmful organisms in quarantine testing; (b) the quarantine testing referred to in (a) shall: (aa) be supervised by the official plant protection organisation of the EU MS concerned and executed by scientifically trained staff of that organisation or of any officially approved body; (bb) be executed at a site provided with appropriate facilities sufficient to contain harmful organisms and maintain the material including indicator plants in such a way as to eliminate any risk of spreading harmful organisms; (cc) be executed on each unit of the material, — by visual examination at regular intervals during the full length of at least one vegetative cycle, having regard to the type of material and its stage of development during the testing programme, for symptoms caused by any harmful organisms, — by testing, in accordance with appropriate methods to be submitted to the Committee referred to in Article 18: — in the case of all potato material at least for a list of organisms including PSTVd; (dd) by appropriate testing on any other symptom observed in the visual examination in order to identify the harmful organisms having caused such symptoms; (c) any material, which has not been found free, under the testing specified under (b) from harmful organisms as specified under (b) shall be immediately destroyed or subjected to procedures which eliminate the harmful organism(s); (d) each organisation or research body holding this material shall inform their official EU MS plant protection service of the material held (point 18.3).
- iii. Plants of *Dendranthema* (DC) Des Moul. intended for planting, other than seeds: official statement that: (a) the plants are no more than third generation stock derived from material which has been found to be free from CSVd during virological tests, or are directly derived from material of which a representative sample of at least 10 % has been found to be free from CSVd during an official inspection carried out at the time of flowering.

The Commission Directive 2008/61/EC<sup>16</sup> defines the conditions under which certain harmful organisms, plants, plant products, and other objects listed in Annexes I to IV to Council Directive 2000/29/EC may be introduced into or moved within the EU territory or certain protected zones thereof, for trial or scientific purposes and for work on varietal selections, and applies also to PSTVd.

Council Directive 92/34/EEC<sup>17</sup> applies to the whole genus *Citrus* and its hybrids. In the Commission Directive 93/48/EEC<sup>18</sup> viroids such as CEVd are included in the list of specific harmful organisms and diseases of quality-affecting significance for *Citrus aurantifolia* (Christm) Swing., *C. limon* L.

<sup>&</sup>lt;sup>15</sup> Council Directive 70/457/EEC of 29 September 1970 on the common catalogue of varieties of agricultural plant species. OJ L 225, 12.10.1970, p. 1-6.

<sup>&</sup>lt;sup>16</sup> Commission Directive 2008/61/EC of 17 June 2008 establishing the conditions under which certain harmful organisms, plants, plant products and other objects listed in Annexes I to V to Council Directive 2000/29/EC may be introduced into or moved within the Community or certain protected zones thereof, for trial or scientific purposes and for work on varietal selections. OJ L 158, 18.6.2008, p. 41-55.

<sup>&</sup>lt;sup>17</sup> Council Directive 92/34/EEC of 28 April 1992 on the marketing of fruit plant propagating material and fruit plants intended for fruit production. OJ L 157, 10.6.1992, p. 1-10.

<sup>&</sup>lt;sup>18</sup> Commission Directive 93/48/EEC of 23 June 1993 setting out the schedule indicating the conditions to be met by fruit plant propagating material and fruit plants intended for fruit production, pursuant to Council Directive 92/34/EEC. OJ L 250, 7.10.1993, p. 1-8.

Burm.F., *C. paradisi* Macf, *C. reticulata* Blanco and *C. sinensis* (L.) Osbeck and, therefore, must be absent from certified citrus plant propagation material.

# 3.1.3. Potential for establishment and spread in the risk assessment area

Seven of the nine solanaceous pospiviroid species plants have been reported in EU MS in protected cultivation (glasshouse) and/or in open fields. Plants which are hosts for these pathogens are widely available in the EU and the eco-climatic conditions are suitable for their establishment and spread.

# **3.1.4.** Potential for consequences/impact (including environmental consequences) in the risk assessment area

While pospiviroids are generally symptomless in solanaceous ornamentals, they have the potential to have considerable impact in potato and tomato crops in the EU.

# **3.1.5.** Conclusions of pest categorisation

Pospiviroids are well-characterized pathogens of crops and, while uncertainties exist over their prevalence and distribution in the EU, they all have the potential to establish, spread and cause considerable losses to some European crops.

MPVd and TPMVd are not reported in the EU territory;

CLVd, TASVd, TCDVd and PCFVd are reported from a limited number of EU MS;

CEVd is widely prevalent in citrus growing regions of the EU but reported only from a limited number of EU MS in solanaceous hosts;

CSVd is reported from a limited number of EU MS in solanaceous hosts, it might be more widely distributed in chrysanthemum but is under regulation in this host (Council Directive 2000/29/EC Annex II Part A)

PSTVd is reported from many EU MS, present in some solanaceous ornamentals and is under official control (Council Directive 2000/29/EC, Annex I Part A, Section I).

As a consequence of the above, all nine solanaceous pospiviroids have the potential to be considered for quarantine listing and therefore are eligible to be included in the PRA.

# **3.2.** Probability of entry: from outside EU

## **3.2.1.** List of pathways

Four pathways have been identified as having the potential to result in the entry of solanaceous pospiviroids in the PRA area:

- 1) True (botanical) seeds of susceptible species
- 2) Seed (potato) tubers
- 3) Plants for planting of susceptible species, including cuttings and rooted ornamental plant species
- 4) Plant products not intended for planting (for food consumption or cut flowers)

## **3.2.2.** Pathway 1: True (botanical) seeds of susceptible species

## 3.2.2.1. Association of the pathogen with the pathway at origin

Seed transmission has been shown for several solanaceous pospiviroids in tomato and potato (see Table 7). In addition, a few outbreaks of pospiviroids in tomato crops have been linked to infection



transmitted through botanical seeds (Candresse et al., 2010; Sansford and Morris, 2010), where simultaneous outbreaks at unrelated sites were traced to seeds from a common seed lot.

Unsuccessful seed transmission experiments have been reported for some pospiviroid species (Singh et al., 1999; Koenraadt et al., 2009), which indicate that seed transmission of pospiviroids could potentially be affected by a number of factors such as viroid strain, host variety or environmental conditions.

From interception records in the Europhyt database (Appendix B Table 18), there is evidence for the presence of PSTVd in tomato seeds imported into Europe from production areas outwith the EU. For those pospiviroids where there is no published evidence of seed transmission in tomato, a similar behaviour is assumed by analogy with those viroid species that are seed transmissible, but in the absence of experimental data, this assessment is associated with medium uncertainty.

The probability of pospiviroids being associated with this pathway involving true (botanical) seeds (potato, tomato and ornamentals) is assessed as low, because of the only few documented cases of outbreaks linked to infected seed lots (e.g. Candresse et al., 2010; Sansford and Morris, 2010) and based on the records of interceptions This assessment is however associated with a high degree of uncertainty as a result of the lack of information on prevalence of pospiviroids outwith the EU territory.

Viroid	Ornamentals	Tomato	Pepper/Others	ers Potato References		
PSTVd	Scopolia sinensis	YES		YES	EUPHRESCO, 2011; Benson and Singh, 1964; Singh, 1970a; Hunter et al., 1969; Singh and Finnie, 1973; Singh et al., 1992a	
CEVd	Verbena sp. Impatiens sp.				Singh et al., 2009	
CLVd		Not proven*			Fox and Monger, 2011;	
PCFVd			YES		Verhoeven et al., 2009a	
TASVd		YES			Antignus et al., 2007	
TCDVd	Vinca minor	YES			Candresse et al., 2010; Singh and Dilworth, 2009	
TPMVd		Not proven			Galindo, 1987	
CSVd	Chrysanthemum	YES			Hollings and Stone, 1973; Monsion et al., 1973; Chung and Pak, 2008	

Table 7:	Published evidence for transmission of solanaceous pospiviroids through botanical (true)
seeds.	

\* Experiments to demonstrate vertical transmission in tomato were unsuccessful, but number of seeds used can not completely rule out low frequency transmission, as was originally the case in the UK outbreak in 2007.

# 3.2.2.2. Survival of the pathogen during transport or storage

From interception records in the Europhyt database (Appendix B Table 18) of true (botanical) seeds entering the PRA area from Third Countries, it is known that pospiviroids can be present in the seed of

suitable host species. The evidence for seed transmission of PSTVd indicates that the pathogen/s will remain viable in dormant seed and can infect the germinating seedling (Hunter et al., 1969; Singh, 1970a; Singh and Finnie, 1973; EUPHRESCO, 2011). PSTVd is shown to remain detectable and viable in excess of twenty years in true potato seeds (Singh et al., 1991). Seed transmission has also been linked to several outbreaks of CLVd (Sansford and Morris, 2010) or TCDVd in the PRA area (Candresse et al., 2010).

Overall the available data indicate that pospiviroids very likely survive transport and storage of infected seeds, and this assessment has a low degree of uncertainty for species for which information is available but with a higher degree of uncertainty for other species.

# 3.2.2.3. Survival of the pathogen to existing management procedures

Viroids can be carried deep within the seed (e.g. Antignus et al., 2007). Commonly used disinfection methods such as acid washing or hypochlorite treatment, which are effective against viral pathogens carried on the seed coat, do not appear to be effective in reducing infection levels of those pospiviroids for which this has been evaluated (PSTVd, TCDVd, TASVd) (Antignus et al., 2007; Singh and Dilworth, 2009; EUPHRESCO, 2011). The pospiviroids are therefore very likely to survive existing management procedures, with low uncertainty (but with a higher degree of uncertainty for pospiviroid species for which info is not available).

# 3.2.2.4. Transfer of the pathogen to a suitable host

In this pathway the imported seeds would be those of a suitable host entering the PRA area, therefore transfer to a suitable host would be immediate.

# **3.2.3.** Pathway 2: Seed (potato) tubers

# 3.2.3.1. Association of the pathogen with the pathway at origin

PSTVd is the only solanaceous pospiviroid species found infecting potato (*S. tuberosum*) under natural conditions. Tubers produced on an infected mother plant will be infected at a very high rate because pospiviroids are known to infect most parts of their host plant (Palukaitis, 1987). PSTVd is present in a number of potato-producing countries, and therefore importing seed tubers carries a significant risk of introducing PSTVd. However, in some seed potato certification schemes (for example USA, Canada) significant and effective efforts have been made to eradicate PSTVd. Depending upon the certification scheme applied in the country of origin, the probability of importing seed potatoes carrying PSTVd ranges from very unlikely to likely.

Natural infection of potato by pospiviroids other than PSTVd has not been reported, but all solanaceous pospiviroids can infect potato under experimental conditions (Table 1). In each case, importation into the EU of seed tubers could carry a significant risk of introducing pospiviroids. However, given the absence of reports of naturally occurring infections in potato, the association of pospiviroids other than PSTVd with this pathway is considered very unlikely, with a medium uncertainty.

# 3.2.3.2. Survival of the pathogen during transport or storage

PSTVd is known to be transmissible from mother tubers to progeny plants (Leclerg et al., 1944). As the pathogen is being transported in living plant organ (tuber), it will survive until the following planting season. Although viroids are generally considered to replicate and accumulate better under high temperature, the ability of TCDVd to survive subzero temperatures (-12 °C) was recently demonstrated (Singh and Dilworth, 2009). Consequently storage under low temperature conditions is unlikely to have an adverse effect on the pathogen survival.

The potential to introduce PSTVd through this pathway is demonstrated by interception records in the Europhyt database. Between 1999 and 2010, PSTVd was found only in one consignment of potato



seed tubers from Third Countries (Appendix B Table 18). The low number of PSTVd interceptions is however also a reflection of the low numbers of these breeding materials entering the PRA region. There is a high probability that PSTVd would survive transport and storage, with a low degree of uncertainty. For other pospiviroids a similar rating is suggested but it is associated with a higher degree of uncertainty.

# 3.2.3.3. Survival of the pathogen to existing management procedures

In the absence of quarantine regulations, the current marketing controls of seed potatoes (Council Directive 2002/56/EC<sup>19</sup>, based on UNECE Standards S1, United Nations, 2010) would only require visual inspection for virus symptoms in field crops. The severity of symptom expression of PSTVd in the growing plant and in daughter tubers is affected by potato variety, PSTVd strain and growing conditions (Pfannensteil and Slack, 1980). Therefore, such a visual inspection does not guarantee detection of all occurrences of PSTVd in imported seed tubers. PSTVd would therefore be expected to survive existing management procedures with a high probability and a low level of uncertainty. For other pospiviroids a similar rating is suggested but associated with a higher degree of uncertainty.

# 3.2.3.4. Transfer of the pathogen to a suitable host

In this pathway, the imported seed potatoes would be those of a suitable host entering the PRA area. Therefore, transfer to a suitable host would be immediate.

# **3.2.4.** Pathway 3: Plants for planting of susceptible species for planting, including cuttings and rooted ornamental plants

## 3.2.4.1. Association of the pathogen with the pathway at origin

It is known that pospiviroids can be present in essentially all tissues of their hosts. Therefore, import in the EU of infected plants for planting, including cuttings and rooted plants of both solanaceous and non-solanaceous ornamental species, represents a distinct pathway for entry. Several host species of this type have been shown to act as conduits for the propagation of solanaceous pospiviroids such as CSVd (Verhoeven et al., 1998 and 2006b), CLVd (Hammond, 2003), TCDVd (Verhoeven et al., 2007a), CEVd, PSTVd and TASVd (Verhoeven et al., 2008a). A more complete list of host species can be found in Appendix B Tables 16 and 17.

There is little information available on the incidence of pospiviroids in ornamentals outwith the EU, except for Bostan et al. (2004), Nie et al. (2005), Singh et al. (2006) and Singh and Dilworth (2009). However, the interception by EU MS of consignments from Third Countries of plants for planting infected with these pathogens (Appendix B, Table 18) clearly indicates the potential for entry through this pathway.

The list of possible host/pospiviroid combinations is also increasing with improving diagnostic technologies (Verhoeven et al., 2008b and 2010b), which means that other as yet unidentified associations may rise to prominence in the future.

Overall, the interception data indicate that the probability of the pospiviroids entering the PRA area via this plant for planting pathway is moderately likely to likely with a medium uncertainty.

## 3.2.4.2. Survival of the pathogen during transport or storage

As the pathogen is being transported in living plant material, it will survive both transport and storage. Records of interceptions in the PRA area (Appendix B, Table 18) demonstrate the regularity of these findings and that viable pathogens have the potential to enter the PRA area via this pathway. Therefore

<sup>&</sup>lt;sup>19</sup> Council Directive 2002/56/EC of 13 June 2002 on the marketing of seed potatoes. OJ L 193, 20.7.2002, p. 60-73.



there is a high probability that the pospiviroids will survive during transport and storage, with low uncertainty.

# 3.2.4.3. Survival of the pathogen to existing management procedures

In the absence of the current quarantine and EU emergency measures, management procedures would consist of visual inspection of plants, which may be infected with pospiviroids. This measure is unlikely to reliably detect infected plants that may be asymptomatically infected due to environmental conditions and other factors affecting symptom expression. Hence, there is a high probability that the pathogens will survive existing management procedures, with only low uncertainty on this assessment.

# 3.2.4.4. Transfer of the pathogen to a suitable host

Within this pathway the viroid is moved into the PRA region through infected suitable plant propagation hosts and transfer to a suitable host would therefore be immediate.

# **3.2.5.** Pathway 4: Plant products not intended for planting (food consumption, cut flowers, pollen, animal feed)

# 3.2.5.1. Association of the pathogen with the pathway at origin

PSTVd is the only solanaceous pospiviroid species found infecting potato (*S. tuberosum*) under natural conditions. Tubers produced on an infected mother plant will be infected at a very high rate because pospiviroids are known to infect most parts of their host plant (Palukaitis, 1987). PSTVd is present in a number of potato-producing countries and therefore, importing tubers for consumption carries a significant risk of introducing PSTVd-infected tubers. Depending upon the country from which tubers originate, the risk of importing potatoes carrying PSTVd infection ranges from low to high, with an overall high level of uncertainty.

Natural infection of potato by other pospiviroids has not been reported, but all of them have been shown to infect potato under experimental conditions (Table 17). In each case importation in the EU of the daughter tubers of infected plants would be assumed to carry a significant risk of introducing pospiviroids.

Given the absence of naturally occurring non-PSTVd pospiviroid infections in potato the probability of introducing them through this pathway is considered very unlikely, with a medium degree of uncertainty.

There is a limited number of reports of pospiviroids in ornamental species for cut flowers and foliage production outside the EU. This may be a reflection of the limited attention given to these pathogens. However, viroids being systemic pathogens, any significant part of an infected plant including cut flowers, foliage, etc. not intended for planting can be considered to be carrying pospiviroids.

Similarly, fruit collected from an infected plant could be considered as carrying pospiviroid both in the fruit tissues and within the seeds (Weideman, 1987; Khoury et al., 1988). Within this pathway fruit and vegetables imported into the PRA area for human consumption may be carrying solanaceous pospiviroids. This reasoning applies not only to fruit of solanaceous crops but also to fruits from CEVd-infected citrus. The associations with pathway at origin would be as for plants for planting, addressed in Section 3.2.4.1.

Significant volumes of ware potatoes, tomatoes, other vegetables, citrus fruit, cut flowers and ornamental foliage are imported into the PRA area, a number of which have the potential to carry solanaceous pospiviroid inoculum. The probability of association of pospiviroids with the corresponding pathway at origin is difficult to evaluate globally, but is estimated as unlikely to moderately likely with a high degree of uncertainty.

# 3.2.5.2. Survival of the pathogens during transport or storage

As the pathogen is being transported in living plant material, there is high probability that the pospiviroids will survive transport and storage with only low uncertainty on this assessment.

# 3.2.5.3. The pathogen surviving the existing management procedures

In the absence of the current quarantine and EU emergency measures, management procedures would consist of visual inspection of imported plant products, which may be infected with pospiviroids. This measure is highly unlikely to reliably detect all infected plant materials. Therefore, there is a high probability that the pathogens will survive existing management procedures, with only low uncertainty on this assessment.

## 3.2.5.4. Transfer of the pathogen to a suitable host

As the produce is intended for human or animal use, generally there will be destruction of the material. However, there are two main potential routes by which pospiviroid inocula could be transferred to suitable hosts: unintended use of foodstuffs for propagation and mechanical- or vector-assisted transfer to susceptible plants.

## Unintended use of foodstuffs for propagation

Material imported for consumption may be inappropriately used for propagation. This may be the planting of ware potatoes for the production of daughter tubers, collection and use of seeds from fruit such as tomato or pepper, discarded or partially composted tubers or fruit with seeds, which then germinate (volunteers). In each case the resulting plants have the potential to carry infection, a scenario which is then comparable to those analyzed for pathways (i) and (ii). However, the plants would tend to be in domestic situations and therefore less likely to come into contact with major crop production sites. The probability of this scenario is assessed as being low associated with a high degree of uncertainty.

## Mechanical and vector assisted transfer to suitable hosts

There are two potential ways for pospiviroids infecting plant materials for consumption to be transferred to susceptible hosts. As the solanaceous pospiviroids are mechanically transmissible (see Section 3.4.2.), any point where infected plant material may come in contact with growing crops leads to a potential pathway. The two obvious points for consideration are: (a) packing houses at production sites, where fruit for market is brought onto site and packed during the growing season to enable year round working of the packhouse; or (b) contamination from pospiviroids picked up on workers hands from consuming contaminated foodstuffs. In this respect, PSTVd remains transmissible from human skin to tomato for at least two hours following exposure to infected plant material of ornamental solanaceous species to tomato (Verhoeven et al., 2010c). The probability of this scenario is assessed as being very unlikely associated with a high degree of uncertainty.

Vector assisted transmission is known for at least some pospiviroids, as described in detail in the Section on spread (Section 3.4.1). This raises the possibility that aphids or bumblebees might transfer pospiviroids from infected fruit or cut flowers to suitable hosts. The probability of this scenario is assessed as being very unlikely associated with a high degree of uncertainty

## **3.2.6.** Conclusions on probability of entry

Four pathways have been identified, three of which implicate propagation material [true/botanical seeds, seed (potato) tubers and plants for planting]. The fourth pathway, plant material not intended for planting, is considered of minor significance due to the low probability of transfer to a suitable host being the limiting factor. For the three main pathways, the probabilities of survival during transport and storage, survival through existing management procedures and transfer to a suitable host are



considered to be overall very high and the only limiting factor is the probability of association with the pathway at origin. In each case these probabilities have been assessed as shown in Table 8.

**Table 8:** Assessment of probability and uncertainty for association of pospiviroids with each entrypathway at origin.

Pathway	Probability	Uncertainty
True (botanical) seeds	Low	High
Seed (potato) tubers	Very unlikely to likely	Medium
Plants for planting	Moderately likely to likely	Medium

As a consequence, trying to balance these ratings across all three main pathways would provide a moderately likely rating for the risk of association of pospiviroids with propagation material at the source of origin.

Because the probability of association with the pathway at origin is the only limiting factor in the assessment of the probability of entry, the probability of entry is considered as equivalent to the probability of association with the pathway and therefore is given as an overall rating of moderately likely with a high uncertainty.

# 3.2.7. Uncertainties

The high levels of uncertainty associated with assessing these pathways arise from a lack of available information concerning many key factors of the different pathways and their relative probabilities of risk. The main area of uncertainty comes from the lack of data on both distribution and prevalence of many of the pospiviroids outwith the EU. At best the availability of this information is inconsistent, arising from countries where control programmes have been implemented to manage the incidence of only some pospiviroids, within a specific commodity crop, such as seed potatoes in the USA or Canada. However, even where this information may be available, it generally amounts to a statement of 'freedom from PSTVd in Seed Potatoes' with no information of prevalence in horticultural crops or ornamental species available. Additionally, the type of systematic survey work carried out under the EU emergency measures (Commission Decision 2007/410/EC) is not available for Third Countries.

With the exception of potato and tomato, the propensity for true (botanical) seed transmission of these pathogens has not been studied for most host/viroid combinations (see Table 7) and is therefore an unknown quantity. Unless information to the contrary is available, it has been assumed that pospiviroids are transmissible to some extent in any given host-viroid combinations. Seed transmission is affected by time of infection and environmental conditions, resulting in highly variable transmission rates. Given that seed transmission records for the most widely studied of these pathogens, PSTVd, range from 0 to 100%, there is an obvious high degree of uncertainty associated.

There is a general lack of data about the incidence of pospiviroids in globally traded produce. There is no information available on the incidence of these pathogens in traded seed potatoes or plants for planting from countries outwith the EU, due to the inconsistent approach to surveillance for the presence of pospiviroids on a global basis. Even within the EU this information tends to focus upon the incidence of PSTVd (EU emergency measures), while other pospiviroids are generally not subject to systematic surveys. The information available from either outwith or within the EU on the incidence of viroids in traded seed lots is also limited. This is due to a lack of consistency in approach to testing of seed lots even between EU MS. Standard protocols for seed sampling and testing for pospiviroids



are not yet available from EPPO<sup>20</sup>, ISHI-Veg<sup>21</sup> or ISTA<sup>22</sup> and this leads to a range of methods and sample sizes being employed by countries on a unilateral basis. Some non-EU countries test seed imports for the presence of PSTVd, but little information on findings is available and these countries are not known to test for the other solanaceous pospiviroids.

There is no information on the incidence of pospiviroids in traded produce entering the PRA area from Third Countries, including tomato fruit and ware potatoes. Surveillance of produce for consumption for these pospiviroids on entry to the EU is not conducted. This lack of surveillance allied to the general lack of information on the presence, distribution and incidence of these pathogens outwith the PRA area leads to a high degree of associated uncertainty. Also, the possibility of transfer from traded food produce to susceptible hosts is associated with a high degree of uncertainty. There is no evidence of this ever having occurred or being associated directly with entry or spread of pospiviroids. However, with other mechanically transmitted viral pathogens, such as *Pepino mosaic virus*, the potential for entry via vegetable packhouses and movement of staff into the growing crop has been tentatively addressed during risk assessment (Jones, 2005). The possibility of discarded plant material resulting in a potential entry is associated with many unknown and unquantifiable factors such as prevailing environment at point of discard, proximity to susceptible species, presence of a suitable vector etc, so that this scenario can only be included in this assessment with an associated high degree of uncertainty.

# 3.3. Probability of establishment

# 3.3.1. Reports of pospiviroids in the European Union

As indicated in the Section 3.1.3.2, with the exception of MPVd and TPMVd, all other solanaceous pospiviroids have been reported from one or more EU countries. In addition, many of them have been intercepted in plant material moving within the EU.

Surveys performed by EU MS in the framework of the emergency measures have documented a high prevalence of asymptomatic PSTVd infection in ornamental solanaceous species and, in particular, in *S. jasminoides* and *Brugmansia* spp. A similar situation applies to old plantations of citrus crops infected with CEVd (Duran-Vila and Semancik, 2003). In addition, the other pospiviroids reported in Europe have been detected in ornamentals hosts but infection rates are less precisely known.

## **3.3.2.** Availability of suitable hosts in the European Union

Tomato and potato, both of which are hosts of solanaceous pospiviroids, are widely planted in all EU MS (Table 9). In the case of tomato, cultivation is mostly under protected conditions (glasshouses) in Northern European Countries but also in open field in many Central and Southern Countries. A similar situation also applies to other vegetable solanaceous species (pepper, eggplant, pepino).

**Table 9:** Area of production (x1000 hectares) of solanaceous vegetables in the EU in 2008 and 2009. EU MS are sorted in the first column alphabetically. Data extracted from Eurostat apro\_cpp\_fruveg-Fruits and vegetables (annual data) on 24/6/2011.

Eggplant Red pepp			Tomatoes		Potatoes			
GEO/TIME	2008	2009	2008	2009	2008	2009	2008	2009
European Union (27								
MSs)	21.0*	16.1*	59.3*	56.0*	265.3*	244.6*	2,043.1*	2,053.4*
Austria	0.0	0.0	0.2	0.2	0.2	0.2	22.8	22.2

<sup>&</sup>lt;sup>20</sup> EPPO (European and Mediterranean Plant Protection Organisation),

http://archives.eppo.org/EPPOStandards/diagnostics.htm

<sup>&</sup>lt;sup>21</sup> ISHI-Veg (International Seed Health Initiative for Vegetable Crops), International Seed Federation,

http://www.worldseed.org/isf/ishi\_vegetable.html

<sup>&</sup>lt;sup>22</sup> ISTA (International Seed Testing Association), http://seedtest.org/en/home.html



Belgium	0.0	0.0	0.1	0.1	0.5	0.5	:	73.7
Bulgaria	0.3	0.7	3.8	5.0	3.5	3.0	21.7	:
Cyprus	0.0	0.0	:	:	0.3	0.2	6.0	5.3
Czech Republic	:	•	:	:	0.3	0.4	29.8	28.7
Denmark	:	•	:	:	:	:	40.7	38.9
Estonia	:	•	:	:	0.0	:	8.7	9.1
Finland	:	•	0.0	0.0	0.1	0.1	26.5	26.4
France	0.4	•	0.6	:	4.1	:	156.2	163.6
Germany	:	:	0.0	0.0	0.3	0.3	259.8	263.7
Greece	2.9	•	3.9	:	25.0	:	33.5	33.5
Hungary	0.1	0.1	3.6	4.0	2.3	2.3	25.4	22.3
Ireland	:	•	:	:	:	:	12.0	12.9
Italy	10.9	9.4	11.7	12.1	115.5	117.1	70.6	70.6
Latvia	:	•	:	:	0.0	0.0	37.8	30.0
Lithuania	:	:	:	:	0.2	0.2	48.0	46.1
Luxembourg	:	:	:	:	0.0	0.0	0.6	0.6
Malta	:	:	:	:	:	:	0.7	0.7
Netherlands	0.1	0.1	1.3	1.3	1.6	1.6	151.9	155.2
Poland	0.0	0.0	1.3	1.4	10.5	11.1	529.5	488.7
Portugal	:	:	:	:	14.3	16.8	38.9	37.9
Romania	6.3	5.8	12.3	12.1	30.3	27.7	255.3	255.2
Slovakia	0.0	0.0	0.4	0.4	0.8	0.8	14.3	11.7
Slovenia	:	••	0.1	0.2	0.2	0.2	:	:
Spain	:	:	20.0	19.2	55.3	62.1	81.9	85.4
Sweden	:	:	:	:	0.0	0.0	26.9	27.0
United Kingdom	:	:	:	:	:	:	143.6	144.0

Note: (:) indicates data not available; (\*) indicates that values were calculated

Although they may be less frequently cultivated in some parts of Northern Europe, ornamental solanaceous species (*S. jasminoides*, *Brugmansia* spp., petunia, etc.) are widely cultivated and commercialised within the EU (e.g.: Flor.As., online; Florcerta, online; Vakblad voor de Bloemisterij, 2009) and likely to be present in all EU MS. The main producers of *S. jasminoides* potted plants are in Germany (5 million plants), Holland (1.5 million plants) and Italy (1.5 million plants) while production of these ornamental species in other EU MS is negligible. *S. jasminoides* is produced in highly specialised operations and few large companies dominate the market. Smaller companies exist too, which often produce more refined products from *S. jasminoides* composing hanging baskets and specialised garland flower plants. In Germany ca. 20 big companies control 70% of *S. jasminoides* potted plants and market half of the production through auction by one organization (R. Schrage, Plant Protection Service, Northrine-Westfalia, Gernany, personal communication, June 2011).

The market for *Brugmansia* is relatively small and in contrast to *S. jasminoides* not centralised. Only few specialied companies exist and, in addition, many producers of exotic and fancy cultivars exist (BGI, online a and b), who produce plants which may also be marketed directly to the end user, over the internet or through exchange.

Large volumes of petunia, calibrachoa and other solanaceous ornamentals are traded in Europe every year and similarly non-solanaceous ornamentals in particular chrysanthemum, host plants for CSVd and other pospiviroids, are widely grown throughout the EU territory. Citrus hosts of CEVd are widely planted in Mediterranean EU MS.

Several solanaceous weed species, including *Solanum nigrum* L. and *Solanum dulcamara* L., are experimental hosts of PSTVd (see Appendix B Table 17) and potential hosts of other pospiviroids.

These species have both a pan-European distribution (RBGE, online a and b). Although not experimentally demonstrated, other solanaceous weeds present in many parts of the PRA area, including *Solanum luteum* Mill. and *Lycium barbarum* L. (RBGE, online c and d), could also be potential hosts of pospiviroids.

Overall, host plants suitable for solanaceous pospiviroids are widely present in all EU MS, although to a lower extent and mostly under protected cultivation conditions in Northern Europe.

# **3.3.3.** Suitability of the environment

The biological functions of pospiviroids are strongly integrated with those of their host plants and there is no indication that their requirements in terms of environment are substantially different from those of their host plants. Although viroids are generally considered to replicate and accumulate better under high temperatures, the ability of TCDVd to survive subzero temperatures (-12 °C) was recently demonstrated (Singh and Dilworth, 2009).

Given that many host plants of solanaceous pospiviroids can be grown in all EU MS, the whole PRA area is considered as having an environment suitable for these pathogens.

# **3.3.4.** Endangered areas

Given the simultaneous presence of suitable host plants and of suitable environmental conditions in the 27 EU MS, the entire EU is considered as an endangered area.

# **3.3.5.** Cultural practices and control measures

Currently used cultural practices across the range of pospiviroids host species are not expected to significantly impede the establishment of solanaceous pospiviroids over the whole EU territory. In particular, no agrochemicals are known that have an anti-viroid preventive or curative activity.

The inability of cultural practices and control measures to significantly reduce the chance of establishment of solanaceous pospiviroids in the 27 EU MS is demonstrated by the numerous reports indicating the presence of one or more pospiviroids in many EU MS.

## 3.3.6. Other characteristics of pospiviroids affecting the probability of the establishment

In at least three types of situations, pospiviroid infections will not be accompanied by obvious symptoms. These concern:

- pospiviroid infection in solanaceous ornamental hosts (Di Serio, 2007; Verhoeven et al., 2008a, b, c, 2010b, d), which is very generally symptomless. This situation also applies to some other non-solanaceous hosts (Querci et al., 1995; Fagoaga and Duran-Vila, 1996; Semancik, 2003)
- pospiviroids or pospiviroid isolates that only induce mild symptoms in at least some of their hosts (Gross et al., 1981; Schnolzer et al., 1985; Fagoaga et al., 1995; Semancik, 2003).
- low temperatures and low light intensity limiting viroid accumulation and symptom expression (Grasmick and Slack, 1985; Handley and Horst, 1988; Semancik, 2003).

As a consequence, pospiviroid infection is likely to go undetected following visual inspection in a number of situations and the corresponding infected plants are unlikely to be removed and destroyed.

# **3.3.7.** Conclusion on the probability of establishment

Given the above elements, the probability of establishment of solanaceous pospiviroids upon entry in the 27 EU MS is considered to be very high. This evaluation is not associated with any significant level of uncertainty.



# 3.4. Probability of spread after establishment

The probability of spread after establishment needs to be considered on three different levels: short distance spread within a crop, short distance spread between crops, and long distance spread. The following Sections will analyse in more detail how pospiviroids may spread by natural means and by human assistance within a crop, between crops and on long distances.

# 3.4.1. Spread by natural means

#### 3.4.1.1. Insect vectors

Initial experiments to examine whether or not PSTVd was insect transmissible within a crop yielded variable results; for example, reports of transmission in potato by the aphids *Myzus persicae* Sulzer and *Macrosiphum euphorbiae* Thomas (Kennedy et al., 1962; Smith, 1972) were not confirmed in later studies with six insect pests of potato including *M. persicae* (Schuman et al., 1980). The efficiency of the PSTVd transmission rate by aphids was very low in tomato (De Bokx and Piron, 1981) and, similarly, TASVd was transmissible from tomato to tomato by some aphid species, albeit at a very low rate (Walter et al., 1980; Walter, 1987). More recent experiments to verify the transmissibility of PSTVd by *M. persicae* in the absence of co-infection with a Polerovirus failed (S. Winter, Leibniz-Institute DSMZ, Germany, personal communication, June 2011).

On the other hand, *M. persicae* has been reported to transmit TPMVd (apparently without an assisting virus) between crops, specifically to tomato after viroid acquisition from wild plants like *Physalis affinis foetens* and *Solanum rostratum* (Galindo, 1989). Transmission rate was reported at 97% after an acquisition period of 24 h. Overall the transmission of pospiviroids by aphids in the absence of an assisting virus is therefore a distinct possibility, despite the conflicting and often negative results reported in the literature for PSTVd under these conditions. Similarly, transmission efficiency, if any, remains to be evaluated for the other pospiviroids.

Co-infection of *Potato leafroll virus* (PLRV, genus *Polerovirus*) and PSTVd in potato provided the first hint that the former might facilitate aphid transmission of the latter (Salazar et al., 1995). *M. persicae* could persistently transmit PSTVd only when the aphids acquired the viroid from potato plants co-infected with PSTVd and PLRV (Querci et al., 1997; Singh and Kurz, 1997). Similar observations were made in experiments with other hosts of PSTVd and PLRV, for instance, for transmission from *P. floridana* to tomato (Syller et al., 1997). PLRV only acted as viroid carrier because experiments using a potato cultivar resistant to PLRV showed that following inoculation with viruliferous aphids only PSTVd was transmitted (Syller and Marczewski, 2001). Other virus-viroid combinations might contribute to natural transmission of viroids (Francki et al., 1986) and, therefore, the possibility that similar situations can exist for other pospiviroids should be considered. Due to the absence of experimental data, this assessment however is accompanied by a high uncertainty.

Bumblebees (*Bombus ignitus* Smith) have been shown to transmit two different pospiviroids, TASVd (Antignus et al., 2007) and TCDVd (Matsuura et al., 2010b) within the same crop (tomato) under greenhouse conditions, but the exact transmission mode remains unknown. In TASVd, pollination by bumblebees has been associated with viroid spread within a greenhouse in which transmission by human activity was excluded (Antignus et al., 2007). Given the apparently non-specific viroid-bumblebee interaction involved, it is possible that other pospiviroids might be similarly transmitted, although this assessment is associated with high uncertainty due to lack of studies.

In summary, PSTVd is aphid-transmitted in the presence of at least one co-infecting virus (PLRV) in potato, and TCDVd and TASVd are transmitted by bumblebees in tomato. In addition, TPMVd could be aphid-transmited from wild species to tomato and TASVd from tomato to tomato. Therefore, transmission of pospiviroids by aphids or bumblebees, within and between crops, has an unlikely to moderately likely probability rating. High uncertainties on this assessment derive from the limited number of virus-viroid-host-vector combinations for which experimental data are available.



# 3.4.1.2. Seed and pollen

Transmission by seed and pollen enables pospiviroid spread exclusively within a plant species. Transmission of PSTVd through seed and/or pollen has been reported in potato (Hunter et al., 1969; Singh, 1970a; Singh et al., 1992a), tomato (Benson and Singh, 1964; Singh, 1970a; Kryczynski et al., 1988) and *Scopolia sinensis*, a wild solanaceous plant (Singh and Finnie, 1973). Seed transmission of PSTVd has not been correlated to specific potato varieties, although reports from different potato collections show large variations in rates of transmission (Fernow et al., 1970; Singh et al., 1993).

Data about other pospiviroids are conflicting: while seed transmission of CSVd has been reported in chrysanthemum (Monsion et al., 1973) and tomato (Kryczynski et al., 1988), no seed transmission of this viroid in chrysanthemum was observed in a previous study (Hollings and Stone, 1973). However, a more recent evaluation has confirmed that CSVd is transmitted in chrysanthemum not only by seed but also by pollen (Chung and Pak, 2008). More specifically, CSVd remained undetected in the non-infected female parent pollinated with infected pollen, but it was transmitted to the progeny (Chung and Pak, 2008). In contrast, systemic CSVd infection was observed in tomato plants pollinated with pollen from diseased plants (Kryczynski et al., 1988).

TCDVd was originally considered non-seed-transmissible in tomato (Singh et al., 1999), but more recently seed transmission has been experimentally verified in this host (Singh and Dilworth, 2009). Furthermore, a recent TCDVd outbreak in tomato in France was likely associated with seed transmission, since testing of 250 batches of 10 seedlings from the original seed lot resulted in two TCDVd-positive pools (Candresse et al., 2010). However, seed contamination, readily detected using sentitive RT-PCR techniques, does not necessarily result in seed transmission Using real-time RT-PCR, Koenraadt et al. (2009) detected TCDVd in all the seeds from TCDVd-infected tomato plants, while TCDVd was not detected by Boonham et al. (2004) when analyising 4000 seedlings batches from 25 infected tomato plants.

In the last few years, seed transmission of other pospiviroids has also been experimentally demonstrated: TASVd in tomato, with a transmission rate up to 80% (Antignus et al., 2007), PCFVd in pepper, with a transmission rate of 19% (Verhoeven et al., 2009a), and CEVd in *Verbena* sp. and *Impatiens* sp. (Singh et al., 2009), suggesting that seed transmission of pospiviroids may be more frequent than would be suggested by former literature.

Results obtained with PSTVd (EUPHRESCO, 2011), TASVd (Antignus et al., 2007) and TCDVd (Singh and Dilworth, 2009) indicate that the viroid accumulates in internal tomato seed tissues and that it most likely remains unaffected (or only partially affected) by conventional disinfection techniques used in the industry to limit spread of seed-transmitted virus diseases (Herrera-Vasquez et al., 2009). These techniques are therefore unable to prevent the spread of pospiviroids through contaminated seeds.

It is generally accepted that seed and pollen transmission favored PSTVd spread among potato germplasm collections all over the word. Moreover, transmission through true (botanical) seeds may contribute to the spreading of pospiviroids within crops, like pepper and tomato, that are seed-propagated.

In summary, PSTVd is seed- and pollen-trasmitted in potato and tomato, TCDVd and TASVd are seed-transmissible in tomato, CSVd in chrysanthemum and tomato, PCFVd in pepper, and CEVd in *Verbena* sp. and *Impatiens* sp. In tomato, seed disinfection techniques are not effective against PSTVd, TASVd and TCDVd. The potential for pospiviroids spread through contaminated seeds and pollen of their hosts should therefore be considered as very high for those host-viroid combinations for which experimental evidence is available (with an associated low uncertainty). For those combinations for which experimental evidence is lacking, a high probability rating is suggested by analogy, but this rating is associated with a high uncertainty.

## **3.4.2.** Spread by human assistance

Most viroids can be transmitted from plant to plant by human activities, either mechanically (providing short-distance spread) or through the production of infected propagation material (also



facilitating long-distance dissemination) (Diener, 1987). While vegetative propagation accounts for pospiviroid spread exclusively within the same species, mechanical transmission may enable transmission within the same species and between different species.

Viroid infection in mother plants results in high rate of infection in their vegetatively propagated progeny plants (Owens and Verhoeven, 2009). Consequently, vegetatively propagated plants are permanent sources of infections for other lots and crops. This infected propagation material can be easily be moved over long distances through trade, thus facilitating the dissemination of pospiviroids. In particular, there is considerable production and trade of the ornamental host species (see Section 3.3.2), providing numerous opportunities for long distance dispersal of pospiviroids. Therefore, vegetative propagation is considered as the main cause of PSTVd spread in potato and of PSTVd and other pospiviroids spread in ornamentals like *Brugmansia* spp., *S. jasminoides* and *L. rantonetti* (Singh et al., 1993; Di Serio, 2007; Owens et al., 2009; Verhoeven et al., 2010b).

Early reports on potato spindle tuber disease already highlighted the efficient mechanical transmission of the causal agent (then unknown) by rubbing together freshly-cut surfaces of healthy and diseased tubers (Bonde and Merriam, 1951), by foliage contacts (Merriam and Bonde, 1954) and by contaminated machinery (Merrian and Bonde, 1954; Manzer and Merriam, 1961). Evidence of mechanical transmission of pospiviroids by crop handling is further supported by the observation that in potato and tomato PSTVd generally spreads along rows in the fields and greenhouses (Verhoeven et al., 2004; Owens and Verhoeven, 2009) and by similar observations with other pospiviroids, including TCDVd, CLVd and CEVd, in tomato (Verhoeven et al., 2004; Matsushita et al., 2008). The possibility that pospiviroid could spread along rows via soil or hydroponic systems has been experimentally excluded because transmission was not observed after adding PSTVd into pots containing healthy tomato plants (Seigner et al., 2008), or after repeatedly adding PSTVd to the rooting medium of tomato plants (Verhoeven et al., 2010c). These results provide further support to the role of crop handling in viroid spreading along the rows of cultivated tomato.

Mechanical transmission may play a major role in pospiviroid transmission both within a given species and between plants of different host species. Fingerprint inoculation (smoothly rubbing non-carborundum dusted leaves with fingertips that had been contaminated by rubbing young leaves of infected source plants) was very successful for transmitting PSTVd from ornamentals to tomato (Seigner et al., 2008). Further studies showed that contaminated fingerprints were able to transmit PSTVd to tomato up to 2 h after acquiring the viroid by rubbing young leaves of PSTVd-infected *S. jasminoides* and *Brugmansia suaveolens*, indicating that PSTVd infectivity may persist for hours outside plants (Verhoeven et al., 2010c). Under the same conditions, fingertip transmission was shown to be much less efficient from ornamentals to potato than to tomato (Verhoeven et al., 2010c) and similar observations were also obtained when using carborundum-mediated inoculation, suggesting that potato is in general less readily infected than tomato. In addition it has been shown that residues of dried tomato sap from infected fruit remain infective for over 8 weeks (Mumford et al, 2004).

Moreover, as previously shown for tomato (Grasmick and Slack, 1985), the transmission efficiency is largely influenced by temperature: while 25 °C favoured PSTVd transmission to both tomato and potato, 15 °C strongly limited PSTVd transmission to both crops (Verhoeven et al., 2010c). Therefore, a temperature of 15°C substantially reduces the chance of PSTVd successful mechanical transmission.

PSTVd was also very efficiently transmitted to tomato by PSTVd-contaminated razor blades that had been contaminated with the viroid by cutting 8 to 10 times leaves and stems of infected *S. jasminoides* or *B. suaveolens* (Verhoeven et al., 2010c). However, in parallel experiments, PSTVd was very poorly transmitted by razor blades within each ornamental species, indicating that the efficiency of this route largely depends on the recipient host (Verhoeven et al., 2010c). The inoculum source may also influence the transmission efficiency. Indeed, PSTVd from *S. jaminoides* was more efficiently transmitted to tomato and potato by diluted-sap and fingertips, and to tomato by razor-blade, than PSTVd from *B. suaveolens*; this difference has been correlated to the host plant rather than to the PSTVd genotype (Verhoeven et al., 2010c).

The finding that PSTVd is transmitted successfully from ornamentals to tomato by contaminated fingertips and razor blades supports the notion that ornamentals could be the origin of natural viroid



infections in tomato reported in the last few years (Navarro et al., 2009; Verhoeven 2010; Verhoeven et al., 2010c).

Tools contaminated by pospiviroids may remain infective for a long time, as shown for CEVd, which is readily transmitted in citrus by contaminated budding tools (Garnsey and Jones, 1967), with the viroid surviving at least 8 days on knife blades (Allen, 1968) and 4 months on contaminated razor blades (Roistacher and Calavan, 1974). Spread of CSVd in chrysanthemum by foliage contact, handling during cultivation and by cutting knives has been also reported (Brierley and Smith, 1941; Keller, 1953).

Chemical disinfection of cutting tools has been shown to reduce the potential for pospiviroids spread. Matsuura et al (2010a) showed that TCDVd-contaminated scalpels were disinfected after dipping the scalpels for 15 seconds in either 3% sodium hypochlorite (NaOCl), or 20% household bleach, lower concentrations not being tested. Singh et al. (1989) even showed 3% of sodium hypochlorite to be effective against PSTVd at the same period of incubation. Also Garnsey and Jones (1967) demonstrated similar effect for CEVd. In addition, Timmermann et al (2001) showed the effectiveness of disinfection by application of a commercial agricultural disinfectant based on benzoic acid.

Since foliage contact has been reported as a possible transmission pathway of several pospiviroids (Brierley and Smith, 1941; Merriam and Bonde, 1954), it cannot be excluded that mechanical transmission without human assistance could partially account for pospiviroid spread in the field within the same species and, possibly, between different species grown in close proximity. However, the relevance of this dissemination pathway seems minor, and the transmission efficiency between different botanical species under field conditions is presumed to be low.

In summary, human activity can contribute to pospiviroid spread. Uncertainties regarding pospiviroid transmission through vegetative propagation are negligible, while those related to mechanical transmission are higher due to variation in data. The importance of mechanical transmission mediated by human activities under field and greenhouse conditions is well established for PSTVd infecting potato and tomato, as well as for several other viroids, including TASVd, CLVd, and TCDVd, in tomato, with a low level of uncertainty. The potential for mechanical transmission of PSTVd from ornamentals to tomato and potato has been experimentally demonstrated, although with a much higher efficiency in tomato. By analogy, similar conclusions can be extended to the other pospiviroids but with medium uncertainty in the absence of specific studies.

# 3.4.3. Recent circumstantial evidence of pospiviroid spread between crops

Tomato and other vegetable and flower crops are increasingly grown in greenhouses and ornamental and vegetable species are sometimes grown in the same compartment. This would increase the potential for transfer of pospiviroids from ornamentals to tomato however there are no precise data that would allow evaluation of the prevalence of such a practice. Despite the quarantine status of PSTVd, several outbreaks in tomato of this and other pospiviroids have been reported in several European countries since 1988. Intriguingly, one of the pospiviroids detected in symptomatic tomato plants was CLVd (Verhoeven et al., 2004; Parrella et al., 2010), previously only found in three asymptomatic ornamental species (Hammond et al., 1989; Singh et al., 1992b; Spieker, 1996b).

PSTVd infections in *S. jasminoides* or *Brugmansia* spp. have been reported in the Netherlands (Verhoeven et al., 2008a; Verhoeven et al., 2010d). Subsequently PSTVd was found in those and in further non-symptomatic ornamentals in several EU MS (Di Serio, 2007; Verhoeven et al., 2008b). In Italy, PSTVd-infected tomatoes were found in close proximity to *S. jasminoides* infected by the same viroid (Navarro et al., 2009). Similarly, the first TCDVd outbreak in Canada was observed in tomatoes grown together with ornamentals in a greenhouse (R.P. Singh, Fredericton NB, Canada, personal communication, March 2011). Evidence from molecular and biological assays supported the idea that PSTVd had been transferred to tomato from the nearby *S. jasminoides*. Together with phylogenetic analyses showing that similar PSTVd genotypes are found in ornamentals and in tomato outbreaks (Navarro et al., 2009; Verhoeven et al., 2010b), these data point to ornamental solanaceous hosts as the source of past PSTVd infections of tomato. There is no evidence for a similar transmission route of PSTVd from ornamentals to potato since PSTVd genotypes from potato form a phylogenetic group



separated from the ornamentals and tomato genotypes (Verhoeven et al., 2010b). The situation is more complex regarding PSTVd transmission within ornamentals: while sequence variants from *Brugmansia* spp., *P. peruviana* and *S. jasminoides* form different phylogenetic groups (Verhoeven et al., 2010b), no specific nucleotide polymorphisms discriminate variants from *S. jasminoides*, *L. rantonetti* and *Streptosolen jamesonii* (Navarro et al., 2009), suggesting a potential transmission between these three species. Similarly, a PSTVd infection in a single plant of *Datura* sp. was assumed to have originated from infected plants of *B. suaveolens* (Verhoeven et al., 2010b). In addition, the close proximity of PSTVd genotypes from New Zealand isolated from pepper, tomato and *P. peruviana* suggest their circulation between these hosts (Lebas et al., 2005; Ward et al., 2010).

It remains unknown whether infections of tomato by other pospiviroids, including TASVd (Antignus et al., 2007), CEVd, CLVd, and TCDVd (Verhoeven et al., 2004) could have originated from other symptomless ornamentals. TCDVd has been found in *Verbena* spp. (Bostan et al., 2004; Singh et al., 2006), *V. minor* (Singh et al., 2009) and *Petunia* spp. (Verhoeven et al. 2007a); CEVd in *Verbena* spp., *Impatiens* spp., *Cestrum* sp., and *L. rantonetti* (Singh et al., 2006; Verhoeven et al., 2008a; Luigi et al., 2011); CEVd and TASVd in *S. jasminoides* (Verhoeven et al., 2008c; Torchetti et al., 2011; Luigi et al., 2011); TASVd in *S. pseudocapsicum* and *Cestrum* sp. (Spieker et al., 1996; Verhoeven et al., 2008b). However, phylogenetic data and transmission experiments support the idea that TCDVd and TASVd have been transfered from ornamentals to tomato (Verhoeven, 2010; Verhoeven and Roenhorst, 2010). In summary, there is evidence that in the last few years PSTVd has been transferred, through unknown mechanism(s), several times from infected ornamentals to tomato, and perhaps to other ornamentals. This might also be the case for CEVd, CLVd, TASVd and TCDVd infecting tomato, as well as for other viroid-host combinations, although alternative scenarios cannot be excluded.

Apart from PSTVd, no other pospiviroid infections have been reported in potato and hence there is no evidence for an existing pathway for spread of pospiviroids from other crops to potato.

## 3.4.4. Conclusion on probability of spread

Within a crop species on a short distance, all four mechanisms (vegetative propagation, mechanical transmission, seed and pollen transmission, insect transmission) contribute to pospiviroid spread. Although there are uncertainties as to whether all mechanisms apply to all pospiviroid/host combinations, at least several of these mechanisms are likely to be effective in any situation so that overall the probability of spread is evaluated as likely to very likely, with low uncertainty.

Between crop species on a short distance, the two mechanisms potentially involved in spread of pospiviroids are mechanical transmission and insect transmission. There is experimental evidence that PSTVd can be spread between crops by mechanical transmission but that potato is a less receptive host than tomato. In parallel, circumstantial evidence and phylogenetic studies show that while PSTVd has likely been transferred from ornamentals to tomato and pepper, no such transfer has occurred to potato. Consequently, different ratings are reached for the probability of spread from symptomless ornamentals to various other hosts:

- Moderately likely with high uncertainty for tomato, based on the evidence for such transfers and on the high receptivity of tomato
- From very unlikely to unlikely for potato, based on its lower receptivity, on the absence of evidence of PSTVd transfer, and on agricultural practices (ornamentals and potato are rarely grown in close proximity). This rating has high uncertainty
- Moderately likely for other vegetables with high uncertainty

There is no evidence that transfer from tomato, other vegetables or potato is more efficient than transfer from ornamentals so that similar ratings are proposed from spread from theses hosts to other crops, although lack of experimental evidence causes a high uncertainty level.

Within a crop species over long distances spread can occur through seed transmission. For ornamentals and potato, vegetative propagation can also play a major role in long distance spread. On

the contrary, the contribution of mechanical transmission and of insect-assisted transmission on long distance spread is expected to be low, even on a limited local scale. Pospiviroids are not known to give rise to large-scale outbreaks in non-vegetatively propagated crops. This is illustrated by the observation that outbreaks of pospiviroids in tomato or pepper crops in the EU have always remained localized to individual plots or glasshouses, or to a restricted group of nearby plots/glasshouses (Mumford et al., 2004; Navarro et al., 2009; Verhoeven, 2004; 2007b and 2009a; Candresse et al., 2010; Nixon et al., 2010), and never reported spreading outside of a limited perimeter.

The probability of spread over long distances is therefore evaluated as likely to very likely for vegetatively propagated species and as moderately likely for non-vegetatively propagated ones.

# 3.4.5. Uncertainties

Uncertainties for the probability of insect, seed and pollen transmission of pospiviroids in commercially grown crops are high, mostly because of the low number of specific cases studied. Uncertainties for the probalility of pospiviroid spread by mechanical transmission within tomato and to a lower extent within potato are low, but pospiviroid spread by this pathway in and between different crops is highly uncertain due to lack of specific data. In addition, the lack of precise data on how frequently solanaceous ornamentals are grown in close proximity to other susceptible crops further adds to uncertainties.

Uncertainties resulting from vegetative propagation and long distance spread are low considering the high number of interceptions (Appendix B, Tables 18 and 19).

## 3.5. Assessment of potential consequences

# **3.5.1.** Direct pest effects

Direct pest effects mainly concern potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*) and pepper (*Capsicum annuum*) and are due to yield and quality loss and to the need to implement prophylactic measures to limit the spread of pospiviroids, as no control options are available. As explained in the general introduction, solanaceous pospiviroids in non-solanaceous hosts (e.g. citrus, chrysanthemum) will not be considered here.

## 3.5.1.1. Potato

In potato, only infections by PSTVd have been recorded in nature. Symptoms were first described by Martin (1922). The shoots of infected plants showed upright growth, reduced branching and were smaller than normal. Leaves were also smaller and more pointed than those of healthy plants. Infected tubers were elongated, had more eves, which sometimes were borne on 'knob-like protuberances'. A year later Folsom (1923) added that: i) symptoms vary per cultivar, ii) the disease may be associated with marginal leaf roll, iii) the colour of the skin of the tubers may be affected, and iv) tubers may show growth cracks. Furthermore, he reported that infection late in the season may not affect the shape of the tubers while the viroid can still be transmitted by these asymptomatic tubers. The latter observation was also reported in Belarus (Blotskaya and Berlinchik, 1999). In 1924, Martin added that symptomatic tubers also showed more eyes than uninfected tubers. In 1925, Gilbert described symptoms for different cultivars i.e., retarded growth, large, coarse stems, or a single stem per plant with somewhat conspicuous axillary shoots, late blossoming, and unusually large tubers which was named 'giant hill'. Similarly, MacLeod (1927) reported more pronounced stem symptoms in Irish Cobbler than Oreen Mountain potatoes. In addition to the mentioned tuber symptoms in the USA, Balashev (1941) reported for Uzbekistan that in cultivars with red or pink tubers the skin colour is paler in tubers originating from diseased plants. Moreover, he reported that the percentage of cracked tubers in five cultivars tested ranged from 14 to 28.2% in healthy against 8.8 to 100% in diseased plants. Hunter and Rich (1964) noticed that sprouts from infected tubers developed more slowly than those from healthy tubers. Murphy et al., (1966) found that fry colour was paler for spindle tuber infected tubers. In addition, they found that the density of tubers from infected plants of cv. Katahdin



was significantly higher than that of tubers from healthy plants, but no differences were found among samples from infected plants of cv. Kennebec.

In greenhouse experiments, Goss (1930) observed that the tuber symptoms became more severe at high soil moisture content or temperature. However, stem symptoms were obscured by these conditions. MacLachlan (1960) found that for various potato cultivars under greenhouse conditions not only stem symptoms were masked but also tuber symptoms.

Disease severity, including both symptomatology and yield reduction, has been reported to vary from mild to severe depending on the strain of PSTVd and on the potato cultivar (Singh et al., 1971; Pfannenstiel and Slack, 1980; Kowalska-Noordam et al., 1987; Nakahara et al., 1997). The identity of the pathogen(s) causing spindle tuber symptoms was equivocal for a long time and as a consequence, there may be some uncertainties concerning symptomatology, especially in old publications.

Furthermore, the frequent association of the viroid with common potato viruses constitutes a significant impediment to the evaluation of precise PSTVd yield losses (Folsom, 1923). It should also be considered that the reported losses were recorded under conditions not directly comparable to current agricultural conditions in the EU. Nevertheless, it can be concluded from the available data that there is clear evidence that the impact of PSTVd in potato can be quite significant, resulting in yield losses in the 10-74% range depending on the particular situation considered.

Country, state	% yield loss	Reference
Canada, New Brunswick	16-64	Singh et al., 1971
China, Heilongjiang	20-30	Cui et al., 1992
Soviet Union	20-35	Leontyeva, 1963
UK	56	Cammack and Richardson, 1963
USA, Maine	10-37	Folsom and Schultz, 1924; Bonde et al., 1943; Murphy et al., 1966
USA, New Hamsphire	65	Hunter and Rich, 1964
USA, New Jersey	46-61	Martin, 1924, 1928
USA, Mississippi	55	Wedgworth, 1928
USA, Oregon	73	McKay and Dykstra, 1932
USA, Florida	74	Burger, 1927
Uzbekistan	24-51	Balashev, 1941

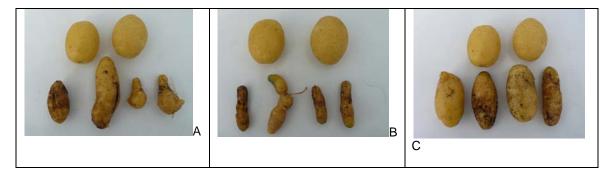
**Table 10:** Reported potato tuber yield reduction due to potato spindle tuber viroid.

Yield losses in a field are related to the number of infected plants. MacLeod (1927) found under controlled conditions that inoculation with the viroid reduced the yield in potato cv. Irish Cobbler and cv. Oreen Mountain by 14 and 9%, respectively (primary infection). The yield of plants grown from infected progeny-tubers was reduced by 36 and 29%, respectively (secondary infection). Leclerg et al. (1944 and 1946) reported a general tendency for potato yields to decrease progressively as the incidence of spindle tuber in the crop increased. A 100% infection rate resulted in yield losses varying from 5-78%, whereas the reduction in yield in the presence of 4 or 8% spindle tuber amounted on the average to 2.6 and 3.7% yield loss, respectively. Balashev (1941) noticed an increase of diseased plants up to 88.3% in four years when tubers were reused as seeds for the next crop. Singh et al. (1971) calculated a loss of 2.6%, considering that PSTVd incidence was 3.8% in the major Canadian



potato cultivars. They estimated that losses caused by the severe strain were approximately three times as high as from the mild strain and that the prevalence of the mild strain was 11 times that of the severe strain. For North America, where efforts have been deployed to curtail PSTVd prevalence, total yield losses due to PSTVd in potato for the period 1922 to 2009 have been calculated to be approximately 1% (Owens and Verhoeven, 2009).

Although no natural infection of potato by other pospiviroids has been reported, experimental evidence suggests that pospiviroids other than PSTVd have the potential to similarly cause significant yield losses in potato. Verhoeven et al. (2004) inoculated potato cv. Nicola with two isolates from PSTVd, two of CLVd and one of CEVd in a greenhouse, and planted ten infected progeny tubers in the field for three successive years. Average yield loss over three years varied for PSTVd between 39 and 79%, for CLVd between 72 and 82% and was 45% for the single isolate of CEVd analyzed. Morever, it was shown by Verhoeven et al. (2009a) that the newly described PCFVd infected the same potato cultivar after mechanical transmission in the greenhouse at 25 °C, and caused similar symptoms as described for CEVd, CLVd and PSTVd (Verhoeven et al., 2004). Under the same experimental greenhouse conditions MPVd, TASVd, TCDVd and TPMVd were also transmitted to potato cv. Nicola. No obvious leaf symptoms were produced but tubers showed PSTVd-like symptoms of varying severity (Figure 1 a, b, c). Under the same conditions, CSVd was occasionally transmitted to potato but tuber symptoms were unclear (J.Th.J. Verhoeven, Plant protection Service of The Netherlands, personal communication, March 2011).



**Figure 1:** Symptoms of MPVd (A), TPMVd (B) and TASVd (C) on potato cv. Nicola; healthy tubers in the top row and infected tubers in the bottom row. © Plant Protection Service, Wageningen, The Netherlands.

Overall, there is clear evidence that PSTVd causes variable but significant symptoms in potato, particularly tuber deformations and yield reduction ranging from 10-74%. Although never reported in natural infections of potato, all other pospiviroids have a similar potential for detrimental effects in this crop. The impact is therefore generally expected to be major and the uncertainty level associated with these evaluations low for PSTVd but medium for the other pospiviroids.

## 3.5.1.2. Tomato

Natural infections by seven pospiviroids have been recorded in tomato (Table 1). In addition, Benson et al. (1965) concluded that the agent causing the tomato bunchy top disease, which previously was described in South Africa (McClean, 1931), had similar biological characteristics to PSTVd and therefore assumed it to be the same pathogen. In addition to the reported natural infections, CSVd (Verhoeven et al., 1998; Mehle et al., 2010) and PCFVd (Verhoeven et al., 2009a) can be transmitted to tomato experimentally.

All pospiviroids incite similar symptoms in tomato, independent of the viroid species. Symptom severity may vary both within and between species but also with the tomato cultivar (Pallás and Flores, 1989; Matousek et al., 2007a; Singh et al. 2010). Symptoms are most conspicuous when plants



become infected at early stages of development and when grown at high temperatures and light intensity. The first symptoms of pospiviroid infection in tomato are growth reduction and chlorosis in the upper leaves. Subsequently, this growth reduction may develop into stunting and bunchy growth, and the chlorosis may become more severe, turning into reddening, purpling and/or necrosis. At this stage, leaves may become deformed and brittle. As stunting begins, flower and fruit initiation stops. Generally, this stunting is permanent; occasionally, plants may either die or partially recover (Figure 2A) (Galindo et al., 1982; EPPO/OEPP, 1999; Singh et al., 1999; Verhoeven et al., 2004). Usually, symptoms are observed along rows in the fields and greenhouses, indicating that the viroid spreads mechanically during crop handling (Figure 2B) (Verhoeven et al., 2004; Matsushita et al., 2008; Ling et al., 2009). For TASVd, Verhoeven et al. (2006a) also reported a delay in the ripening of the fruit and a reduction in their storage life from 3 weeks to 1 week.



**Figure 2:** TCDVd: Initial and advanched symptoms (A) and symptoms spreading along a row (B) © Plant Protection Service, Wageningen, The Netherlands.

As fruit production generally stops on infected plants, yield loss is strongly dependent on the age at which plants become infected. Early infection, before fruit setting, will result in close to 100% loss, while losses associated with later infections are more variable, since fruits initiated before the onset of foliar symptoms may still develop to a marketable size (J.Th.J. Verhoeven, Plant protection Service of The Netherlands, personal communication, March 2011).

Very variable infection rates have been observed in pospiviroids outbreaks in glasshouses, inducing in turn very variable yield losses when assessing them at the glasshouse level. Verhoeven et al. (2004) reported infections rates below 2% – even below 1% in most cases – but also two cases of rates of 30 and > 90% respectively. Also in other cases the infections rates often were either low i.e. < 5% (e.g. Ling et al., 2009; Verhoeven et al., 2007b) or high i.e. > 20% (Candresse et al., 2010; Verhoeven et al., 2006a). In several cases, high infection rates are due to a failure to recognize the disease early and/or to take adequate measures (Verhoeven et al., 2004, 2007b).

Overall there is ample evidence that significant yield losses may result from pospiviroid infections in tomato and the impact is therefore expected to be major, with low uncertainty.



# 3.5.1.3. Pepper

In pepper natural infections have been recorded for only two pospiviroids i.e., PCFVd and PSTVd. PCFVd was recorded in single pepper greenhouses in the Netherlands and Canada, respectively (Verhoeven et al., 2009a, 2011a). In both outbreaks the infections spread along the rows. Fruit size of the infected plants was reduced up to 50% (Figure 3), plant growth was slightly reduced and leaves slightly pale. The portion of infected plants in the two identified PCFVd outbreaks was below 3%, so that total losses per greenhouse were still limited.



**Figure 3:** A healthy fruit of pepper cv. Lamborgini (left) and two small fruits naturally infected by PCFVd (right). © Plant Protection Service, Wageningen, The Netherlands.

PSTVd has been recorded in three pepper crops in New Zealand (Lebas et al., 2005). The infected pepper plants displayed only very mild symptoms i.e., a certain "waviness" or distortion of the leaf margins near the top of the plants. However, after artificial, mechanical inoculation of PSTVd to pepper cv. Yolo Wonder, fruit size was significantly reduced (Verhoeven et al., 2009a).

There are no data about the potential damage that other pospiviroids could cause in pepper.

PSTVd and PCFVd have therefore the potential to respectively cause minor (PSTVd) to significant (PCFVd) damage in pepper and the impact is therefore expected to be minor to major, with low to medium uncertainty. The other pospiviroids might have a significant potential but such an assessment would be associated with a very high uncertainty.

#### 3.5.1.4. Other fruit and vegetable crops

Infections by PSTVd have been reported from pepino (*Solanum muricatum*) (Puchta et al., 1990; Shamloul et al., 1997), avocado (*Persea americanum*) (Querci et al., 1995) and Cape gooseberry (*P. peruviana*) (Verhoeven et al., 2009b; Ward et al., 2010). None of the infected plant showed symptoms, except for plants of Cape gooseberry raised from seeds in New Zealand (Ward et al., 2010). However, seedlings and cuttings of this crop infected by a closely related PSTVd genotype did not exhibit symptoms (Verhoeven et al., 2009b). This apparent contradiction might have been caused by the different environmental conditions or genotypes of *P. peruviana* used. Generally, however, direct losses caused by pospiviroids in these hosts should be considered minimal. Natural symptomless CEVd infections have been reported in other vegetable crops, i.e., carrot (*Daucus carota*), aubergine (*Solanum melongena*) and turnip (*Brassica napus*) (Fagoaga and Duran-Vila, 1996). In addition to the natural infections by pospiviroids, PCFVd causes symptomless infections after mechanical inoculation in aubergine cv. Black Beauty (Verhoeven et al., 2009a).

In summary, the impact of pospiviroids in vegetables other than potato, tomato and pepper is therefore expected to be minimal to minor, with medium uncertainty.

## 3.5.1.5. Ornamentals

Recently, many new host plants, particularly ornamental plant species, naturally infected by pospiviroid have been discovered (see Appendix B Table 16). However, all these infections are asymptomatic (Nie et al., 2005; Di Serio, 2007; Verhoeven et al., 2008a, 2010d). In addition to these natural infections by pospiviroids, PCFVd was also shown to cause symptomless infections after experimental mechanical inoculation in *B. suaveolens* cv. Geel, *Lycianthes rantonnetii* and *S. jasminoides* (Verhoeven et al., 2009a).

As a conclusion, the impact of pospiviroids in ornamental solanaceous species is expected to be minimal, with low uncertainty.

## **3.5.2.** Indirect pest effects

Pospiviroids do not cause relevant indirect environmental or social effects. However, a number of non-EU Countries have regulations to prevent the import and spread of PSTVd (see Section 3.1.3.1). Outbreaks of PSTVd in the seed potato production of EU MS are therefore expected to result in loss of export trade, with significant impact on the concerned seed potato industries. A similar situation may also apply to other crops, but the impact is expected to be less severe due the more limited trade in these other commodities.

In addition, pospiviroid infected lots of propagation material (tubers, cuttings, micro plants and seeds) of all solanaceous crops are sources of infection for new cultivations of these crops. As a consequence, these lots may loose their value as high quality propagation material. This holds especially true for crops suffering direct pest effects such as potato and tomato.

# **3.5.3.** Conclusion of the assessment of consequences (with specifications for the endangered area if different from the risk assessment area)

Direct pest effects are expected to be markedly different for the various host plant species. The impact of solanaceous pospiviroids is expected to be major on potato and tomato, and moderate on pepper. The uncertainty associated to these evaluations is low in the case of potato and tomato but medium (PSTVd and PCFVd) to high (other pospiviroids) in the case of pepper.

The impact on other vegetables (*sensu lato*) is expected to be minimal to minor and that on ornamental species minimal. The associated uncertainties are medium and low, respectively.

Indirect pest effects are expected to be minimal, with the exception of the impact on industries producing and commercializing plant propagation materials (seed potato tubers, true botanical seeds, plants for planting) that might encounter trade restrictions due to phytosanitary regulations of receiving countries and reduced interest by clients.

## 3.5.4. Uncertainties

Uncertainties for the potential direct impact of pospiviroids on potato, tomato and ornamentals are low to medium, whereas the uncertainties for other vegetables are medium to high. Uncertainties for the potential indirect impact of pospiviroids concerning environmental or social effects are low, whereas uncertainty for the indirect impact on industry is medium.

## **3.6.** Conclusion on risk assessment (including uncertainties)

## 3.6.1. Entry

Four pathways have been identified, three of which implicate propagation material [True (botanical) seeds, Seed (potato) tubers and Plants for planting]. The fourth pathway involves plant materials not intended for planting and is considered of minor significance due to the perceived low probability of transfer to a suitable host. The uncertainties associated with this evaluation concern mostly the probability of association of the pathogens with the pathway at origin (due to the limited information



available on geographical distribution and prevalence of the pospiviroids) and with the probability of transfer to a suitable host, due to the numerous parameters involved.

For the three main pathways, probabilities of survival through transfer and storage, of survival through management procedures and of transfer to a suitable host are considered to be high or very high and there is little uncertainty associated with these ratings. The only limiting factor is therefore the probability of association with the pathway at origin, which as for the pathway involving plant materials not intended for planting, carries a medium uncertainty level.

Overall, the probability of entry of solanaceous pospiviroids in the EU territory through the effects of all identified pathways is considered as moderately likely.

# 3.6.2. Establishment

Given previous reports of pospiviroids in many EU MS, the wide availability of suitable hosts, the suitability of the EU area for these agents and the inability of cultural practices and control measures to decrease the chance of establishment, the probability of establishment of solanaceous pospiviroids upon entry in the 27 EU MS is considered to be very high (certain or close to certain). This evaluation is not associated with any significant level of uncertainty.

## 3.6.3. Spread

Within a crop species on a short distance, the probability of spread is overall evaluated as likely to very likely, with only low uncertainty.

The probability for transfer between crop species on a short distance is generally evaluated as being moderately likely, with high uncertainty. However, due to its lower receptivity and to agricultural practices that limits its contacts with other susceptible crops, the probability to potato is rated as very unlikely to unlikely, but with an associated high uncertainty.

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.

## **3.6.4.** Impact

Direct pest effects are expected to be markedly different for the various host plant species. The impact of solanaceous pospiviroids is expected to be major on potato, tomato and moderate on pepper. The uncertainty associated to these evaluations is low in the case of potato and tomato but medium (PSTVd and PCFVd) to high (other pospiviroids) in the case of pepper.

The impact on other vegetables (*sensu lato*) is expected to be minimal to minor and that on ornamental species to be minimal. The associated uncertainties are medium and low, respectively.

Indirect pest effects are expected to be minimal with low uncertainties, with the exception of the impact on industries producing and commercializing plant propagation materials (seed potato tubers, true botanical seeds, plants for planting) with medium uncertainties.

## 4. Identification of management options

#### 4.1. Identification of management options to reduce the probability of entry

The management options to reduce the probability of entry have been identified distinguishing between those that would be applied at the country of origin (pre-entry measures) and those applied at the point of entry (import control measures).



#### 4.1.1. Identification of pre-entry measures

#### 4.1.1.1. Banning the introduction of plants and plant products

Banning imports of pospiviroid host plants simultaneously prevents the entry of pospiviroids. The ban could be directed to all types of planting material and plant parts or restricted to some elements. Derogation of the import ban can be considered for specific goals e.g. breeding, by allowing plants to enter in quarantine. Material can be released from quarantine when freedom of pospiviroids has been shown adequately.

#### 4.1.1.2. Import requirements for the consignment

Guarantees concerning the consignments to be imported can be required from exporting countries to reflect that the consignment originates from a country, area, or place of production that is free of pospiviroids. Alternatively, pospiviroid freedom can be required for the consignment only. The importing country can specify the way in which the exporting country can satisfy its requirement.

#### 4.1.1.3. Quality requirements (private standards) for the consignment

Companies can require guarantees of freedom from pospiviroids in imported consignments. These guarantees can be best supported by certification systems e.g. the Council Directive 2002/56/EC. Quality requirements are also indicated as "private standards" and they are under discussion at the Sanitary and Phytosanitary (SPS) Measures Committee of the World Trade Organisation (WTO, online).

#### 4.1.1.4. Treatment of the commodity

No treatments are available to eliminate pospiviroids present in host plants. However, some measures can be applied to prevent the use of commodities for specific uses and consequently reduce the chance of establishment. For example, potato tubers can be treated by chemicals to suppress germination. This measure still allows consumption of the tubers but prevents usage for propagation and consequently establishment of pospiviroids.

#### 4.1.2. Identification of import control measures

#### 4.1.2.1. Visual inspection

Visual inspection of imported plants may detect pospiviroid-infected plants that show symptoms. Plants and tubers of potato and plants of tomato may show symptoms. Symptomatic plants could be tested and if infection status is confirmed the consigment could be refused or its use could be limited (see 4.1.2.3).

#### 4.1.2.2. Testing

Pospiviroids can be detected by adequate testing (see Section 3.1.1.). In case of a limited number of plants, leaves of all plants could be tested. In case of large number of plants, however, only random samples can be tested. If infection is found, the consigment could be refused or its use could be limited (see 4.1.2.3).

#### 4.1.2.3. Limiting the use of the imported consignment

To reduce the chance of entry and establishment of pospiviroids from countries where these viroids occurr, the use of imported consignments can be limited; e.g. ornamental plants or potato tubers may be allowed for sale to consumers but not for propagation. Additional measures may be needed to guarantee the limited use e.g. chemical treatment of tubers to suppress germination.



#### 4.1.2.4. Post-entry quarantine

Post-entry quarantine can be applied when banned commodities are imported under derogation and imply the growing of plants under quarantine conditions until they are shown to be free from pospiviroids. For example, the import of potatoes from non-EU countries is prohibited (2000/29/EC) but for specific purposes the ban can be derogated under the condition that the imported plants will be placed in quarantine and tested for quarantine organisms (2008/61/EC).

#### 4.2. Identification of management options to reduce the probability of establishment

No management options have been identified to specifically reduce the probability of establishment.

# 4.3. Identification of management options to reduce the probability of spread and potential consequences

The management options to reduce the probability of spread and its potential consequencs have been identified distinguishing among measures to be taken before cultivation, during cultivation and in case of an outbreak.

# **4.3.1.** Identification of management options to reduce the probability of spread and potential consequences before cultivation starts

Starting new cultivations with healthy plants is a prerequisite for producing crops free of pospiviroids. As no natural resistance of practical impact has been reported in any of the cultivated hosts, use of resistant crops is not currently a viable management option and was therefore not adressed.

#### 4.3.1.1. Visual selection of healthy plants

In case of crops in which pospiviroids cause obvious symptoms, visual selection contributes to obtaining planting material and true seeds free of pospiviroids.

#### 4.3.1.2. Testing of plant material

Testing of plants and seeds, individually or in bulked samples, allows detection of pospiviroids in the planting material. Infected or contaminated lots can be discarded.

#### 4.3.1.3. Certification of planting material

Certification programs based on selection of healthy mother plants after visual inspections and/or testing, followed by visual inspections and testing of propagations are mainly used for vegetatively propagated crops, e.g. potatoes, fruit crops and some ornamentals. Although there is a large variation of certification programs, they are usually based on the same principal (Shepard and Claflin, 1975; Oosterveld, 1987; EPPO certification schemes, n.d.; Council Directive 2002/56/EC). After visual inspections and/or testing, healthy mother plants are selected for propagation under favourable conditions to prevent re-infections. After one or few propagation cycles, during which visual inspections and random testing take place, planting material is produced for delivery to growers of production crops. A characteristic of most certification programs is also the downgrading of plants after each propagation cycle.

#### 4.3.1.4. Official surveillance in nurseries

Visual inspections of nurseries by official bodies may contribute to starting new cultivation free of pospiviroids for crops expressing symptoms. Similarly surveillance in nurseries by testing contributes for all crops equally.



# **4.3.2.** Identification of management options to reduce the probability of spread and potential consequences during cultivation

#### 4.3.2.1. Separation of host plant cultivations

Separation of host plant cultivations reduces the chance of spread to other crops after pospiviroid outbreaks. This holds true for spread both by human assistance and natural means. Host plant cultivations can be separated by growing different crops at different fields and greenhouses or at specified minimum distances from each other. Compartments within greenhouses further reduce the chance of spread. Moreover, avoidance of plants species (e.g. private plants) other than the main cultivated crops would also reduce the probability of spread to alternate hosts.

#### 4.3.2.2. Hygiene best practice

The following list of management principles are all recognised measures for containing the spread of pospiviroids and other contact transmitted pathogens. If implemented, they can help to reduce both the probability of outbreak and the spread following an outbreak. Consequently the overall impact of an outbreak would be reduced.

- **Trained staff**: staff should be trained in plant pathology (basics of symptomatology, epidemiology and control measures) and best practice procedures (hygiene and plant handling). Employment of trained staff contributes to prevention of outbreaks and to prompt recognition of pospiviroid symptoms.
- **Banning consumption on the premises of fruit of susceptible species:** growers and employees eating fruit of susceptible species touch fruits that might be infected with pospiviroids and their hands may thus become contaminated, consequently raising the risk of human-assisted viroid transmission to crops. Although, the risk of introducing a pospiviroid in this way is considered low, banning fruit consumption (e.g. tomato) reduces the chance of a pospiviroid outbreak. Fruit produced in the premises do not present a risk of further spread.
- Banning sorting/packing of fruit produced from other companies/locations: some tomato and pepper producing companies utilize their facilities for sorting and packing fruits produced by other companies. If pospiviroid infected fruits are sorted, both machinery and people handling the fruits will be contaminated. If people sorting the fruits also work in the cultivations pospiviroids may be spread (Verhoeven et al., 2010c). So preventing the introduction of potentially infected fruit on premises where fruit packing and production of solanaceous crops are done at the same location may reduce the chance of transmission of pospiviroids.
- Using disposable clothes: similar to *Potato virus X* (PVX) pospiviroids are expected to be transmitted via contaminated clothes. This measure includes the use of clothes (including disposable overshoes) that will be destroyed or washed after usage. Application reduces the risk of both introducing pospiviroids from outside the field/greenhouse and spreading pospiviroids already present to other locations.
- Using disposable gloves: disposable gloves can be used either between different cultivations or even within different areas of a single cultivation, even up to changing gloves between individual plants. Application of this measure reduces the risk of both introducing pospiviroids from outside the field/greenhouse and spreading pospiviroids already present to other locations.
- Washing of hands between crops: washing hands (30 seconds) using soap before entering a new field, greenhouse or compartment and after leaving it, will reduce the chance that pospiviroids spread may occur when touching other plants.



- **Restricting the use of equipment to one location**: especially knives have been shown to effectively spread pospiviroids (Garnsey and Jones, 1967; Allen, 1968; Verhoeven et al., 2010c). Therefore, restricting the use of equipment (small tools e.g. knives) to one field, greenhouse, compartment or a smaller area prevents the spread of pospiviroids via equipment to crops grown at other locations.
- Chemical disinfection of knives and pruning instruments: knives and pruning instruments are effective means of mechanically transmission of pospiviroids within and between crops (see Section 3.4.2.). Regular disinfection of cutting tools with chemical treatments (e.g. while moving from plant to plant, from row to row or from crop to crop) may therefore reduce the spread of pospiviroids.
- Cleaning and disinfection of machinery between different crops: PSTVd and other pospiviroids can be spread by cultivating and hilling machineries, and tractor wheels (see Section 3.4.2.). To reduce the chance of pospiviroid spread between crops in this way, cleaning of machinery (tractors, hilling equipment, spraying apparatuses, picking cars etc) followed by disinfection may be applied. Machinery can be cleaned by high water-pressure, steam cleaners or comparable ways. Additional disinfection may be achieved by application of chemicals such as sodium hypochlorite (NaOCl), household bleach and commercial agricultural desinfectants or by UV irradiation.
- **Consistent working direction**: human assisted spread of pathogens will take place in the same direction as the humans are working. Always working in the same direction limits this way of spread to a single direction, and as such, reduces the spread of the pathogen. As a consequence, measures to be taken can be more concentrated.
- Limiting the access to the place of production: the fewer people are entering a cultivation of pospiviroid host plants, the smaller the chance is of introduction or spread of pospiviroids via human assistance. Therefore, access to the cultivation should be restricted to people working in the specific cultivation. Further restrictions of access should be applied as much as possible by having employees always working in the same area or number of adjacent rows.
- **Crop rotation**: it is common practice in agriculture to rotate crops through a cycle of species. This is primarily for soil nutrition purposes e.g. by including a leguminous crop in a cycle to fix nitrogen in the soil. However, in certification schemes for crops such as seed potatoes fixed minimum rotations of between four to six years are applied to help reduce the risk of pests and diseases establishing in the field e.g. for potato cyst nematode. In some horticultural production systems a similar practice is also applied following outbreaks of persistent diseases to break the cycle of disease, e.g. *Cucumber green mottle mosaic virus* (CGMMV) in cucumber.

## 4.3.2.3. Monitoring for pospiviroid symptoms

Knowing the health status of their crops is essential for growers to promptly take adequate measures. For crops showing pospiviroid symptoms, monitoring is a basic tool for acquiring information on the health status of their cultivations.

## 4.3.2.4. Testing

In addition to monitoring for symptoms, testing provides information on the health status of a crop including symptomless crops e.g. ornamentals.

## 4.3.2.5. Aphid vector control

Aphids have occasionally been reported to transmit pospiviroids, especially when the viroid has been acquired from plants coinfected with PLRV (see Section 3.4.1.1.) As a consequence, controlling

aphids, especially those transmitting PLRV, in crops that are host for both pospiviroids and PLRV, could contribute to reduce the spread of pospiviroids.

#### 4.3.2.6. Control of weeds and volunteer plants

Volunteer plants originating from infected plants, especially those propagated vegetatively, may act as sources of inoculum. For potato, this will be the case in the next crop. Tomato and pepper seeds, however, may germinate immediately and therefore, volunteer plants may emerge during the same cultivation. Controlling these plants will reduce the sources of inoculum.

The table of experimental pospiviroid host plant species (Appendix B Table 17) lists several weed hosts, which grow widely in the PRA area. Therefore, weeds should be considered as potential hosts of pospiviroids too. Perennial weeds pose a greater risk than annual weeds because in the first case the weeds are permanent sources of inoculum whereas in the latter case a limited number of the offspring will be infected at maximum.

For potato, volunteer plants and weeds may be controlled mechanically (hoeing machine, cultivator), chemically (herbicides) and by cultural practices (thorough lifting of potatoes, soil treatment to increase frost damage to volunteer tuber). For tomato and pepper, volunteer plants and weeds can also be controlled manually (roguing). The occurrence of volunteer plants is not known for the main ornamental solanaceous species.

#### 4.3.2.7. Avoiding bumblebees for pollination of tomato

Transmission by bumblebees has been reported for TASVd and TCDVd (see Section 3.4.1.1). In tomato, however, bumblebees are frequently used for pollination. Not using bumblebees may reduce the chance of pospiviroid spread but would simultaneously reduce fruit yield. As an alternative to bumblebee pollination, human-assisted pollination using sticks for vibration of flowers can be applied. It is however less efficient than bumblebees and therefore has negative impact on yield and, in addition, carries a risk of promoting mechanical transmission of pospiviroids.

# 4.3.3. Identification of management options to reduce the probability of spread and potential consequences following pospiviroid outbreak

Following a pospiviroid outbreak measures can be implemented to further reduce the spread and impact of the infection. In addition to the measures listed above which should be applied prohylactically and post-outbreak, the following measures can also be implemented. The end of cultivation is the optimal period to fully eradicate any pospiviroid infection in the previous cultivation. All efforts should be generated to eradicate the viroid at that time, to achieve a start of new cultivations without infections originating from previous cultivations.

#### 4.3.3.1. Tracing sources of infection

Knowledge of the source of infection supports taking adequate measures to prevent new outbreaks from the same source.

## 4.3.3.2. Destruction of infected plants following outbreaks

Infected plants cannot be cured. Therefore, destruction of infected plants is the only way to reduce the number of sources of inoculum within a specific cultivation. For up to a few infected plants at a single spot, destruction could be restricted to the symptomatic plants and a limited number of plants adjacent to the infected ones. In case outbreaks occur at various locations within a cultivation, destruction of all plants should be considered because the amount of pospiviroid inoculum in the cultivation is likely to be too high for adequate control without destruction of the whole crop. For crops not showing pospiviroid symptoms, all plants should be eradicated for effective control, even if infection was found in a single sample. Destruction of infected plants should always be followed by additional measures (see Sections 4.3.3.4 to 4.3.3.6).

In case of cultivations for breeding and propagation, it could be considered to apply total destruction of plants even in case of an outbreak at a single spot because of the type of plant material. However, for plants for breeding or mother plants, searching for healthy plants for propagation by repeated testing under strict conditions could also be considered. Destruction of all plants from a field/greenhouse with a pospiviroid infection in the previous cultivation is a way to eliminate the potential sources of inoculum. In this scenario, "all plants" include the plants from the main crop, plants of other host crop species that may be present, volunteer plants, and weeds.

# 4.3.3.3. Application of restricted areas and adaptation of working direction

To prevent further spread of pospiviroids in case of an outbreak, the row and some neighbouring rows (e.g. 3 rows in the working direction and 1 row in the opposite direction) should be marked (restricted area) and treated separately, preferably at the end of the working day. Within the restricted area the outer rows should be handled first and the row with the infected plant(s) last. Working in the restricted zone should be limited to a single person who should do all required activities only at the end of working days.

## 4.3.3.4. Destroying disposable materials

Pospiviroids may remain at non-plant surfaces for long time and still keep their infectivity (Mumford et al., 2004). Therefore, not only infected plants should be removed, but also disposable materials used during the cultivation e.g. ground cover, hydroponics etc. These items will mainly be used in greenhouse-grown crops.

## 4.3.3.5. Sanitation of production location

Sanitation of the production location includes thorough cleaning of the premises and non-disposable materials e.g. gutters, watering system and heating pipes. Generally, this type of sanitation is restricted to greenhouses. The premises and outer parts of non-disposable material can be cleaned with high water-pressure, steam cleaners or comparable ways. A scrub brush should be used for parts that are difficult to clean. A regular acid treatment can be used for internal cleaning of watering tubes and for drippers. Additional disinfection can be achieved by the application of chemicals such as sodium hypochlorite (NaOCI), household bleach and commercial agricultural disinfectants.

## 4.3.3.6. Cleaning and disinfection of machinery and equipment

Cleaning and disinfection machinery (tractors, hilling equipment, spraying apparatuses, picking cars etc) followed by disinfection will reduce the chance that pospiviroids will be spread between cultivations by machinery. Machinery can be cleaned by high water-pressure, steam cleaners or comparable ways. Additional disinfection may be achieved by application of chemicals such as sodium hypochlorite (NaOCl), household bleach and commercial agricultural desinfectants, or by UV irradiation.

## 4.3.3.7. Control of volunteer plants and weeds

Whilst it is considered good practice to control plants and weeds during cultivation as a general measure, the importance of this measure is greater following an outbreak of pospiviroids (see Section 2.3.2.6).

Volunteer plants originating from infected plants, especially those propagated vegetatively, may act as sources of inoculum. For potato, this will be the case in the next cultivation. Tomato and pepper seeds, however, may germinate immediately and therefore volunteer plants may emerge during the same cultivation. Controlling these plants will reduce the sources of inoculum.

The table of experimental pospiviroid host plant species (Appendix B Table 17) lists several weed hosts, which grow widely in the PRA area. Therefore, weeds should be considered as potential hosts of pospiviroids too. Perennial weeds pose a greater risk than annual weeds because in the first case the



weeds are permanent sources of inoculum whereas in the latter case a limited number of the offspring will be infected at maximum.

For potato, volunteer plants and weeds may be controlled mechanically (hoeing machine, cultivator), chemically (herbicides) and by cultural practices (thorough lifting of potatoes, soil treatment to increase frost damage to volunteer tuber). For tomato and pepper, volunteer plants and weeds can also be controlled manually (roguing). The occurrence of volunteer plants is not known for the main ornamental solanaceous species.

#### 4.3.3.8. Banning interplanting/continuous cropping

Some tomato growers already plant the young plants of the new cultivation next to plants of the old cultivation, in order to reduce the period without producing marketable fruits. This way of producing may be profitable in the absence of pospiviroids and other serious disease agents; however, in case of pospiviroid outbreaks in the old cultivation, this approach will certainly lead to substantial infections rates in the new cultivation.

#### 4.3.3.9. Temporary ban (scheduling) of cultivation of host plants

Following pospiviroid outbreak rotating to a non-host plant reduces the chance that a pospiviroid will survive at a production location. A change for a single production cycle is considered satisfactory for all crops, except potato. The latter is due to the fact that potato volunteer plants may survive a longer period, without taking adequate additional measures.

For optimizing the effect of potato volunteer plant control, a temporary ban of growing potato would contribute to the control of pospiviroids. During the ban, volunteer plants and alternative hosts should be controlled intensely. The longer the banning period persists, the higher the effect to be expected.

#### 4.4. Description of EU current emergency measures (Commission Decision 2007/410/EC).

In June 2007 the EU Commission adopted emergency measures to further prevent the introduction into and the spread within the EU territory of PSTVd (Commission Decision 2007/410/EC). These measures apply to plants of the genus *Brugmansia* and of the species *S. jasminoides*, intended for planting, including seeds. The Commission Decision defines additional measures for import and movement of the specified plants within the EU territory and requires the EU MS to conduct official surveys and to notify the results to the Commission.

In particular, for import into and movement within the EU, plants for planting and seeds of *Brugmansia* spp. and *S. jasminoides* must originate and be grown always in a place of production:

- in countries where PSTVd is not known to occur or
- in a pest free area or
- where all lots have been tested and found free of PSTVd prior to movement or
- where all associated mother plants of the specified plants have been tested and found free from PSTVd prior to movement of the specified plants. After testing, the growing conditions are such that associated mother plants and the specified plants will remain free from PSTVd prior to movement.

In addition to this, imported plants of *Brugmansia* spp. and *S. jasminoides* have to be inspected at entry into EU, tested and found free of PSTVd.

For movement within EU, the plants of *Brugmansia* spp. and *S. jasminoides* need to be accompanied by a plant passport and have been grown always or since their introduction into the EU in a place of



production as specified above. Only exceptions are the small quantities of plants for use by the owner or recipient for non-commercial purposes, provided that there is no risk of spreading of PSTVd.

These measures partially correspond to what described above in Sections 4.1.1.2, 4.1.1.3, 4.2.1.2 and 4.3.1.2.

## 5. Evaluation of management options

#### 5.1. Evaluation of management options to reduce the probability of entry

#### 5.1.1. Evaluation of pre-entry measures

The pre-entry measures are evaluated in the Sections 5.1.1.1 to 5.1.1.4 and results are summarised in section 5.1.1.5.

#### 5.1.1.1. Banning the introduction of plants and plant products

Currently under Council Directive 2000/29/EC tubers of *Solanum spp*. for propagation purposes (i.e. seed potatoes) are prohibited entry to the EU from Third Countries other than Switzerland. Derogation is available under Commission Directive  $97/46/EC^{23}$  for entry but only following post entry quarantine testing of material for potentially harmful organisms. Therefore for potato this measure is considered to have a high feasibility and to also be highly effective at reducing the probability of infected potato material entering the PRA area.

Introducing similar measures against pospiviroids in propagation material of other host plants would also be assessed as being highly effective. However the impact of such a measure for PSTVd in ornamentals would probably be low given the known wide presence of PSTVd in EU in these plants (see Section 3.1.3.2). In addition, the uncertainties on the host range of pospiviroids (see Sections 3.1.2 and 3.2.4.1) would also limit the effectiveness of this measure for ornamentals.

The impact of such measure for plants products not for planting (e.g. fruit) would be similarly low given the minor significance of this pathway (see Section 3.2.6).

Feasibility would be high for ornamentals for planting and moderate in the other cases given the respective volumes of traded materials and the high dependence of the EU on imported seeds.

#### 5.1.1.2. Import requirements for the consignment

The official guarantee for freedom of quarantine organisms is generally considered a solid basis for safely importing plant material, but it is highly dependent on tests and inspections carried out to ensure the absence of the pathogen. In the case of pospiviroids, latent infections in therefore symptomless plants might be missed and consequently still be present in places/consignments considered to be free. The limited knowledge with regard to the range of host species could potentially lead to asymptomatic plants of unreported host species entering the EU.

If based on visual inspection, the efficiency of the measure would be low. If based on well designed and validated testing, the efficiency would be high, with the exception of ornamentals due to uncertainties on the host range of pospiviroids (see Sections 3.1.2 and 3.2.4.1). The feasibility of the measure is high.

<sup>&</sup>lt;sup>23</sup> Commission Directive 97/46/EC of 25 July 1997 amending Directive 95/44/EC establishing the conditions under which certain harmful organisms, plants, plant products and other objects listed in Annexes I to V to Council Directive 77/93/EEC may be introduced into or moved within the Community or certain protected zones thereof, for trial or scientific purposes and for work on varietal selections. OJ L 204, 31.7.1997, p. 43-46.



#### 5.1.1.3. Quality requirements (private standards) for the consignment

Certification schemes still may provide a measure for limiting the risk of pospiviroids entering the EU. The efficacy of this option would depend on the monitoring measures taken in the certified material production process (e.g. is freedom guarantee arising from a visual inspection and/or from laboratory based testing? What sampling strategy is used? etc.). Visual inspection alone would not be adequate nor would test regimes based on confirmation of symptomatic plants. In all cases testing would need to be statistically robust to allow for detection of symptomless infection in the consignment. Testing of mother plants would give a greater guarantee of compliance with import quality requirements, and this could be further enhanced by additional testing of consignments prior to export from Third Countries. However, this latter issue would in effect mean instigating certification schemes for all host crops on a global basis. Therefore, the effectiveness of this measure is estimated to be lower than that afforded by the two previous ones and is rated as moderate. Feasibility is considered low to moderate, in those cases where international certification guidances exist.

With regards to private standards, concerns were expressed at the Sanitary and Phytosanitary (SPS) Measures Committee of the World Trade Organisation (WTO, online) considering that: private standards are not always based on science; they may deviate from international standards or from official governmental requirements; they are numerous and not harmonized; they may be costly for suppliers complying with them and seeking certification for their products; they are set up without transparency, consultation or systems for appealing; they prescribe how measures should be applied rather than what the outcome should be, ignoring the principle that equivalent outcomes achieved by different means should be recognized; they pose disproportionate burdens on small- and medium-sized producers and exporters in developing countries. On the opposite view, other members of the SPS Measures Committee see some benefits in private standards, i.e. to help suppliers comply with national and international standards, to promote best practices and improved productivity, to improve brands reputation and to help suppliers access to markets and credit. Also, private standards can address emerging risks in a rapid manner, fill gaps and make it easier for international standards to eventually be adopted.

#### 5.1.1.4. Treatment of the commodity

Therapeutic treatments that eliminate pospiviroids from the growing crop are not available. Treatments to sanitize botanical seeds are also of little use in preventing seed borne transmission of pospiviroids (see Section3.2.2.3). Therefore the effectiveness of this management option is negligible.

Ware potato tubers can be treated chemically to suppress germination. The effect of the measure is only temporary, which implies that tubers could still be planted after several months of forced dormancy, although the seed-potato quality will have decreased. Therefore, the effectiveness of the measure is considered moderate with a high feasibility to control the risk of inappropriate use of ware potato for planting. However, as this type of pathway has been identified as having only minor significance, the effect of this management option will be further limited. This management option would, therefore, have a low effectiveness for managing the overall risk of pospiviroids entering the PRA area.

#### 5.1.1.5. Summary on evaluation of pre-entry measures

The results of the evaluation of pre-entry measures are summarised in Table 11.

No single pre-entry management option identified offers an overall solution to minimise the risk of pospiviroids entering the PRA area. Whilst an import ban on host commodities has been identified as being highly effective against pospiviroid entry in some hosts, such as potato, it has an associated moderate feasibility on a broader basis due to lack of knowledge of the full host range of all pospiviroids and due to dependence of EU agriculture on seed import. Other identified measures have a perceived lower effectiveness, although they may still provide some level of protection against entry.



Quality requirements based upon certification schemes would, in effect, be difficult to implement due to the need for these schemes to have international/global coverage outside the reaches of EU legislation. Direct treatment of commodities is not a feasible option for any of the major entry pathways. It would only be applicable in table/ware potatoes and even then is only a temporary measure affecting a minor entry pathway

**Table 11:** Summary table for the evaluation of pre-entry management options for risk mitigation against pospiviroids

Identified Measure	Effectiveness	Feasibility	Comments
Banning the introduction of plants and plant products	High	Low to high	Generally high effective unless for minor entry pathways as plant products. High feasibility on potato, due to existing restrictions, and on solanaceous ornamentals, due to limited trade volume. Low feasibility for other plants and plant products due to high trade volumes, particularly for seeds.
Import requirements for the consignment	Low to high	High	Effectiveness would vary depending whether it is based on visual inspection or on well designed and validated testing.
Quality requirements for the consigment	Moderate	Low to moderate	Feasibility would be moderate for those crops for which international certification guidances already exist.
Treatment of the commodity	Negligible to moderate	Negligible to high	No direct treatment exists for pospiviroids in plants. Treatments of botanical seeds are not effective. Suppression of germination in seed potatoes is feasible but its effetiveness is moderate having only a temporary effect.

#### 5.1.2. Evaluation of import control measures

The import control measures at the point of entry are evaluated in the Sections 5.1.2.1 to 5.1.2.4 and results are summarised in Section 5.1.2.5.

#### 5.1.2.1. Visual inspection

Pospiviroid host plants may not express symptoms at the time of trade e.g. tomato and pepper, or do not express symptoms at all, e.g. ornamentals and true seeds. Therefore, the effectiveness of this measure is considered low. Its feasibility is high because visual inspection is common practice for import control.

# 5.1.2.2. Testing

There are widely validated methods for symptomatic diagnosis or screening asymptomatic growing plants for pospiviroids including both 'generic'/'universal' tests covering many target species and species specific tests (see Section 3.1.1). However, there are few validated tests for detecting seed-borne pospiviroid infections and limited experience in screening botanical seeds for the full range of solanaceous pospiviroids. A further limitation of this measure is the lack of international seed

sampling standards and test methods for detecting viruses or viroids in any host matrix, especially in testing botanical seeds. Nevertheless, the answers received to the questionnaire sent by EFSA to EU MS indicate that some of them are already testing for PSTVd (and sometimes for other solanaceous pospiviroids) in commercial seed lots of various species (see Appendix C). Highest efficiency is only achieved with individual testing of plants and seeds. However such measure can only be applied to low numbers of plants of identified species entering the PRA area. It is difficult to apply these procedures to late generation material with high numbers of plants, for example in the case of ornamental species.

The effectiveness of testing is high in case of leaf testing of all imported plants. In case of destructive tests e.g. seeds, or imports of large quantities of material only a limited number can be tested, which decreases the effectiveness to moderate.

In case no standardised methods are available yet e.g., seeds for several viroids and potato tubers for all viroids, the effectiveness of the method is moderate. The feasibility of testing of a limited number of entities is considered moderate but it decreases to low for large numbers.

# 5.1.2.3. Limiting the use of the imported consignment

Limiting the use of imported consignment to use by consumers only reduces the chance of entry of pospiviroids. This measure can be applied for tubers of potato, which may be imported only for human consumption. For ornamentals the limitation to use by consumers only is less effective as the material would still be entering the PRA area and could potentially be distributed more widely than material destined for a single point of propagation. The overall effectiveness is low because this option affects only the pathways identified as having the lowest significance. Its feasibility is rated as moderate.

## 5.1.2.4. Post-entry quarantine

The effectiveness of post-entry quarantine is high to very high when all plants in post-entry quarantine are tested for pospiviroids. Its feasibility is high for nuclear stocks for vegetative propagation as these measures are already applied across the PRA area for potato, However, feasibility is low for late generation/plants for planting material since it can only be applied to a very limited number of plants, For seed propagated crops, this option is only feasible for very small lots of breeding material but cannot obviously be applied to large commercial seed lots.

## 5.1.2.5. Summary on evaluation of import control measures

The results of the evaluation of import control measures are summarised in Table 12.

The management option which is currently most commonly applied is visual inspection of consignments at point of entry. However, this measure has low efficacy when applied to the major pathways of entry. In many vegetatively propagated hosts pospiviroid infections are asymptomatic and seed-borne infections can only be detected using laboratory based testing methods.

<b>Table 12:</b>	Summary	table	for	the	evaluation	of	management	options	for	risk	mitigation	against
pospiviroid	s at 'point	of entr	y'									

Identified Measure	Effectiveness	Feasibility	Comments
Visual inspection	Low	High	Host plant may not yet show symptoms at import or be asymptomatic hosts.
Testing	Moderate to high	Low to moderate	For leaf testing of plants, high effectiveness. For seeds, moderate effectiveness because of sampling and lack of



			standardised methods. For seed potatoes effectiveness also moderate due to lack of standardised methods. Feasibility will depend on number of tests/sample size.
Limiting use of the inported consignment	Low	Moderate	Applicable only to potato tubers imported for human consumption or to ornamentals for use by private consumers only (less effective).
Post entry quarantine	High to very high	Low to high	Very effective if all plants are tested. High feasibility for nuclear stocks for vegetative propagation, low for late generation planting material.

#### 5.2. Evaluation of management options to reduce the probability of establishment

No management options have been identified to reduce the probability of establishment.

# 5.3. Evaluation of management options to reduce the probability of spread and potential consequence

# **5.3.1.** Evaluation of management options to reduce the probability of spread and potential consequences before cultivation starts

The management options to reduce the probability of spread and potential consequences before cultivation starts (pre-planting) are evaluated in the Sections 5.3.1.1 to 5.3.1.4 and results are summarised in Section 5.3.1.5.

## 5.3.1.1. Visual selection of healthy plants

Pospiviroids can only be detected in plant species showing obvious symptoms, such as potato and tomato. In these crops, selection of symptomless plants contributes to starting with healthy plant material. However, its effectiveness is limited by the fact that i) seeds do not show symptoms, ii) not all infected plants show symptoms, iii) viroid strain differences and iv) plants may still be too young to produce detectable symptoms at the time of planting (e.g. tomato). In plant species that remain symptomless after infections (e.g. ornamentals), selection based on visual inspections does not contribute at all to start with healthy planting material. The effectiveness of the measure varies from low to moderate (potato and tomato) and from negligible to low (other crops). Its feasibility is high.

#### 5.3.1.2. Testing of plant material

Adequate tests are available for the detection of pospiviroids in planting material (Boonham et al., 2004; Verhoeven et al., 2004; Monger et al., 2010). However, in case of testing of planting material often large numbers of plants will be planted. This implies that only random samples can be tested, except for small quantity lots (e.g. nuclear stock plants). So far, there is only little experience with tomato seed testing for CLVd (Fox and Monger, 2011), PSTVd and TCDVd (Koenraadt et al., 2009), and there are no published data for other pospiviroids. In contrast to *Pepino mosaic virus*, for which a non-destructive seed test is available (Mumford et al., 2006), true seed testing for pospiviroids will destroy the tested seeds, which limits the possible role of this type of testing. The effectiveness of the measure varies from very high (testing all nuclear stock/mother plants) to moderate/high (random

samples) and moderate (seeds) and its feasibility varies similarly from low (for bulk testing of seeds/propogation material) to very high for nuclear stock/mother plants.

## 5.3.1.3. Certification of planting material

In the USA and Canada PSTVd was successfully eradicated from the seed potato industry mainly based of certification systems including testing of nuclear stock of potato variety clones and visual inspections during seed potato propagation (Sun et al., 2004; De Boer and De Haan, 2005). In the Netherlands, a certification program for *Brugmansia* spp. and *Solanum jasminoides* including testing of all nuclear stock and regular random testing of propagation crops enabled eradication of PSTVd from both crops in one year (De Hoop et al., 2008). So, certification systems have been proven to successfully eradicate PSTVd from vegetatively propagated crops. Simultaneous certification for *Potato leaf roll virus* (PLRV) further reduces the chance for natural transmission of PSTVd and possibly other pospiviroids by aphids.

In seed-propagated crops similar systems could be developed including 100% testing of plants to be used for breeding, visual inspection when relevant (e.g. tomato) and random to thorough testing of plants used for seed production. The effectiveness of the measure is high to very high and its feasibility is moderate (ornamentals vegetatively propagated) to low (seed propagated tomato and pepper) to high (potato).

## 5.3.1.4. Official surveillance in nurseries

If based on visual inspection, official surveillance of nurseries for pospiviroids is unlikely to be effective because ornamentals generally do not display symtoms and because other host plants may not display symptoms at seedling stage.

If surveillance is accompanied by sampling and testing, effectiveness is increased but still lower than that of a certification schemes because only a fraction of plants will be evaluated and only at a given generation.

The feasibility of the measure is high only for visual inspection but low to moderate if testing is performed given the high number of premises and plants involved.

5.3.1.5. Summary on evaluation of management options to reduce the probability of spread and potential consequences before cultivation starts

The results of the evaluation of management options to reduce the probability of spread and potential consequences before cultivation starts are summarised in Table 13.

Visual selection of healthy plants from breeding lines or from mother stocks through successive generations is the basis on which most european seed potato certification schemes are conducted, Large numbers of plants can be rapidly screened by trained staff or inspectors, however, visual selection alone is of low efficacy due to the nature of pospiviroid symptomatology. This would have some effect in production systems for potato and tomato, but limited use in systems for the production of ornamental species due to the high numbers of asymptomatic hosts.

Testing as a stand alone measure can be highly effective if applied to mother plants at the early generation stages e.g. nuclear stocks, when it is feasible to test all plants. However, at later generations or in testing imported seed stocks, only a random selection of individuals in any population can be tested, limiting the effectiveness of this measure. This issue is further compounded when testing botanical seeds due to the limitations of viroid detection technology and the lack of experience with testing true seeds. International cooperation in developing effective seed sampling and detection protocols would be required to ensure any level of feasibility to applying these measures.

In effect, certification schemes combine visual inspection with testing of either symptomatic plant material or randomly taken samples to ensure mother stocks with a high plant health status. However,

even with these schemes it is difficult to ensure true freedom from disease. These principles are already in place throughout Europe in seed potato production, and in some cases could be applied in other vegetatively produced species. The feasibility for this measure decreases for seed produced crops such as tomato or pepper. Due to the international supply chains for seed of vegetable crops schemes would require cooperation from Third Countries to be both effective and feasible.

Official surveillance of sites producing propagation material may be limited in feasibility and effect when restricted to only one pospiviroid species (PSTVd), considering that all solanaceous pospiviroids pose a comparable threat to tomato and other susceptible crops.

Overall, the strategy which presents the highest efficiency and feasibility is certification. Strategies that rely on visual inspection have the lowest efficiency due to the inherent limitations of this approach. Strategies that rely on testing have high efficiency and feasibility for small lots of plants or seeds, but efficiency and feasibility decrease when the number of plants or seeds increases, as it is the case in commercial lots.

**Table 13:** Summary table for the evaluation of management options to reduce the probability of spread prior to cultivation (pre-planting)

Identified Measure	Effectiveness pre-cultivation	Feasibility	Comments
Visual selection of healthy plants	Negligible to moderate	High	Due to symptoms expression, effectiveness is low to moderate for potato and tomato, and from negligible to low for other crops.
Testing of plant material	Moderate to very high	Low to very high	Very high effectiveness and feasibility when testing nuclear stocks or mother plants. Moderate to high effectiveness for sample testing, moderate for seeds. Similarly feasibility will be low for bulk testing and very high for nuclear stocks or mother plants.
Certification of planting material	High to very high	Low to high	For seed potatoes, certification schemes are already in place across EU, therefore feasibility is high. For vegetatively propagated crops, EU certification schemes would need to be initiated, although already applied within some EU MS, therefore feasibility would be moderate. For seed propagated crops, seed production is generally outwith the EU, therefore feasibility would be low.
Official surveillance of nurseries	Low to moderate	Low to high	If based only on visual inspection, low effectiveness and high feasibility. If accompanied by sampling and testing, moderately



	effective but less feasible.

# 5.3.2. Evaluation of management options to reduce the probability of spread and potential consequences during cultivation

The management options to reduce the probability of spread and potential consequences during cultivation are evaluated in the Sections 5.3.2.1 to 5.3.2.9 and results are summarised in Section 5.3.2.10.

#### 5.3.2.1. Separation of host plant crops

Similarly to viruses, separating high-grade (nuclear stocks) seed potato fields or areas from low-grade (late generation) seed-potato and/or ware potato productions fields or areas reduces the risk of pospiviroid outbreaks in (high-grade) seed potatoes and would further limit the spread of pospiviroids in any of the potato growing locations. All greenhouse-grown cultivations of any crop are separated from cultivations grown in other greenhouses, which helps preventing or reducing the chance of spread between cultivations. Use of greenhouses with compartments further reduces the chance of pospiviroids spread all over the greenhouse. Different crops are preferably grown in different greenhouses or at least in different compartments. For crops grown outside greenhouses, spread will also be reduced when growing cultivations of host plants apart from each other. The effectiveness of the measure is moderate to high for field-grown cultivations and high for greenhouse-grown cultivations. The feasibility of the measure is considered high for seed potatoes, high for greenhouse-grown crops and low to moderate for other field-grown crops.

#### 5.3.2.2. Hygiene best practices

The efficiency and feasibility of hygiene best practices are evaluated globally below, after discussion of each individual practice.

- **Trained staff** helps to prevent outbreaks and minimises the potential for inadvertent transmission of the pathogen during symptomless infections by ensuring that careful plant handling and hygiene best practice measures are followed. Trained staff should also be able to recognize symptoms of infection as they appear, thus allowing earlier response and reducing the spread of pospiviroids.
- **Banning consumption on the premises of fruit of susceptible species** originating from locations with unknown status could efficiently control pospiviroid transfer from contaminated fruits to plants grown in the facilities. This measure is applied in tomato nurseries to prevent the introduction of *Pepino mosaic virus*.
- **Banning sorting/packing of fruit produced at other companies/locations** prevents the introduction of pospiviroids by this way. Therefore, the measure is very effective for each individual facility.
- Using disposable clothes (including overshoes) eliminates the chance that pospiviroids will be spread to other cultivations of host plants by clothing. Some companies are already applying this strategy.
- Using disposable gloves eliminates the chance that pospiviroids will be spread by human hands (see Section 3.4.2.) to (other) cultivations of host plants or if applicable within a cultivation.
- Washing hands between crops reduces the chance that pospiviroids will be spread by human hands to (other) cultivations of host plants. The feasibility will be low to moderate in fields and high in greenhouses.



- **Restricting the use of equipment to one location** will prevent the spread of pospiviroids via equipment to other locations. Feasibility is low to moderate in fields and high in greenhouses.
- Chemical disinfection of equipment: a range of effective techniques is available for chemical disinfection of small equipment, such as knives, to prevent spread of pospiviroids (see Section 3.4.2). This method has a potential for use when equipment, e.g. knives, is used in various cultivations or parts of specific cultivations. For use between touching individual plants, the incubation period of 15 seconds is too long, but when used between rows the method would prevent spread by equipment between rows. As such the method would be highly effective. Its feasibility is moderate in fields and high in greenhouses.
- Cleaning and disinfection of machinery between different crops reduces the chance that machinery will spread pospiviroids between cultivations. The effectiveness of the measure is high. For those growers already using cleaning and disinfection against bacteria the feasibility is high; for others feasibility will be low to moderate.
- Working always in the same direction is used in protected crops such as tomato and pepper, to reduce spread of mechanically transmitted viruses such as tobamoviruses and *Pepino mosaic virus*. This measure similarly applies to all crops requiring considerable crop handling by humans (e.g. ornamentals, potato for breeding). Feasibility is considered high in greenhouse-grown crops and moderate to high in field-grown crops.
- Limiting access to the place of production reduces the possibility of transfer of pospiviroids both between and within crops. Moreover, by this measure the access can be restricted to trained staff. The effectiveness can be further increased by restricting the access of staff to only work in a defined area in the cultivation. The feasibility of the measure is high for protected crops and moderate to high for field-grown crops.
- **Crop rotation**: except for potato (because of volunteers, see Sections 4.3.2.6, 4.3.3.7 and 4.3.3.9), the cultivation of a non-host for one production cycle is considered highly effective to eliminate the chance that pospiviroids survive a period between host plant crops. The feasibility of the measure should be considered low to moderate for tomato and pepper growers because many of them are specialized in a single crop. For others the feasibility is moderate to high.

The measures listed above are components of sound cultural practices. Many of these measures are of low efficacy if implemented as stand alone measures, e.g. wearing gloves, without decontaminating cutting knives. However, taken as a suite of management options and applied as such they provide an effective barrier against outbreaks. Given the nature of pospiviroid outbreaks with a possible extended period of latent infection prior to symptoms becoming evident, it is advisable to apply all these measures prophylactically. All measures are relatively straightforward with few of the measures entailing feasibility concerns in glasshouse crops. Feasibility is however somewhat lower for field grown crops. As a suite of options, they are rated as being highly effective, with a moderate to high feasibility, as the only major barriers to implementation would be in changing staff behaviours and working patterns and meeting any additional costs.

# 5.3.2.3. Monitoring for pospiviroid symptoms

Monitoring for pospiviroid symptoms is only meaningful in cultivations of symptomatic plants, i.e., potato, tomato and pepper, although also for these crops symptomlessly infected plants should be considered, especially related to the variety grown. In addition to the symptom expression of the variety grown, also the intensity of monitoring contributes to the effectiveness of the measure.

For breeding, seed potato propagation, and propagation of true seeds of tomato and pepper, intensive monitoring for symptoms of diseases is common practice. Therefore, the effectiveness of the measure



is considered to vary from moderate to high and its feasibility for these types of cultivations is very high.

For the production of ware potatoes and marketable fruits of tomato and pepper, monitoring usually also will be performed, though at various frequencies and accuracies. Therefore, here the effectiveness of the measure is moderate, with a moderate to high rating for feasibility. For symptomlessly infected crops the effectiveness of the measure is negligible.

## 5.3.2.4. Testing

Testing is very effective for detecting pospiviroids in plant samples and the sensitivity of the available methods (Boonham et al., 2004; Verhoeven et al., 2004; Monger et al., 2010) even allows bulking of samples from several plants. For obvious reasons this technical practice can only be applied during cultivation of propagation material. The effectiveness of the measure varies from very high (testing all nuclear stock/mother plants) to moderate/high (random samples) and its feasibility is from moderate to high.

#### 5.3.2.5. Aphid vector control

Potato is the main host of PLRV and therefore, the potential role of aphids vectors is considered most relevant in this crop. Indeed, in potato propagation, aphid control is common practice, but, given the existence of other mechanisms of spread, the overall effectiveness of any aphid control strategy on pospiviroid spread is considered low. The feasibility of the measure is medium with the exception of seed potato production for which it is very high because it already is common practice. In other situations (e.g. glasshouse production, ware potatoes and ornamental crops) aphid control is not a common practice to limit virus or viroid spread. Consequently prophylactic aphid control measures may only be taken infrequently, if at all. In some IPM production systems, small populations of aphids may even be 'seeded' into glasshouses to provide a food source for populations of predatory insect.

## 5.3.2.6. Control of weeds and volunteer plants

The role of weeds in the natural spread of pospiviroids is not proven. Therefore the systematic control of weeds is expected to be of low impact. Volunteer potato plants may contribute to spread but their control will be nearly impossible during cultivations of potato, because the volunteer plants are very hard to dicriminate from the cultivated ones. As a consequence, both the effectiveness and the feasibility of the measure are negligible.

## 5.3.2.7. Avoiding bumblebees for pollination of tomato

Not assisting pollination in tomato glasshouse crops cannot be an option due to the consequential yield losses. Human-assisted pollination (e.g. with vibrating sticks) would carry a similar risk of spreading the disease but with higher technical difficulties and would impact yield. Therefore, avoiding bumble bees for pollination should not be considered as a viable management option.

5.3.2.8. Summary on evaluation of management options to reduce the probability of spread and potential consequences during cultivation.

The results of the evaluation of management options to reduce the probability of spread and potential consequences during cultivation are summarised in Table 14.

The prophylactic application of the measures listed throughout this section is probably the best way to minimise the risk of pospiviroid outbreaks in crops. The added advantage is that should an outbreak occur these measures will minimise the risk of spread of the pathogen and also minimise the potential impact on the crop. They should be considered as a best practice protocol for crop production to minimise any pathogen outbreak, not just pospiviroids.

These measures are most effective when applied as a suite of management options. Individually each measure has a low effect, however applied together they are a powerful tool, in many cases have low



cost and do not require statutory intervention. However, they do require effective support on the ground and some degree of training and changes in behaviour from staff at sites of production. There is low uncertainty as to the benefits that can be obtained from the use of this panel of prophylaptic options. However it is difficult to ascertain the individual benefits of each measure, and no investigations have been carried out to quantify which or how many of these measures should be considered as strategic.

 Table 14:
 Summary table of management options for reducing the probability of spread during cultivation

Identified Measure	Effectiveness	Feasibility	Comments
Separation of host plant crops	Moderate to high	Low to high	Effectiveness and feasibility are high for greenhouse-grown crops. For field-grown crops effectiveness is moderate to high and feasibility low to moderate. Feasibility is high for seed potatoes.
Hygiene best practice	High	Moderate to high	Measures to be applied together. Higher feasibility for greenhouse-grown cultivations than for field-grown crops.
Monitoring for pospiviroid symptoms	Negligible to high	Moderate to very high	For plant propagation material, effectiveness is moderate to high and feasibility is very high. For production of ware potatoes and vegetables, effectivness is moderate with feasibility moderate to high. For symptomless crops effectiveness is negligible.
Testing	Moderate to very high	Moderate to high	High effectiveness and feasibility for nuclear stocks of plant propagation material.
Aphid vector control	Low	Moderate to high	Generally moderate feasibility except for seed potatoes for which it is high.
Control of volunteer plants and weeds	Very low	Very low	Role of weeds in spread of pospiviroids not proven. For volunteers in potato both effectiveness and feasibility are very low.
Avoiding bumblebees for pollination of tomato		NOT FEASIBLE	

# 5.3.3. Evaluation of management options to reduce the probability of spread and potential consequences following pospiviroid outbreak

The management options to reduce the probability of spread and potential consequences following a pospiviroid outbreak are evaluated in the Sections 5.3.3.1 to 5.3.3.9 and results are summarised in Section 5.3.3.10. The management options listed below should be considered as supplementary to the prophylactic management measures evaluated above (see Section 5.3.2).

## 5.3.3.1. Tracing sources of infection

For growers there may be a few ways to trace the possible source of infection, as often additional testing, including sequence analysis of the viroid, will be needed for confirmation. Therefore for single growers the effectiveness is low to moderate, with a low to moderate feasibility.

Statutory tracing would have higher chances of success because of easier access to information. However, since finding the most likely source of infection has often appeared complicated in the past, the effectiveness of the measure is still moderate, but its feasibility is high.

#### 5.3.3.2. Destruction of infected plants

Destruction of all plants of a plot with one or more infected plants is very effective to prevent further spread, because it would eliminate all potential sources of inoculum. As a consequence, the effectiveness and feasibility of the measure are high, with the possible exception of potato due to the difficult control of volunteer tubers.

If applied at a early stage of an outbreak, containment may be achieved by 'roguing' those plants known to be infected and neighboring plants, followed by increased monitoring throughout the glasshouse for the rest of the growing crop and further removal of diseased plant as they appear (Verhoeven et al 2007a). However, destruction of only a limited number of plants carries a risk of being less effective due to the fact that the pospiviroid may have been spread further unnoticed because symptoms have not yet appeared in infected plants.

#### 5.3.3.3. Application of restricted areas and adaptation of working direction

Application of restricted zones in combination with adaptation of the working direction is useful in case of a pospiviroid outbreak when infection(s) were found at a single spot and a limited number of plants have been eradicated. The measure, as a supplement to partial destruction of the crop, is moderately effective but its effectiveness may be further improved by testing the plants in the rows next to the pospiviroid outbreak. Its feasibility is high.

#### 5.3.3.4. Destroying disposable materials

Removing and destroying all disposable material after a pospiviroid outbreak may contribute to limit the risk of recurrence but the specific contribution of this particular measure and of those described below (see Sections 5.3.3.5 to 5.3.3.6) carries some uncertainties. Feasibility is generally high.

#### 5.3.3.5. Sanitation of production location

Taking adequate time to thoroughly clean the premises and the non-disposable materials is important to obtain the required effects of disinfectants. The effectiveness of proper cleaning and disinfecting is expected to be high. Most growers already apply sanitation of the premises between crops. Since the sanitation after an outbreak should be stricter and will be more time consuming, the feasibility of the measure is moderate to high.

#### 5.3.3.6. Cleaning and disinfections of machinery and equipment

Cleaning followed by disinfection of machinery reduces the chance that machinery will spread pospiviroids between cultivations. The effectiveness of the measure is moderate. For those growers already using cleaning and disinfection against bacteria the feasibility is high; for others feasibility will be low to moderate.

#### 5.3.3.7. Control of volunteer plants and weeds in the following crop

The usefulness of controlling volunteer plants and weeds in the crop cycle subsequent to an outbreak is unclear (with the exception of the control of volunteer potatoes), but nevertheless it may be considered as an additional measure.

Control of volunteer potatoes is complicated because not all volunteer tubers will emerge at the same time and because they may emerge repeatedly. Furthermore, volunteer plants may grow from true seeds from infected potato plants even after several years of dormancy. In conclusion, the effectiveness of this measure is at best moderate. The feasibility of the measure is considered moderate.

#### 5.3.3.8. Banning interplanting/continuous cropping

Interplanting the next cultivation of a susceptible crop, while the previous crop is still in place, includes a very high chance for transmission of pospiviroids from the old cultivation to the new one. The effectiveness of banning interplanting, therefore, is very high. Generally, the feasibility is high since this measure is common practice at many companies.

#### 5.3.3.9. Temporary ban of cultivation of host plants

In Canada a two year ban of potato crop has been successfully applied to eradicate PSTVd infected potato volunteer plants (R.P. Singh, Fredericton NB, Canada, personal communication, March 2011). This measure is therefore considered highly effective. In some countries seed potatoes crops is already restricted to be grown only once every few years for control of potato cyst nematode<sup>24</sup>, so feasibility is considered very high.

For other crops, the potential impact is less clear but a break in cultivation that could supplement the disinfection and sanitation strategies outlined above may still prove valuable. The feasibility would be high.

5.3.3.10. Summary on evaluation of management options to reduce the probability of spread and potential consequences following a pospiviroid outbreak

The results of the evaluation of management options to reduce the probability of spread and potential consequences following a pospiviroid outbreak are summarised in Table 15.

After a viroid outbreak it is essential to remove inoculum sources to minimise the risk of further spread of disease. Application of "best practice" measures (as identified in Section 5.3.2) will help to contain an outbreak and limit the spread of the pathogen both within the affected cultivation and to other crops/cultivations. Due to the persistent nature of pospiviroids, it is essential to apply thorough post-outbreak clean up procedures, and dispose of any material which may have come into contact with affected plants. It may also be necessary to impose a temporary break in production of susceptible hosts to further minimise the risk of spread from contaminated surfaces or infected volunteer plants.

Generally speaking, pospiviroids are one group of pathogens for which control and elimination have been successfully achieved in a number of cases through the use of the above discussed management options. Their combined effectiveness is therefore considered high.

**Table 15:** Summary table of management options identified for reducing the probability of spread following a pospiviroid outbreak.

Identified Measure	Effectiveness	Feasibility	Comments
Tracing sources of infection	Low to moderate	Low to high	Higher effectiveness and feasibility for statutory tracing
Destruction of infected plants	High	High	Less effective for potato due to volunteers.

<sup>&</sup>lt;sup>24</sup> http://www.legislation.gov.uk/ssi/2005/613/contents/made



			Less effective in case of partial destruction of the crop.
Application of restricted areas and adaptation of working direction	Moderate	High	
Destroying disposable materials		High	
Sanitation of production location	High	Moderate to high	
Cleaning and disinfection of machinery and equipment	Moderate	Low to high	High feasibility for growers applying it already for bacterial diseases.
Control of volunteer plants and weeds	Moderate	Moderate	
Banning interplanting/continuous cropping	Very high	High	
Temporary ban of cultivation of host plants	High	High to very high	Particularly effective and feasible for seed potatoes

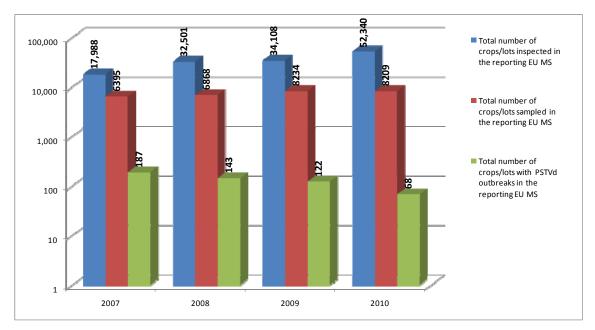
#### 5.3.4. Evaluation of current emergency measures

The evaluation of current emergengy measures on PSTVd is conducted with respect to their effects towards PSTVd situation in solanaceous ornamentals in the EU MS (Section 5.3.4.1), spread within the EU (Section 5.3.4.2) and import into the EU territory (Section 5.3.4.3). Considerations regarding the situation of PSTVd in other host plants are presented in Section 5.3.4.4 and considerations on other pospiviroids in Section 5.3.4.5.

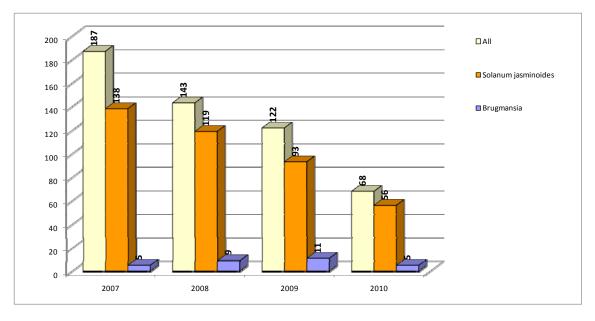
5.3.4.1. Evaluation of emergency measures with respect to PSTVd situation in *S. jasminoides* and *Brugmansia* spp. in the EU MS

As the current emergency measures were implemented in light of findings of PSTVd in the ornamental plants of S. jasminoides and Brugmansia spp., the first evaluation of the efficacy of these measures is the effect they have had on the level of residual inoculum of PSTVd in these species within the EU MS. During the period under which the EU emergency measures have been in place, FVO survey data shows there has been a decline in the number of crops/lots found to be infected with PSTVd (Figure 4). During this period the number of inspections of target species increased by a factor of 3, the number of lot sampled increased about 1.3-fold, and the number of findings of the viroid dropped to around 36% of the 2007 level. There has been a reduction in overall incidence per lot sampled from 2.9% in 2007 to 0.8% in 2010. These figures are by no means a complete picture as the implementation of the emergency measures is to some extent left to the interpretation of each EU MS (intensity of the testing/sampling effort, adaptation of sampling strategy to the structure of the national production/commercialisation of target species, intensity of eradication efforts following a positive detection, etc.) leading to some uncertainties about what is sampled and tested during an inspection. This is important as for the ornamental species under consideration PSTVd infections remain asymptomatic and visual inspection as a stand alone measure is inadequate to detect the presence of this pathogen.





**Figure 4:** PSTVd survey data for the period 2007-2010 (Appendix D) showing the total number of crops/lots inspected, the total number of crops/lots sampled and the total number of crops/lots with PSTVd outbreaks in the reporting EU MS. Logarithmic scale applied to show increase in inspections, slight increase in testing and decrease in findings.



**Figure 5:** PSTVd survey data for the period 2007-2010 (Appendix D) showing the total number of crops/lots with PSTVd outbreaks for all species sampled (All), for *S. jasminoides* and for *Brugmansia* spp. in the reporting EU MS.

Further analysis of the FVO data shows that *S. jasminoides* made a more significant contribution to the number of recorded outbreaks than *Brugmansia* spp., and that the drop in numbers of outbreaks of PSTVd over the period directly relates to the decrease in findings from *S. jasminoides* (Figure 5). During the same period, a similar pattern was not observed in *Brugmansia* spp. with the number of findings increasing slightly during the four years period and returning to their 2007 level by 2010.



However, this small number of findings has little influence on the general impact of the emergency measures. In summary, it is clear that the emergency measures have had the effect of raising awareness of the widespread presence of PSTVd in *S. jasminoides* and *Brugmansia* spp., thus increasing the number of inspections for this pathogen. Against this background of increased vigilance, the number of outbreaks/findings of PSTVd in the targeted ornamental species has greatly diminished. The reduction in findings may not have been equal in both targeted species, with a more limited effect in *Brugmansia* spp. However, it is also clear from these figures that, with regard to both species, PSTVd has not been fully eradicated from the EU.

The effectiveness of the current emergency measures to prevent the introduction and spread of PSTVd in solanaceous ornamentals has been addressed in the questionnaire sent to EU MS NPPOs (Appendix C). The PSTVd situation in the EU MS which replied to the EFSA questionnaire is described in Section 3.1.3.2 and in Tables 3 and 4. In the questionnaire it was also asked whether the phytosanitary measures taken against PSTVd in solanaceous ornamentals were deemed effective (Appendix C). Seventeen NPPOs replied to the questionnaire but only thirteen reported the detection of PSTVd in solanaceous ornamentals currently or in the past. Out of these, ten considered the phytosanitary measures as effective (with a range of comments: e.g. effective, effective eradication of outbreaks, no new findings, decrease of the percentage of infected plants in commercial lots) and three did not reply to this specific question.

The emergency measures have therefore significantly impacted the presence of PSTVd in the EU traded lots of *S. jasminoides* and, to a lesser extent, of *Brugmansia* spp. Despite this general trend, there is country to country variation (see Appendix D) These measures have likely contributed to eradication of PSTVd in nuclear stock in the most industrial production schemes of *S. jasminoides* in some EU MS (see data for Countries producing high volumes of *S. jasminoides* in the left part of Figure 7 in Appendix D). However, the pathogen appears to have already been broadly distributed in these species and is widely present in domestic situations (see right hand side of Figure 7 in Appendix D), thus limiting the potential for total eradication.

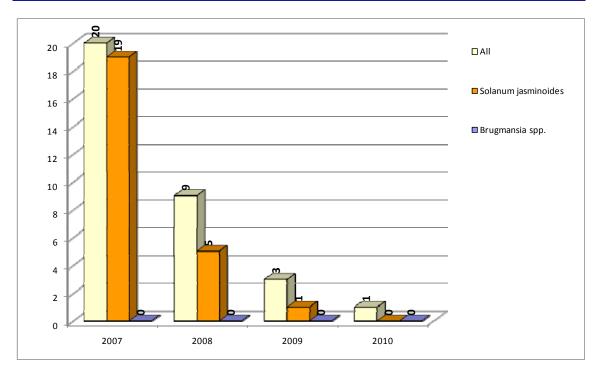
## 5.3.4.2. Spread of PSTVd within the EU

Following the decrease in PSTVd findings from the EU MS internal surveys, there has been a corresponding marked decrease in the number of interceptions in plant material of the targeted species moving within the EU (Figure 6). These data are taken from Table 20, Appendix B, where the data are further sub-divided into different host plant findings by year.

This supports the conclusion that the EU production chain for *S. jasminoides* and *Brugmansia* spp. has been significantly 'flushed through' of infected plant material and is now largely producing PSTVd-free propagation plants. This trend is confirmed also by the reduction of PSTVd incidence in samples taken from internal EU trade (from 14,5% of positive samples/tested samples in 2007 to 8,1% in 2010), as reported by FVO (FVO, 2011).

There are uncertainties related to the interpretation of both the data extacted from Europhyt and the data of the FVO report (2011), as a precise description and size of the intercepted consignment as well as the sampling methodology are not available. This can lead to difficulties in the interpretation of the prevalence of PSTVd in the EU. Notwithstanding, both datasets show that PSTVd is intercepted at a low rate in intra-EU trade, and that the incidence of these interceptions has been declining over the period of implementation of the Emergency Measures.





**Figure 6:** Total number of consignments of *S. jasminoides* and *Brugmansia* spp.intercepted because of PSTVd from within EU trade for period 2007-2010 (Source: Europhyt;, see Appendix B, Table 20).

5.3.4.3. Import of PSTVd into the EU territory

Both Europhyt (Table 18, Appendix B) and FVO reports (FVO, 2011) show a decline in the number of findings of PSTVd in plant material being imported into the EU MS from Third Countries. The same uncertainties apply to these data as mentioned above. However, the declining number of interceptions provides evidence for a lower incidence of PSTVd entering the EU MS from outside the EU territory. The emergency measures have raised awareness of the risk of this pathogen entering the area, which is evident from the increase in samples taken in 2008 and in 2009 from material entering the EU (FVO, 2011).

## 5.3.4.4. Considerations regarding the situation of PSTVd in other crops

There are two aspects to assess the effects of the EU emergency measures in crops other than *S. jasminoides* and *Brugmansia* spp. These are the effect on other ornamental species which are known hosts of PSTVd (e.g. *Petunia* sp., *Calibrachoa* sp. and *L. rantonnetii*) and the effect on outbreaks of PSTVd in food crops, mostly potato and tomato within the EU territory.

By raising awareness of asymptomatic PSTVd infections in *S. jasminoides* and *Brugmansia*, the emergency measures have had the effect of increasing research and bringing a focus on other potential hosts. Emergency measures have not specifically targeted these other hosts, and data available to cover them is consequently weaker. Extending the emergency measures to these other known hosts would likely reduce, as for *S. jasminoides* and *Brugmansia*, the prevalence of PSTVd and provide protection against this potential entry route. However, there may be other, as yet unidentified, hosts which pose a bigger potential risk than those currently identified. Alternatively, implementing measures for testing for PSTVd all Solanaceae plant material entering the EU could be difficult to justify in light of current imperfect knowledge on PSTVd host range.

There were only few recorded outbreaks of PSTVd in tomato and none in commercial potato crops during the last decade. As the number of outbreaks is very low, it is not possible to draw any meaningful conclusion on the impact of emergency measures on outbreaks in tomato.

# 5.3.4.5. Considerations on other pospiviroids

Europhyt data, as presented in Tables 19 and 21 in Appendix B, indicate that there may be a potentially greater problem with pospiviroids other than PSTVd entering the EU and being distributed within the EU territory. However, it is difficult to draw meaningful conclusions from the data presented here due to the phytosanitary status of the other pospiviroids within the EU. Since only PSTVd has a quarantine status in solanaceous crops<sup>25</sup>, the obligation to report the pathogen refers to PSTVd only. Therefore these data may be incomplete and findings of viroids other than PSTVd may be under reported to either plant health authorities in EU MS or, by plant health authorities to Europhyt or FVO.

From the replies to the EFSA questionnaire (Appendix C), five EU MS conduct surveys on one or more of the other pospiviroids, in addition to PSTVd, but this is not the general rule so that only an incomplete picture of the prevalence and interception of these viroids is available (see Section 3.1.3.2).

There have been several reports of non-PSTVd pospiviroid outbreaks in tomato within the period covered by the EU emergency measures, with no clear direct link between the implementation of the emergency measures and the reporting of these findings. However, it is probably the case that, in some countries at least, the emergency measures have raised the awareness for the presence of other pospiviroids, as shown by the increased number of reports in Europhyt. This is further supported by the fact that, in the FVO surveys 2010 report, outbreaks of TASVd in *S. jasminoides* and *Brugmansia* were reported in one EU MS and of TCDVd in *Petunia* in two EU MS.

## 5.4. Conclusion

#### 5.4.1. Conclusions on risk management options in general

The identified and evaluated management options described in Sections 4 and 5 aim at reducing the risk of entry and spread of pospiviroids within the EU and at limiting their impact. Effectiveness of the identified measures and their feasibility are compiled in Tables 11 to 15. A unique feature of pospiviroid infections in solanaceous ornamentals is the lack of visible symptoms hence complicating surveillance and phytosanitary control of production processes and consignments, whereas in potato and tomato pospiviroid infections result in variable symptoms. Natural infections of potato were only reported for PSTVd, however all solanaceous pospiviroids are infectious to potato and tomato hence present a threat similar to PSTVd for both crops.

In general given the very similar biology of pospiviroids, all analyzed management option are expected to have a very similar effectiveness on all these agents. In addition, if applied against a particular pospiviroid, most management options are expected to have an impact on any other one that might simultaneously be present.

*Sensu stricto*, pospiviroid pathogens are established with the cultivation of an infected plant hence there is no management option that can prevent establishment other than exclusion, avoidance and destruction of the infected plants. Efficient implementation of these management options requires to take into account the difficulties presented by these pathogens and their host plants or products thereof (seeds) for inspection and testing. The following key conclusions were reached:

- Pre-entry measures include import requirements comprising plant materials originating from pest free areas of production and certified free of PSTVd and other solanaceous pospiviroids.
- Implementation of import requirements has a high effectiveness and feasibility only when inspections and viroid tests are conducted with prudence following standardized procedures.

<sup>&</sup>lt;sup>25</sup> CSVd is regulated in chrysanthemum.



- Testing for pospiviroids at points of entry is highly effective with the possible exception of seed testing due to the potentially low numbers of seeds infected and to the lack of standardized seed testing methods.
- Post entry quarantine would also be highly effective for vegetative propagation material but only when all plants are tested, such as for nuclear propagation stocks.
- Subsequent surveillance and targeted inspection for pospiviroids would assure efficiency of the measure and freedom from pospiviroids.
- No management options were identified to reduce the likelihood of establishment.
- Management options to reduce probability of spread before cultivation starts and during cultivation most significantly would prescribe the use of planting material (certified) free from pospiviroids.
- Integrated crop management including disease/ pest/ vector control provides a set of measures to reduce disease spreading significantly.
- Official surveillance in nurseries accompanied by targeted testing for pospiviroids assures freedom of these pathogens in plant stocks.
- Hygiene best practice as a suite of prophylactic measures and best practices in crop management, including the use of healthy planting material certified for viroid freedom and best hygiene practices and sanitation before, during and after cultivation to reduce occurrence and spread can provide an effective control of pospiviroids. Although some individual measures can be very effective (e.g. use of certified planting material) most other measures would individually have only a partial effectiveness but their concurrent implementation will reduce the risk of pospiviroid infections to a manageable level and reduce the impact of the disease.
- Options to reduce the probability of spread and to reduce impact after an outbreak comprise destruction of infected plant materials, sanitation, cleaning, disinfection which when applied as a routine and with prudence effectively eliminate pospiviroids.
- Banning of continuous cropping and intercropping, weed and volunteer control and temporary ban of host plants are rigorous measures to prevent reinfection from alternative hosts and to keep crops free from pospiviroid infections.

As illustrated by examples from Canada to manage outbreaks of PSTVd in potato (Singh and Crowley, 1985), the rigiditiy with which specific measures are applied is crucial for successful risk management in potato. Successful eradication of PSTVd in *S. jasminoides* (De Hoop et al., 2008) shows a similar pattern. Following outbreaks, the strict adherence to prescribed measures (destruction of infected source plants, including materials for distribution, sanitation, temporary ban of host plants, etc.) and their timely implementation is most decisive for successful outbreak management.

## 5.4.2. Conclusions on the provisional emergency measures for PSTVd

The emergency measures were concerned with findings of PSTVd in the ornamental species *S. jasminoides* and *Brugmansia* spp. The available data indicate that, although surveys of different intensities have been performed by EU MS, the emergency measures have resulted in significant increases in inspection activity, focused at the reduction of this pathogen in the targeted species. There does not appear, however, to have been a corresponding increase in the numbers of samples tested. Although these activities appear to have significantly reduced the levels of PSTVd inoculum in these species, this reduction is mostly represented by a reduction in findings of infected *S. jasminoides* 



plants and not necessarily by a similar level of reduction for plants of *Brugmansia* spp. In terms of the overall level of PSTVd circulating within the EU territory, the measures significantly reduced the incidence of this pathogen, eventhough this effect has so far not been complete.

Due to the extremely low incidence of PSTVd in food crops of potato, tomato and pepper in the EU MS, it is not possible to conclude whether the reduction in PSTVd prevalence in ornamentals as a consequence of the emergency measures has also led to a reduction of outbreaks in these food crops.

A side effect of the emergency measures was to raise the general level of awareness about other pospiviroids infecting ornamentals. Reported findings of viroids such as TCDVd, TASVd, CSVd and CEVd in ornamental species now far exceed the number of PSTVd records (see Tables 20 and 21 concerning findings in internal EU trade). This is probably a result of the increased vigilance combined with the wide use of generic detection methods with broad specificity towards a range of pospiviroids. It would appear that these viroids are now increasing in their prevalence and importance within the EU, eventhough all findings may not be reported as a consequence of the non-quarantine status of non-PSTVd pospiviroids. On the other hand, this increase in reporting may also result from an increased interest on pospiviroids research during the past few years.

## 5.5. Uncertainties

### 5.5.1. Uncertainties on risk management options

Uncertainties exist over the effectiveness of inspection, sampling and testing measures at points of entry since pathogens in low concentrations in seeds and in symptomless or mixedly infected crops can escape detection. Since the relative significance of the two main pathways for pospiviroids contamination of tomato and potato crops (seed borne infection and transmission from symptomlessly infected ornamentals] is not known, uncertainty exists on the ultimate effectiveness of a strategy targeting only one of these two pathways. In particular uncertainties exist therefore over the effect of disease management in ornamentals to prevent occurrence of PSTVd in tomato and potato. There is low uncertainty on the overall effectiveness and feasibility management options to reduce impact of pospiviroids: while the effectiveness of each individual hygiene best practice measure is likely to be low, if applied as a suite of measures, they are considered to be effective, with only low uncertainty.

### 5.5.2. Uncertainties on the provisional emergency measures for PSTVd

The major area of uncertainty regarding the EU emergency measures is the interpretation and application of these measures within individual EU Member States, in particular concerning the intensity of efforts aimed at eradication following detection of PSTVd in ornamentals. It also concerns the voluntary extension by EU Member States of the emergency measures to other host plants or to other viroids. National surveys data compiled by FVO give the number of inspections conducted and number of samples tested but this does not always directly relate to crops or lots being inspected. An area of uncertainty arising from this is that although the number of inspections carried out increased three-fold, this measure alone would not have been adequate to ensure freedom from PSTVd due to the asymptomatic nature of infection in the species targeted by the measures. The number of tests carried out increased slightly, but not proportionally to the increase in number of inspections. Without knowing the number and type of samples taken and how this relates to inspections the effectiveness of surveillance measures cannot be properly evaluated.

There are also uncertainties regarding missing data in the FVO reports and missing replies to the EFSA questionnaire for some countries. Additional uncertainties exist on the side effect of emergency measures on other solanaceous pospiviroids as not all EU MS report their findings of pospiviroids other than PSTVd through the FVO surveys or Europhyt.

A high uncertainty also concerns the protection afforded by the emergency measures to EU tomato and potato crops. This results from the very limited number of PSTVd outbreaks in these crops (none



in potato), which does not allow to draw any meaningful comparisons between pre- and postemergency measures periods.

## CONCLUSIONS

After consideration of the evidence, the Panel reached the following conclusions:

#### With regard to the assessment of the risk of solanaceous pospiviroids for the EU territory:

- Four entry pathways have been identified, three of which implicate propagation material [True (botanical) seeds, Seed (potato) tubers and Plants for planting]. The fourth pathway involves plant materials not intended for planting and is considered of minor significance due to the perceived low probability of transfer to a suitable host. The uncertainties associated with this evaluation concern mostly the probability of association of the pathogens with the pathway at origin (due to the limited information available on geographical distribution and prevalence of the pospiviroids) and with the probability of transfer to a suitable host, due to the numerous parameters involved. For the three main pathways, probabilities of survival through transfer and storage, of survival through management procedures and of transfer to a suitable host are considered to be high or very high and there is little uncertainty associated with these ratings. The only limiting factor is the probability of association with the pathway at origin, which as for the pathway involving plant materials not intended for planting, carries a medium uncertainty level. Overall, the probability of entry of solanaceous pospiviroids in the EU territory through the effects of all identified pathways is considered as moderately likely.
- Given previous reports of pospiviroids in many EU Members States, the wide availability of suitable hosts, the suitability of the EU area for these agents and the inability of cultural practices and control measures to decrease the chance of establishment, the probability of establishment of solanaceous pospiviroids upon entry in the 27 EU MS is considered to be very high (certain or close to certain). This evaluation is not associated with any significant level of uncertainty.
- Within a crop species on a short distance, the probability of spread is overall evaluated as likely to very likely, with low uncertainty. The probability for transfer between crop species on a short distance is generally evaluated as being moderately likely, with high uncertainty. However, due to the lower receptivity of the potato crop and to agricultural practices that limit potato crop contacts with other susceptible crops, the probability of spread to potato is rated as very unlikely to unlikely, but with an associated high uncertainty. 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.
- Direct pest effects are expected to be markedly different for the various host plant species. The impact of solanaceous pospiviroids is expected to be major on potato, tomato and moderate on pepper. The uncertainty associated to these evaluations is low in the case of potato and tomato but medium (PSTVd and PCFVd) to high (other pospiviroids) in the case of pepper. The impact on other vegetables is expected to be minimal to minor and that on ornamental species to be minimal. The associated uncertainties are medium and low, respectively. Indirect pest effects are expected to be minimal with low uncertainties, with the exception of the impact on industries producing and commercializing plant propagation materials (seed potato tubers, true botanical seeds, plants for planting) with medium uncertainties.

With regard to the identification and evaluation of management options, these aim at reducing the risk of entry and spread of pospiviroids within the EU and to limit their impact. A unique feature of

pospiviroid infections in solanaceous ornamentals is the lack of visible symptoms hence complicating surveillance and phytosanitary control of production processes and consignments, whereas in potato and tomato pospiviroid infections result in variable symptoms. Natural infections of potato were only reported for PSTVd, however all pospiviroids are infectious to potato and tomato hence present a threat similar to PSTVd for both crops. In general given the very similar biology of pospiviroids, all analyzed management options are expected to have a very similar effectiveness against all solanaceous pospiviroids. In addition, if applied against a particular pospiviroid, most management options are established with the cultivation of an infected plant hence there is no management option that can prevent establishment other than exclusion, avoidance and destruction of the infected plants. Efficient implementation of these management options requires to take into account the difficulties presented by these pathogens and their host plants or products thereof (seeds) for inspection and testing. The following key conclusions were reached:

- Pre-entry measures include import requirements comprising plant materials originating from pest free areas of production and certified free of PSTVd and other solanaceous pospiviroids.
- Implementation of import requirements has a high effectiveness and feasibility only when inspections and viroid tests are conducted with prudence following standardized procedures.
- Testing for pospiviroids at points of entry is highly effective with the possible exception of seed testing due to the potentially low numbers of seeds infected and to the lack of standardized seed testing methods.
- Post entry quarantine would also be highly effective for vegetative propagation material but only when all plants are tested, such as for nuclear propagation stocks.
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As illustrated by examples from Canada to manage outbreaks of PSTVd in potato, the rigiditiy with which specific measures are applied is crucial for successful risk management in potato. Successful eradication of PSTVd in *S. jasminoides* show similar pattern. Following outbreaks, the strict adherence to prescribed measures (destruction of infected source plants, including materials for distribution, sanitation, temporary ban of host plants etc.) and their timely implementation is most decisive for successful outbreak management.

Uncertainties exist over the effectiveness of inspection, sampling and testing measures at points of entry since pathogens in low concentrations in seeds and symptomless crops can escape detection. Since the relative significance of the two main pathways for pospiviroids contamination of tomato and potato crops (seed borne infection and transmission from symptomlessly infected ornamentals] is not known, uncertainty exists on the ultimate effectiveness of a strategy targeting only one of these two pathways. In particular uncertainties exist therefore over the effect of disease management in ornamentals to prevent occurrence of PSTVd in tomato and potato. There is low uncertainty on the overall effectiveness of each individual hygiene best practice measure is likely to be low, if applied as a suite of measures, they are considered to be effective, with only low uncertainty.

With regard to the evaluation of the effectiveness of the provisional emergency measures, these were concerned with findings of PSTVd in the ornamental species *S. jasminoides* and *Brugmansia* spp. The available data indicate that, although surveys of different intensities have been performed by EU MS, the emergency measures have resulted in significant increases in inspection activities focused at the reduction of this pathogen in the targeted species. There does not appear, however, to have been a corresponding increase in the numbers of samples tested. Although these activities appear to have significantly reduced the levels of PSTVd inoculum in these species, this reduction is mostly represented by a reduction in findings of infected *S. jasminoides* plants and not necessarily by a similar level of reduction for plants of *Brugmansia* spp. In terms of the overall level of PSTVd circulating within the EU territory the measures significantly reduced the incidence of this pathogen, eventhough this effect has so far not been complete.

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A side effect of the emergency measures was to raise the general level of awareness about other pospiviroids infecting ornamentals. Reported findings of viroids such as TCDVd, TASVd, CSVd and CEVd in ornamental species now far exceed the numbers of PSTVd records. This is probably a result of the increased vigilance combined with the wide use of generic detection methods with broad specificity towards a range of pospiviroids. It would appear that these viroids are now increasing in their prevalence and importance within the EU, eventhough all findings may not be reported as a consequence of the non-quarantine status of non-PSTVd pospiviroids. On the other hand, this increase in reporting may also result from an increased interest on pospiviroids research during the past few years.

The major area of uncertainty regarding the EU emergency measures is the interpretation and application of these measures within individual EU Member States, in particular concerning the intensity of efforts aimed at eradication following detection of PSTVd in ornamentals. It also concerns the voluntary extension by EU Member States of the emergency measures to other host plants or to other viroids. National surveys data compiled by FVO give the number of inspections conducted and number of samples tested but this does not always directly relate to crops or lots being inspected. An area of uncertainty arising from this is that although the number of inspections carried out increased

three-fold, this measure alone would not have been adequate to ensure freedom from PSTVd due to the asymptomatic nature of infection in the species targeted by the measures. The number of tests carried out increased slightly, but not proportionally to the increase in number of inspections. Without knowing the number and type of samples taken and how this relates to inspections the effectiveness of surveillance measures cannot be properly evaluated. There are also uncertainties regarding missing data in the FVO reports and missing replies to the EFSA questionnaire for some Countries. Additional uncertainties exist on the side effect of emergency measures on other solanaceous pospiviroids because not all EU Member States report to FVO or Europhyt findings of pospiviroids other than PSTVd. High uncertainty also concerns the protection afforded by the emergency measures to the EU tomato and potato crops. This is because of the very limited number of PSTVd outbreaks in these crops (none in potato), which does not allow to draw meaningful comparisons between pre and postemergency measures periods.

#### **DOCUMENTATION PROVIDED TO EFSA**

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## APPENDICES

#### A. SCORING SYSTEM

In order to follow the principle of transparency as described under Paragraph 3.1 of the Guidance document on the harmonised framework for risk assessment [EFSA Panel on Plant Health (PLH), 2010] – "...*Transparency requires that the scoring system to be used is described in advance. This includes the number of ratings, the description of each rating.... the Panel recognises the need for further development..."*– the Plant Health Panel has developed specifically for this opinion rating descriptors to provide clear justification when a rating is given.

#### Ratings used in the conclusion of the pest risk assessment

In this opinion of EFSA's Plant health Panel for the risk assessment of solanaceous Pospiviroids and the evaluation of the effectiveness of the management options, a rating system of five levels with their respective descriptors has been used to formulate separately the conclusions on entry, establishment, spread, and impact as described in the following tables.

#### Rating of probability of entry

Rating for entry via a pathway	Descriptors for solanaceous pospiviroids
Very unlikely	<ul> <li>The likelihood of entry would be very low because the pest: <ol> <li>is not or only very rarely associated with the pathway at the origin</li> <li>cannot survive during transport or storage</li> <li>cannot survive the current pest management procedures existing in the risk assessment area</li> <li>cannot transfer to a suitable host in the risk assessment area.</li> </ol> </li> </ul>
Unlikely	<ul> <li>The likelihood of entry would be low because the pest:</li> <li>1. is rarely associated with the pathway at the origin;</li> <li>2. can survive at very low rate during transport or storage;</li> <li>3. is strongly limited by the current pest management procedures existing in the risk assessment area;</li> <li>4. has effective limitations for transfer to a suitable host in the risk assessment area.</li> </ul>
Moderat ely likely	<ul> <li>The likelihood of entry would be moderate because the pest:</li> <li>1. is occasionally associated with the pathway at the origin;</li> <li>2. can survive at low rate during transport or storage;</li> <li>3. is limited by the current pest management procedures existing in the risk assessment area;</li> <li>4. has some limitations for transfer to a suitable host in the risk assessment area.</li> </ul>
Likely	<ul> <li>The likelihood of entry would be high because the pest:</li> <li>1. is frequently associated with the pathway at the origin;</li> <li>2. can survive during transport or storage;</li> <li>3. is unlikely to be limited by the current pest management procedures existing in the risk assessment area</li> <li>4. has very few limitations for transfer to a suitable host in the risk assessment area</li> </ul>
Very likely	The likelihood of entry would be very high because the pest: 1. is always or almost always associated with the pathway at the



<ul> <li>origin;</li> <li>2. always survives during transport or storage;</li> <li>3. is not limited by the current pest management procedures existing in the risk assessment area; and/or</li> <li>4. has no limitations for transfer to a suitable host in the risk</li> </ul>	
assessment area.	

# Rating of probability of establishment

Rating for establish ment	Descriptors for solanaceous pospiviroids
Very unlikely	The likelihood of establishment would be very low because of: absence or very limited availability of host plants; unsuitable environmental conditions; occurrence of other considerable obstacles preventing establishment.
Unlikely	The likelihood of establishment would be low because of limited availability of host plants; unsuitable environmental conditions over the majority of the risk assessment area; occurrence of other obstacles preventing establishment.
Moderat ely likely	The likelihood of establishment would be moderate because hosts plants are abundant in few areas of the risk assessment area; environmental conditions are suitable in few areas of the risk assessment area; no obstacles to establishment occur.
Likely	The likelihood of establishment would be high because hosts plants are widely distributed in some areas of the risk assessment area; environmental conditions are suitable in some areas of the risk assessment area; no obstacles to establishment occur. Alternatively, the pest has already established in some areas of the risk assessment area
Very likely	The likelihood of establishment would be very high because: hosts plants are widely distributed; environmental conditions are suitable over the majority of the risk assessment area; no obstacles to establishment occur. Alternatively, the pest has already established in the risk assessment area.

# Rating of probability of spread

Rating for spread	Descriptors for solanaceous pospiviroids	
Very	The likelihood of spread would be very low because the pest:	
unlikely	i) has only one, specific way to spread (e.g., a specific vector, specific	
	assisting virus) which is not present in the risk assessment area,	
	ii) highly effective barriers to spread exist,	
	iii) the hosts are not or very rarely present in the area of possible spread	
Unlikely	The likelihood of spread would be low because the pest:	
	i) has one to few, specific ways to spread (e.g., specific vectors, specific	
	assisting virus) and their occurrence in the risk assessment area is rare,	
	ii) effective barriers to spread exist,	
	iii) the hosts are occasionally present	
Moderately	The likelihood of spread would be moderate because the pest:	
likely	i) has few, specific ways to spread (e.g., specific vectors, specific assisting	
	virus) and their occurrence in the risk assessment area is limited,	
	ii) partially effective barriers to spread exist,	
	iii) the hosts are abundant in few parts of the risk assessment area.	



Likely	The likelihood of spread would be high because the pest:	
	iv) has some, non-specific ways to spread (mechanical transmission),	
	which occur in the risk assessment area,	
	v) no effective barriers to spread exist,	
	vi) the hosts are widely present in some parts of the risk assessment area	
Very likely	The likelihood of spread would be very high because the pest:	
	i) has multiple, non-specific ways to spread (mechanical transmission),	
	which all occur in the risk assessment area,	
	ii) no effective barriers to spread exist,	
	iii) the hosts are widely present in the whole risk assessment area	

Rating of magnitude of the potential consequences on crops (environmental consequences not included)

Rating of	Descriptors for solanaceous pospiviroids
potential	
conseque	
nces	
Minimal	Differences in crop production (saleable fruits, tubers, plants for planting, seed) are within normal day to day variation; no additional control measures are required.
Minor	Crop production (saleable fruits, tubers, plants for planting, seed) is rarely reduced or at a limited level; additional control measures are rarely necessary.
Moderat e	Crop production (saleable fruits, tubers, plants for planting, seed) is occasionally reduced at a limited level; additional control measures are occasionally necessary.
Major	Crop production (saleable fruits, tubers, plants for planting, seed) is frequently reduced at a significant level; additional control measures are frequently necessary
Massive	Crop production (saleable fruits, tubers, plants for planting, seed) is always or almost always reduced at a very significant level (severe crop losses which compromise the harvest); additional control measures are always necessary.

### Ratings used for the evaluation of the management options

The Panel developed the following ratings with their corresponding descriptors for evaluating the effectiveness of the risk management options to reduce the level of risk.

Rating of the effectiveness of risk management options

Rating	Descriptors for solanaceous Pospiviroids	
Negligibl	The management option has no practical effect in reducing the probability of	
е	entry or establishment or spread, or the potential consequences.	
Low	The management option reduces the probability of entry or establishment or	
	spread, or the potential consequences by a limited extent.	
Moderat	The management option reduces the probability of entry or establishment or	
е	spread, or the potential consequences by a substantial extent.	
High	The management option reduces the probability of entry or establishment or	
	spread, or the potential consequences by a major extent.	
Very	The management option essentially eliminates the probability of entry or	



#### *High* establishment or spread, or any potential consequences.

Rating of the technical feasibility of risk management options

Rating	Descriptors for solanaceous Pospiviroids	
Negligible	The management option is not in use in the risk assessment area, and the many technical difficulties they has(e.g., changing or abandoning the current practices, implement new practices and or measures) make its implementation practically impossible.	
Low	The management option is not in use in the risk assessment area, and the many technical difficulties they have (e.g., changing or abandoning the current practices, implement new practices and or measures) make its implementation very difficult or nearly impossible.	
Moderate	The management option is not in use in the risk assessment area, and it can be implemented (e.g., changing or abandoning the current practices, implement new practices and or measures) with some technical difficulties.	
High	The management option is not in use in the risk assessment area, but it can be readily implemented (e.g., changing or abandoning the current practices, implement new practices and or measures) with limited technical difficulties.	
Very high	The management option is already in use in the risk assessment area or can be easily implemented.with no technical difficulties	

# **Rating for uncertainty**

Rating	Descriptors	
Low	No or few information or data are missing, incomplete, inconsistent or conflicti No subjective judgement is introduced. No unpublished data are used.	
Medium	Some information or data are missing, incomplete, inconsistent or conflicting. Subjective judgement is introduced with supporting evidence. Unpublished data are sometimes used.	
High	Most parts of information or data are missing, incomplete, inconsistent or conflicting. Subjective judgement may be introduced without supporting evidence. Unpublished data are frequently used.	



## **B.** TABLES

 Table 16:
 Natural host plants of solanaceous pospiviroids

Potato spindle tuber viroid	Brugmansia sanguinea	Verhoeven et al., 2010c
	Brugmansia suaveolens	Verhoeven et al., 2008a
	Brugmansia x candida	Verhoeven et al., 2010c
	Brugmansia x flava	Verhoeven et al., 2010c
	Capsicum annuum	Lebas et al., 2005
	Chrysanthemum sp.	Lemmetty et al., 2011
	Petunia sp.	Mertelik et al., 2010
	Physalis peruviana	Verhoeven et al., 2009a
	Solanum lycopersicum	Puchta et al., 1990
	Solanum muricatum	Shamloul et al., 1997
	Solanum pseudocapsicum	Lemmetty et al., 2011
	Solanum tuberosum	Diener and Raymer, 1967
	Streptosolen jamesonii	Verhoeven et al., 2008b
	Calibrachoa sp.	Verhoeven, 2010
	Lycianthes rantonnetii	Di Serio, 2007
	Datura sp.	Verhoeven et al., 2010c
	Solanum jasminoides	Verhoeven et al., 2008a
Chrysanthemum stunt viroid	Ageratum sp.	NCBI sequence database <sup>26</sup>
	Argyranthemum frutescens	Menzel and Maiss, 2000
	Chrysanthemum x morifolium	Diener & Lawson, 1973
	Dahlia sp.	NCBI sequence data <sup>27</sup>
	Pericallis x hybrida	Verhoeven, 2010
	Petunia sp.	Verhoeven et al., 1998
	Solanum jasminoides	Verhoeven et al., 2006b
	Verbena sp.	Bostan et al., 2004
	Vinca major	Bostan et al., 2004; Nie et al., 2005
Citrus exocortis viroid	Brassica napus	Fagoaga & Duran-Vila, 1996
	Cestrum sp.	Luigi et al., 2011
	Citrus spp.	Diener, 1979

 <sup>&</sup>lt;sup>26</sup> <u>http://www.ncbi.nlm.nih.gov/nuccore/1122271</u>
 <sup>27</sup> <u>http://www.ncbi.nlm.nih.gov/nuccore/AB255880.1</u>



	Daucus carota	Fagoaga and Duran-Vila, 1996
	Vicia faba	Fagoaga et al., 1995
	Glandularia pulchetla	Singh et al., 2006
	Impatiens sp.	Bostan et al., 2004; Nie et al., 2005
	Lycianthes rantonnetii	Luigi et al. 2011
	Solanum jasminoides	Verhoeven et al., 2008c; Torchetti et al., 2011
	Solanum lycopersicum	Mishra et al., 1991
	Solanum melongena	Fagoaga and Duran-Vila, 1996
	Verbena sp.	Singh et al., 2006
Columnea latent viroid	Brunfelsia undulata	Spieker, 1996b
	Columnea erythrophye	Hammond et al., 1989
	Gloxinia gymnostoma	Nielsen and Nicolaisen, 2010
	Gloxinia nematanthodes	Nielsen and Nicolaisen, 2010
	Gloxinia purpurascens	Nielsen and Nicolaisen, 2010
	Nematanthus wettsteinii	Singh et al., 1992b
	Solanum lycopersicum	Verhoeven et al., 2004
	Solanum stramonifolium	Genbank database <sup>28</sup>
Mexican papita viroid	Solanum cardiophyllum	(Martinez-Soriano et al., 1996)
	Solanum lycopersicum	Ling & Bledsoe, 2009
Pepper chat fruit viroid	Capsicum annuum	Verhoeven et al., 2009b
Tomato apical stunt viroid	Cestrum sp.	Verhoeven et al., 2008a
	Lycianthes rantonnetii	Verhoeven et al., 2010b
	Solanum jasminoides	Verhoeven et al., 2008
	Solanum lycopersicum	Candresse et al., 1987; Walter, 1981
	Solanum pseudocapsicum	Spieker et al., 1996
	Streptosolen jamesonii	Verhoeven et al., 2010b
Tomato chlorotic dwarf viroid	Brugmansia sanguinea	Verhoeven et al., 2010c
	Petunia hybrida	Verhoeven et al., 2007b
	Pittosporum tobira	Verhoeven, 2010
	Verbena sp.	Singh et al., 2006

<sup>&</sup>lt;sup>28</sup> <u>http://www.ncbi.nlm.nih.gov/nuccore/JF742634.1</u>



	Vinca minor	Singh & Dilworth, 2009
	Solanum lycopersicum	Singh et al., 1999
Tomato planta macho viroid	Solanum lycopersicum	Galindo et al., 1982

Table 17:	Experimental host plants (susceptible hosts), other than tomato and potato, of solanaceous
pospiviroid	ls

Chrysanthemum stunt viroid	Achillea millefolium	Diener, 1979
	Achillea ptarmiea	Diener, 1979
	Ambrosia trifida	Diener, 1979
	Anthemis tinetoria	Diener, 1979
	Centaurea cyanus	Diener, 1979
	Chrysanthemum carinatum	Diener, 1979
	Chrysanthemum cinerariaefolium	Diener, 1979
	Chrysanthemllm coccineum	Diener, 1979
	Chrysanthemum coronarium	Diener, 1979
	Chrysanthemum corymbosum	Diener, 1979
	Chrysanthemum frutescens	Diener, 1979
	Chrysanthemum hortorum	Diener, 1979
	Chrysanthemum lacustre	Diener, 1979
	Chrysanthemum leucanthemum	Diener, 1979
	Chrysanthemum majus	Diener, 1979
	Chrysanthemum maximum	Diener, 1979
	Chrysanthemum morifolium	Diener, 1979
	Chrysanthemum myconis	Diener, 1979
	Chrysanthemum nivetlei	Diener, 1979
	Chrysanthemum parthenium	Diener, 1979
	Chrysanthemum parthenium f. flosculosum	Diener, 1979
	Chrysanthemum praealtum	Diener, 1979
	Chrysanthemum sp.	Diener, 1979
	Chrysanthemum viscosum	Diener, 1979
	Dahlia pinnata	Diener, 1979
	Dahlia variabilis	Diener, 1979



	Echinacea purpurea	Diener, 1979
	Emilia sagittata	Diener, 1979
	Hetiopsis pitcheriana	Diener, 1979
	Liatris pycnostachia	Diener, 1979
	Liatris spicata	Diener, 1979
	Sanvitalia procumbens	Diener, 1979
	Senecio cruentus	Diener, 1979
	Senecio glastifolius	Diener, 1979
	Senecio mikanioides	Diener, 1979
	Solanum esculentum	Verhoeven et al., 1998
	Solanum tuberosum	J.Th.J. Verhoeven, Plant protection Service of The Netherlands, personal communication, March 2011
	Sonchus asper	Diener, 1979
	Tanacetum boreale	Diener, 1979
	Tanacetum camphoratum	Diener, 1979
	Tanacetum vulgare	Diener, 1979
	Tithonia rotundifolia	Diener, 1979
	Venidium fastuosum	Diener, 1979
	Yerbesina encetioides	Diener, 1979
	Zinnia etegans	Diener, 1979
Citrus exocortis viroid	Gynura aurantiaca	Diener, 1979
	Gynura sarmentosa	Diener, 1979
	Petunia axillaris	Diener, 1979
	Petunia hybrida	Diener, 1979
	Petunia violacea	Diener, 1979
	Physalis floridana	Diener, 1979
	Physalis ixocarpa	Diener, 1979
	Physalis peruviana	Diener, 1979
	Scopolia carniolica	Diener, 1979
	Scopolia lurida	Diener, 1979
	Scopolia physaloides	Diener, 1979
	Scopolia sinensis	Diener, 1979



	Scopolia stramonifolia	Diener, 1979
	Scopolia tangutica	Diener, 1979
	Solanum aculeatissimum	Diener, 1979
	Solanum dulcamara	Diener, 1979
	Solanum hispidum	Diener, 1979
	Solanum integrifolium	Diener, 1979
	Solanum marginatum	Diener, 1979
	Solanum quitoense	Diener, 1979
	Solanum topiro	Diener, 1979
	Solanum tuberosum	Semancik et al., 1973;Verhoeven et al., 2004
Columnea latent viroid	Amaranthus retroflexus	Matousek et al., 2007b
	Anthemis arvensis	Matousek et al., 2007b
	Datura stramonium	Verhoeven et al., 2011b
	Cucumis sativus	Hammond et al., 1989
	Nicandra physaloides	Verhoeven et al., 2011b
	Nicotiana benthamiana	Verhoeven et al., 2011b
	Nicotina glutinosa	Verhoeven et al., 2011b
	Solanum tuberosum	Verhoeven et al., 2004
Mexican papita viroid	Datura stramonium	Verhoeven et al., 2011b
	Nicandra physaloides	Verhoeven et al., 2011b
	Nicotina benthamiana	Verhoeven et al., 2011b
	Nicotiana glutinosa	Martinez-Soriano et al., 1996
	Solanum tuberosum	J.Th.J. Verhoeven, Plant protection Service of The Netherlands, personal communication, March 2011
Pepper chat fruit viroid	Brugmansia suaveolens	Verhoeven et al., 2009b
	Lycianthes rantonnetii	Verhoeven et al., 2009b
	Solanum lycopersicum	Verhoeven et al., 2009b
	Solanum jasminoides	Verhoeven et al., 2009b
	Solanum melongena	Verhoeven et al., 2009b
	Solanum tuberosum	Verhoeven et al., 2009b



Potato spindle tuber viroid	Amaranthus retroflexus	Matousek et al., 2007b
	Anthemis arvensis	Itaya et al., 2007; Matousek et al., 2007b
	Arabidopsis thaliana	Diener, 1979; Matousek et al., 2004
	Atropa betladonna	Diener, 1979
	Browallia demissa	Diener, 1979
	Browallia grandiflora	Diener, 1979
	Browallia speciosa	Diener, 1979
	Browallia viscosa	Diener, 1979
	Datura stramonium	Diener, 1979
	Dianthus barbatus	Diener, 1979
	Diascia barberae	Diener, 1979
	Gomphrena globosa	Diener, 1979
	Gynura aurantiaca	Penaiglesias and Vecino, 1986
	Lycianthes rantonnetii	Diener, 1979
	Lycopersicon glandulosum	Singh and Obrien, 1970
	Lycopersicon hirsutum	Singh and Obrien, 1970
	Lycopersicon peruvianum	Singh and Obrien, 1970
	Lycopersicon pimpinetlifolium	Diener, 1979
	Matricaria chamomilla	Matousek et al., 2007b
	Myosotis Jylvotica	Diener, 1979
	Nemesia foetens	Diener, 1979
	Nemesia sp. "Carnival"	Diener, 1979
	Nicandra physaloides	Diener, 1979
	Nicotiana tecana	Diener, 1979
	Nicotiana alata	Diener, 1979
	Nicotiana benthamiana	Verhoeven et al., 2011b
	Nicotiana bonariensis	Diener, 1979
	Nicotiana chinensis	Diener, 1979
	Nicotiana clevetandii	Diener, 1979
	Nicotiana clevelandii x Nicotiana glutinosa	Diener, 1979
	Nicotiana debneyi	Diener, 1979
	Nicotiana glauca	Diener, 1979



Nicotiana glutinosa	Diener, 1979
 Nicotiana knightiana	Diener, 1979
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 Nicotiana langdsorffii	Diener, 1979
Nicotiana longiflora	Diener, 1979
Nicotiana megalosiphon	Diener, 1979
Nicotiana nudicaulis	Diener, 1979
Nicotiana paniculata	Diener, 1979
Nicotiana plumbaginifolia	Diener, 1979
Nicotiana quadrivalvis	Diener, 1979
Nicotiana raimondii	Diener, 1979
Nicotiana repanda	Diener, 1979
Nicotiana rotundifolia	Diener, 1979
Nicotiana rustica	Diener, 1979
Nicotiana solanifolia	Diener, 1979
Nicotiana tabacum	Matousek et al., 1993; Wassenegger et al., 1996
Nicotiana viscosa	Diener, 1979
Nicotiana x sanderae	Diener, 1979
Nicotiana goodspeedii	Diener, 1979
Nicotina sylvestris	Diener, 1979
Nicotiana bigetovii	Diener, 1979
Nierembergia coerulea	Diener, 1979
Nolana sp. "Lavender Gown"	Diener, 1979
Penstemon richartsonii	Diener, 1979
Penstemon sp.	Diener, 1979
Petunia axillaris	Diener, 1979
Petunia hybrida	Diener, 1979
Petunia inflata	Diener, 1979
Petunia violacea	Diener, 1979
Physais pruinosa	Diener, 1979
Physalis alkekengi	Diener, 1979
Physalis angulata	Diener, 1979
Physalis floridana	Diener, 1979
Physalis franchetii	Diener, 1979
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Physalis heterophylla	Diener, 1979
Physalis ixocarpa	Diener, 1979
Physalis minima	Diener, 1979
Physalis parviflora	Diener, 1979
Physalis peruviana	Diener, 1979
Physalis philadetphica	Diener, 1979
Physalis somnlifera	Diener, 1979
Physalis viscosa	Diener, 1979
Raphanus sativa	Matousek et al., 2004
Salanum memphiticum	Diener, 1979
Salpiglossis sinuata	Diener, 1979
Salpiglossis spinescens	Diener, 1979
Saracha jaltomata	Diener, 1979
Saracha umbetlata	Diener, 1979
Scabiosa japonica	Diener, 1979
Schizanthus pinnatus	Diener, 1979
Schizanthus retusus	Diener, 1979
Scopolia anomala	Diener, 1979
Scopolia corniolica	Diener, 1979
Scopolia corniolica	Diener, 1979
Scopolia lurida	Singh, 1970b; Singh, 1973
Scopolia physaloides	Diener, 1979
Scopolia sinensis	Singh, 1970b; Singh, 1973
Scopolia stramonifolia	Singh, 1973
Scopolia tangutica	Singh, 1973
Solanum acaule	Singh, 1973
Solanum aethiopicum	Diener, 1979
Solanum alatum	Diener, 1979
 Solanum americanum	Diener, 1979
Solanum atriplicifolium	Diener, 1979
Solanum auriculatum	Diener, 1979
Solanum aviculare	Singh, 1970b;Singh, 1973
Solanum berthaulii	Easton and Merriam, 1963; Singh and Obrien, 1970



Solanum boliviense	Easton and Merriam, 1963;
Solanum bonariense	Singh and Obrien, 1970 Diener, 1979
Solanum bulbocastanum	Easton and Merriam, 1963
 Solanum carolinense	Diener, 1979
 Solanum cervantesii	Diener, 1979
Solanum chlorocarpum	Diener, 1979
Solanum ciliatum	Diener, 1979
Solanum cornutum	Diener, 1979
Solanum depilatum	Singh, 1970b;Singh, 1973
Solanum diflorum	Diener 1979
Solanum dulcamara	Diener 1979
Solanum famatinae	Easton and Merriam, 1963; Singh and Obrien, 1970
Solanum goniocalyx	Easton and Merriam, 1963; Singh and Obrien, 1970
Solanum gracile	Diener, 1979
Solanum guineense	Diener, 1979
Solanum hendersonii	Diener, 1979
Solanum hibiscifolium	Diener, 1979
Solanum humile	Diener, 1979
Solanum judaicum	Diener, 1979
Solanum kitaibetii	Diener, 1979
Solanum kurtzianum	Easton and Merriam, 1963; Singh and Obrien, 1970
Solanum laciniatum	Diener, 1979
Solanum luteum	Diener, 1979
Solanum maglia	Easton and Merriam, 1963; Singh and Obrien, 1970
Solanum maritimum	Diener, 1979
Solanum nigrum	Diener, 1979
Solanum nitidibaccatum	Diener, 1979
Solanum nodiflorum	Diener, 1979
Solanum ochroleucum	Diener, 1979
Solanum olgae	Diener, 1979
Solanum ottonis	Diener, 1979
Solanum papita	Diener, 1979



	Solanum paranense	Diener, 1979	
	Solanum persicum	Diener, 1979	
	Solanum polyadenium	Easton and Merriam, 1963; Singh and Obrien, 1970	
	Solanum polytrichon	Easton and Merriam, 1963; Singh and Obrien, 1970	
	Solanum pseudo-capsicum	Diener, 1979	
	Solanum pyracanthum	Diener, 1979	
	Solanum rostratum	Diener, 1979	
	Solanum saponaceum	Diener, 1979	
	Solanum sinaicum	Diener, 1979	
	Solanum sisymbrifolium	Diener, 1979	
	Solanum sodomeun	Diener, 1979	
	Solanum surattense	Diener, 1979	
	Solanum tomentosum	Diener, 1979	
	Solanum tripartitum	Singh, 1970b; Singh, 1973	
	Solanum umbetlatum	Diener, 1979	
	Solanum verbascifolium	Diener, 1979	
	Solanum vernei	Easton and Merriam, 1963; Singh and Obrien, 1970	
	Solanum capsicastrum	Diener, 1979	
	Soltlnum decipiens	Diener, 1979	
	Valeriana officinalis	Diener, 1979	
	Veronica agrestis	Matousek et al., 2007b	
Tomato apical stunt viroid	Nicotiana benthamiana	Antignus et al., 2002	
	Nicotiana sylvestris	Antignus et al., 2002	
	Nicotiana rustica	Antignus et al., 2002	
	Nicotiana tabacum cv. Samsun	Antignus et al., 2002	
	Nicotiana tabacum cv. White Burley	Antignus et al., 2002	
	Physalis floridana	Antignus et al., 2002	
	Solanum tuberosum	Verhoeven and Roenhorst, 2010	
Tomato chlorotic dwarf viroid	Capsicum annuum	Matsushita et al. 2009	
	Chrysanthemum coronarium	Matsushita et al., 2009	



	Datura metel	Singh et al., 1999		
	Datura stramonium	Verhoeven et al., 2011b		
	Gomphrena globosa	Singh et al., 1999		
	Nicotiana clevelandii	Matsushita et al., 2009		
	Nicotiana debneyi	Singh et al., 1999		
	Nicotiana glutinosa	Matsushita et al., 2009		
	Nicotiana occidentalis	Matsushita et al., 2009		
	Nicotiana physaloides	Singh et al., 1999		
	Nicotiana rustica	Matsushita et al., 2009		
	Nicotiana tabacum cv. Samsun	Matsushita et al., 2009		
	Nicotiana tabacum Xanthi-nc	Matsushita et al., 2009		
	Nicandra physaloides	Singh et al., 1999		
	Nicotiana benthamiana	Matsushita et al., 2009		
	Nicotiana debneyi	Singh et al., 1999		
	Nicotiana glutinosa	Matsushita et al., 2009		
	Nicotiana tabacum cv. White Burley	Verhoeven et al., 2011b		
	Nicotina benthamiana	Matsushita et al., 2009		
	Petunia floridana	Matsushita et al., 2009		
	Petunia hybrida	Matsushita et al., 2009		
	Physalis angulata	Singh et al., 1999		
	Physalis floridana	Matsushita et al., 2009		
	Scopolia sinensis	Singh et al., 1999		
	Leucanthemum paludosum 'North Pole'	Matsushita et al., 2009		
	Solanum melongena	Matsushita et al., 2009		
	Solanum mammosum	Matsushita et al., 2009		
	Solanum nigrum	Matsushita et al., 2009		
	Solanum demissum	Singh et al., 1999		
	Solanum carolinense	Matsushita et al., 2009		
	Solanum tuberosum	Verhoeven and Roenhorst, 2010		
Tomato planta macho viroid	Datura stramonium	Verhoeven et al., 2011b		
	Gomphrena globosa	MartinezSoriano et al., 1996		



Gynura aurantiaca	Galindo et al., 1982
Nicandra physaloides	Verhoeven et al., 2011b
Nicotiana benthamiana	Verhoeven et al., 2011b
Nicotiana glutinosa	Verhoeven et al., 2011b
Solanum tuberosum	J.Th.J. Verhoeven, Plant protection Service of The Netherlands, personal communication, March 2011



Species	Matrix	Pospiviroid	oid Number of consignments intercepted from Third Countries at EU entry points				
			Before 2007	2007	2008	2009	2010
Calibrachoa sp.	Cuttings	PSTVd	-	-	-	1	-
Cestrum sp.	not yet planted	PSTVd	-	2	-	-	-
Lycopersicon esculentum (tomato)	Seeds	PSTVd	4	2	-	-	-
Petunia sp.	Cuttings	PSTVd	-	-	1	1	3
Solanum jasminoides	Cuttings/ not yet planted	PSTVd	3	4	-	-	-
Solanum sp.	already planted	PSTVd	-	1	-	-	-
Solanum tuberosum	potato tubers for breeding	PSTVd	1 (+ PVX)	-	-	-	-
Total		PSTVd	8	9	1	1	3

**Table 18:** Interceptions of PSTVd from Third Countries reported by EU MS in the Europhyt database (period 1997-2010).

**Table 19:** Interceptions of pospiviroids other than PSTVd from Third Countries reported by EU MS in the Europhyt database (period 1997-2010).

Species	Matrix	Pospiviroid	Number of consignments intercepted from Third Countries at EU entry points				
			Before 2007	2007	2008	2009	2010
Petunia	Cuttings	TCDVd	-	-	-	10	10
<i>Dendranthema</i> sp. and <i>Chrysanthemum</i> sp.	Cuttings/already planted	CSVd	3	-	-	-	1
Total			3	-	-	10	11



Species	Matrix	Pospiviroid	Number of consignments intercepted from other EU MS					
			Before 2007	2007	2008	2009	2010	
Brugmansia spp.	Already planted	PSTVd	1	-	-	-	-	
Datura arborea	Already plantes	PSTVd	-	-	-	1	1	
Lycopersicon sp.	Already planted	PSTVd	-	-	1	-	-	
Petunia	Already planted	PSTVd	-	-	2	-	-	
Solanum jasminoides	Already planted/not yet planted	PSTVd	1	19	5	1	-	
Lycianthes rantonnetii	Already planted	PSTVd	-	1	1	1	-	
Solanum sp.	Already planted	PSTVd	1	-	-	-	-	
Total		PSTVd	3	20	9	3	1	

**Table 20:** Interceptions of PSTVd from other EU MS Countries reported by EU MS in the Europhyt database (period 1997-2010).

Table 21: Interceptions of pospiviroids other than PSTVd from other EU MS Countries reported by EU MS in the Europhyt database (period 1997-2010).

Species	Matrix	Pospiviroid	Number of consignments intercepted from other EU MS					
			Before 2007	2007	2008	2009	2010	
Solanum jasminoides	Not yet planted	CEVd	-	-	-	-	1	
Dendranthema sp./	Already planted/not yet	CSVd	4	-	-	1	-	
Chrysanthemum sp.	planted/cuttings							
Solanum jasminoides	Already planted/not yet	CSVd	-	-	-	-	1	
	planted							
Brugmansia spp.	Already planted	TASVd	-	-	-	-	2	
Solanum jasminoides	Already planted	TASVd	-	-	-	-	14	
Petunia	Cuttings	TCDVd	-	-	-	-	2	
Total			4	-	-	1	20	



# C. SUMMARIES OF REPLIES TO THE QUESTIONNAIRE TO EU MS NPPOS ON SOLANACEOUS POSPIVIROIDS

**Table 22:** Questions and summary of replies on presence and prevalence of solanaceous pospiviroids in EU MS.

Pospiviroid species	Crop/crop group					Prese	ence in y	our country	7						Prev	valence	
		Please indicate for each crop group whether the viroid is present your country (currently present; present in the past but no longer present; never detected)					ner the v	group pleas iroid was do yes/no/unkr	etected in	indic	ate whe tected ir	rop group J ther the vir 1 the greenl 0/unknown	odi was house	each vir localiz r	crop gro oid is wi ed to so arely pr	oup whe desprea	ons (L) or few
		Currently present	Present in the past, no longer present	Never detected	No info provided	Yes	No	Unknown	No info provided	Yes	No	Unknown	No info provided	W	L	R	No info provided
Potato spindle	potato	0	4	13	0	2	3	0	12	0	4	0	13	0	0	2	15
tuber viroid	potato seeds	0	1	15	1	0	4	0	13	1	2	0	14	0	0	0	17
(PSTVd)	tomato	0	5	12	0	0	6	0	11	4	2	0	11	0	0	0	17
	other solanaceous vegetables	1	2	13	1	0	5	0	12	2	2	0	13	0	0	0	17
	solanaceous ornamentals	6	7	4	0	1	10	0	6	13	1	0	3	0	2	8	7
	other hosts (please specify)	0	1	14	2	0	2	1	14	1	1	1	14	0	0	0	17
Citrus e0ocortis	potato	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
viroid (CEVd)	potato seeds	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	tomato	0	1	14	2	0	1	0	16	1	0	0	16	0	0	0	17
	other solanaceous vegetables	1	0	14	2	0	0	0	17	0	0	0	17	0	0	0	17
	solanaceous ornamentals	5	1	9	2	0	5	0	12	5	1	0	11	0	1	4	12
	citrus	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	other hosts (please specify)	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
Chrysanthemum	potato	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
stunt viroid (CSVd)	potato seeds	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
(CSVU)	tomato	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	other solanaceous vegetables	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	solanaceous ornamentals	3	3	9	2	0	5	0	12	6	0	0	11	0	1	2	14
	chrysanthemum	5	4	7	1	1	4	1	11	9	0	0	8	0	2	4	11
	other hosts (please specify)	2	1	11	3	0	1	2	14	3	0	0	14	0	1	2	14
Columnea latent	potato	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
viroid (CLVd)	potato seeds	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17



	tomato	0	3	11	3	0	3	0	14	2	1	0	14	0	0	0	17
	other solanaceous vegetables	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	solanaceous ornamentals	0	1	14	2	0	1	0	16	1	0	0	16	0	0	0	17
	other hosts (please specify)	1	0	13	3	0	0	1	16	1	0	0	16	0	1	0	16
Me0ican papita	potato	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
viroid (MPVd)	potato seeds	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	tomato	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	other solanaceous vegetables	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	solanaceous ornamentals	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	other hosts (please specify)	0	0	14	3	0	0	0	17	0	0	0	17	0	0	0	17
Pepper chat	potato	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
fruit viroid	potato seeds	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
(PCFVd)	tomato	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	other solanaceous vegetables	0	1	14	2	0	1	0	16	1	0	0	17	0	0	0	17
	solanaceous ornamentals	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	other hosts (please specify)	0	0	14	3	0	0	0	17	0	0	0	17	0	0	0	17
Tomato apical	potato	0	0	14	3	0	0	0	17	0	0	0	17	0	0	0	17
stunt viroid	potato seeds	0	0	14	3	0	0	0	17	0	0	0	17	0	0	0	17
(TASVd)	tomato	0	0	14	3	0	0	0	17	0	0	0	17	0	0	0	17
	other solanaceous vegetables	0	0	14	3	0	0	0	17	0	0	0	17	0	0	0	17
	solanaceous ornamentals	4	2	8	3	0	5	0	12	6	0	0	11	1	0	3	13
	other hosts (please specify)	0	0	13	4	0	0	0	17	0	0	0	17	0	0	0	17
Tomato	potato	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
chlorotic dwarf	potato seeds	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
viroid (TCDVd)	tomato	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	other solanaceous vegetables	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	solanaceous ornamentals	3	4	8	2	0	6	0	11	7	0	0	10	0	1	2	14
	other hosts (please specify)	3	0	10	4	0	2	1	14	3	0	0	14	0	1	2	14
Tomato planta	potato	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
macho viroid (TPMVd)	potato seeds	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
(1 PM V 0)	tomato	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	other solanaceous vegetables	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	solanaceous ornamentals	0	0	15	2	0	0	0	17	0	0	0	17	0	0	0	17
	other hosts (please specify)	0	0	14	3	0	0	0	17	0	0	0	17	0	0	0	17



Pospiviroid	Crop / crop group			]	Eradication						ytosanitary			ide your eval	
species		indi whe			please group s been KNOWN)	indica grou vi eradio	up whet roid is u cation (Y	ach crop her the	<i>pospiv</i> cr	viroid sp op/crop (YES/)	NO)			ess of the mea	sure
		Yes	No	Unknown	No info provided	Yes	No	No info provided	Yes	No	No info provided	Effective	Not effective	No experience	No info provided
Potato spindle	potato	3	0	1	13	0	1	16	6	0	11	2	0	2	2
tuber viroid	potato seeds	1	0	0	16	0	0	17	5	0	12	1	0	1	3
(PSTVd)	tomato	4	0	0	13	0	0	17	7	0	10	4	0	0	3
	other solanaceous vegetables	2	0	0	15	0	0	17	4	1	12	2	0	0	2
	solanaceous ornamentals	10	0	0	7	4	0	13	13	1	3	10	0	0	3
	other hosts (please specify)	1	0	0	16	0	0	17	4	0	13	0	0	1	3
Citrus exocortis	potato	0	0	0	17	0	0	17	2	1	14	0	0	1	1
viroid (CEVd)	potato seeds	0	0	0	17	0	0	17	1	2	14	0	0	1	0
	tomato	1	0	0	16	0	0	17	0	3	14	0	0	0	0
	other solanaceous vegetables	0	0	0	17	0	0	17	0	3	14	0	0	0	0
	solanaceous ornamentals	0	1	2	14	2	2	13	3	5	9	1	1	1	0
	citrus	0	0	0	17	0	0	17	0	3	14	0	0	0	0
	other hosts (please specify)	0	0	0	17	0	0	17	0	3	14	0	0	0	0
Chrysanthemum	potato	0	0	0	17	0	0	17	2	1	14	1	0	1	0
stunt viroid	potato seeds	0	0	0	17	0	0	17	2	1	14	0	0	1	1
(CSVd)	tomato	0	0	0	17	0	0	17	1	2	14	0	0	0	1
	other solanaceous vegetables	0	0	0	17	0	0	17	1	2	14	0	0	0	1
	solanaceous ornamentals	2	1	1	13	3	0	14	5	2	10	2	1	1	1
	chrysanthemum	3	0	0	14	4	1	12	8	2	7	5	0	0	3
	other hosts (please specify)	1	0	0	16	0	2	15	2	3	12	1	0	0	1
Columnea	potato	0	0	0	17	0	0	17	1	2	14	0	0	1	0
latent viroid	potato seeds	0	0	0	17	0	0	17	1	2	14	0	0	1	0
(CLVd)	tomato	3	0	0	14	0	0	17	2	3	12	2	0	0	0
	other solanaceous vegetables	0	0	0	17	0	0	17	0	3	14	0	0	0	0
	solanaceous ornamentals	1	0	0	16	0	0	17	0	4	13	0	0	0	0
	other hosts (please specify)	0	0	0	17	0	1	16	0	3	14	0	0	0	0
Mexican papita	potato	0	0	0	17	0	0	17	1	2	14	0	0	1	0
viroid (MPVd)	potato seeds	0	0	0	17	0	0	17	1	2	14	0	0	1	0
	tomato	0	0	0	17	0	0	17	0	3	14	0	0	0	0
	other solanaceous vegetables	0	0	0	17	0	0	17	0	3	14	0	0	0	0
	solanaceous ornamentals	0	0	0	17	0	0	17	0	3	14	0	0	0	0
	other hosts (please specify)	0	0	0	17	0	0	17	0	3	14	0	0	0	0

 Table 23:
 Questions and summary of replies on eradication and phytosanitary measures against solanaceous pospiviroids in EU MS.



Pepper chat	potato	0	0	0	17	0	0	17	1	2	14	0	0	1	0
fruit viroid	potato seeds	0	0	0	17	0	0	17	1	2	14	0	0	1	0
(PCFVd)	tomato	0	0	0	17	0	0	17	0	3	14	0	0	0	0
	other solanaceous vegetables	1	0	0	16	0	0	17	0	3	14	0	0	0	0
	solanaceous ornamentals	0	0	0	17	0	0	17	0	3	14	0	0	0	0
	other hosts (please specify)	0	0	0	17	0	0	17	0	3	14	0	0	0	0
Tomato apical	potato	0	0	0	17	0	0	17	2	1	14	0	0	1	1
stunt viroid	potato seeds	0	0	0	17	0	0	17	2	1	14	0	0	1	1
(TASVd)	tomato	0	0	0	17	0	0	17	1	2	14	0	0	0	1
	other solanaceous vegetables	0	0	0	17	0	0	17	1	2	14	0	0	0	1
	solanaceous ornamentals	1	0	2	14	2	2	13	4	3	10	2	1	1	0
	other hosts (please specify)	0	0	0	17	0	0	17	1	2	14	0	0	0	1
Tomato	potato	0	0	0	17	0	0	17	2	1	14	0	0	1	1
chlorotic dwarf	potato seeds	0	0	0	17	0	0	17	2	1	14	0	0	1	1
viroid (TCDVd)	tomato	0	0	0	17	0	0	17	1	2	14	0	0	0	1
	other solanaceous vegetables	0	0	0	17	0	0	17	1	2	14	0	0	0	1
	solanaceous ornamentals	4	0	1	12	1	2	14	5	3	9	3	1	1	0
	other hosts (please specify)	0	0	1	16	1	2	14	3	2	12	0	1	1	1
Tomato planta	potato	0	0	0	17	0	0	17	1	2	14	0	0	1	0
macho viroid	potato seeds	0	0	0	17	0	0	17	1	2	14	0	0	1	0
(TPMVd)	tomato	0	0	0	17	0	0	17	0	3	14	0	0	0	0
	other solanaceous vegetables	0	0	0	17	0	0	17	0	3	14	0	0	0	0
	solanaceous ornamentals	0	0	0	17	0	0	17	0	3	14	0	0	0	0
	other hosts (please specify)	0	0	0	17	0	0	17	0	3	14	0	0	0	0



Pospiviroid species	Crop/crop group		u perform ction? (YE			report inte ophyt? (Y		When was your most recent national survey on this viroid/crop combination? (No survey done; 2010; 2009; 2008; 2007; 2006; 2005; before 2005).					
		Yes	No	No info provided	Yes	No	No info provided	No survey done	2008	2009	2010	No info provided	
Potato spindle tuber viroid	potato	9	7	1	12	2	3	1	1	2	12	1	
(PSTVd)	potato seeds	8	7	2	11	2	4	4	0	0	10	3	
	tomato	8	9	0	11	3	3	0	0	1	14	2	
	other solanaceous vegetables	7	9	1	10	14	3	6	0	1	7	3	
	solanaceous ornamentals	9	8	0	12	4	1	1	0	1	14	1	
	other hosts (please specify)	5	9	3	9	3	5	3	0	2	6	6	
Citrus exocortis viroid	potato	3	12	2	4	4	9	9	1	0	2	5	
(CEVd)	potato seeds	3	12	2	4	4	9	8	0	0	3	6	
	tomato	2	13	2	3	5	9	7	0	1	4	5	
	other solanaceous vegetables	2	13	2	3	5	9	9	0	0	2	6	
	solanaceous ornamentals	2	13	2	3	5	9	6	0	1	5	5	
	citrus	3	12	2	3	5	9	9	0	0	1	7	
	other hosts (please specify)	2	12	3	2	5	10	8	0	1	2	6	
Chrysanthemum stunt viroid	potato	2	13	2	5	3	9	9	1	0	1	6	
(CSVd)	potato seeds	2	13	2	5	3	9	8	0	0	2	7	
	tomato	1	14	2	4	4	9	7	0	1	3	6	
	other solanaceous vegetables	1	14	2	4	4	9	9	0	0	1	7	
	solanaceous ornamentals	1	14	2	4	4	9	7	0	1	3	6	
	chrysanthemum	8	7	2	10	2	5	8	0	0	4	5	
	other hosts (please specify)	1	13	3	3	4	10	8	0	1	1	7	
Columnea latent viroid	potato	3	12	2	5	4	8	9	1	0	2	5	
(CLVd)	potato seeds	3	12	2	5	4	8	8	0	0	3	6	
	tomato	2	13	2	4	5	8	7	0	1	4	5	
	other solanaceous vegetables	2	13	2	4	5	8	9	0	0	2	6	
	solanaceous ornamentals	2	13	2	4	5	8	7	0	1	4	5	
	other hosts (please specify)	2	12	3	3	5	9	8	0	1	2	6	
Mexican papita viroid	potato	3	12	2	4	4	9	9	0	0	2	6	
(MPVd)	potato seeds	3	12	2	4	4	9	8	0	0	3	6	
	tomato	2	13	2	3	5	9	7	0	1	3	6	
	other solanaceous vegetables	2	13	2	3	5	9	9	0	0	2	6	
	solanaceous ornamentals	2	13	2	3	5	9	7	0	1	3	6	

# Table 24: Questions and summary of replies on inspections and surveys for solanaceous pospiviroids in EU MS.



	other hosts (please specify)	2	12	3	2	5	10	8	0	1	2	6
Pepper chat fruit viroid	potato	2	13	2	3	5	9	10	0	0	1	6
(PCFVd)	potato seeds	2	13	2	3	5	9	9	0	0	2	6
	tomato	1	14	2	2	6	9	8	0	1	2	6
	other solanaceous vegetables	1	14	2	2	6	9	9	0	0	2	6
	solanaceous ornamentals	1	14	2	2	6	9	8	0	1	2	6
	other hosts (please specify)	1	13	3	1	6	10	8	0	1	2	6
Tomato apical stunt viroid	potato	3	12	2	5	4	8	9	1	0	2	5
(TASVd)	potato seeds	3	12	2	5	4	8	8	0	0	3	6
	tomato	2	13	2	4	5	8	7	0	1	4	5
	other solanaceous vegetables	2	13	2	4	5	8	9	0	0	2	6
	solanaceous ornamentals	2	13	2	4	6	7	6	0	1	5	5
	other hosts (please specify)	2	12	3	3	5	9	8	0	1	2	6
Tomato chlorotic dwarf	potato	3	12	2	5	4	8	9	1	0	2	5
viroid (TCDVd)	potato seeds	3	12	2	5	4	8	8	0	0	3	6
	tomato	2	13	2	4	5	8	7	0	1	4	5
	other solanaceous vegetables	2	13	2	4	5	8	9	0	0	2	6
	solanaceous ornamentals	2	13	2	4	5	8	6	0	1	5	5
	other hosts (please specify)	2	12	3	3	5	9	8	0	1	2	6
Tomato planta macho viroid	potato	3	12	2	5	4	8	9	0	0	2	6
(TPMVd)	potato seeds	3	12	2	5	4	8	8	0	0	3	6
	tomato	2	13	2	4	5	8	7	0	1	3	6
	other solanaceous vegetables	2	13	2	4	5	8	9	0	0	2	6
	solanaceous ornamentals	2	13	2	4	5	8	7	0	1	3	6
	other hosts (please specify)	2	12	3	3	5	9	8	0	1	2	6



# **Table 25:** Questions and summary of replies on seed detection for solanaceous pospiviroids in EUMS.

Pospiviroid species	Crop/crop group		m detection of t this crop group	
		Yes	No	No info provided
Potato spindle tuber	true potato seeds	4	13	0
viroid (PSTVd)	tomato seeds	6	11	0
	seeds of other solanaceous vegetables	1	16	0
	seeds of solanaceous ornamentals	3	14	0
	seeds of other hosts	0	16	1
Citrus exocortis viroid	true potato seeds	2	13	2
(CEVd)	tomato seeds	1	14	2
	seeds of other solanaceous vegetables (please specify)	0	15	2
	seeds of solanaceous ornamentals (please specify)	0	15	2
	citrus seeds	0	15	2
	seeds of other hosts (please specify)	0	15	2
Chrysanthemum stunt	true potato seeds	1	15	1
viroid (CSVd)	tomato seeds	1	15	1
	seeds of other solanaceous vegetables (please specify)	0	16	1
	seeds of solanaceous ornamentals (please specify)	0	16	1
	chrysanthemum seeds	0	16	1
	seeds of other hosts (please specify)	0	16	1
Columnea latent viroid	true potato seeds	2	13	2
(CLVd)	tomato seeds	2	13	2
	seeds of other solanaceous vegetables (please specify)	0	15	2
	seeds of solanaceous ornamentals (please specify)	0	15	2
		0	15	2
M	seeds of other hosts (please specify)	2	_	2
<i>Mexican papita viroid</i> (MPVd)	true potato seeds		13	
(ivii vu)	tomato seeds	1	14	2
	seeds of other solanaceous vegetables (please specify)	0	15	2
	seeds of solanaceous ornamentals (please specify)	0	15	2
	seeds of other hosts (please specify)	0	14	3
Pepper chat fruit	true potato seeds	2	13	2
viroid (PCFVd)	tomato seeds	0	15	2
	seeds of other solanaceous vegetables (please specify)	0	15	2
	seeds of solanaceous ornamentals (please specify)	0	15	2
	seeds of other hosts (please specify)	0	15	2
Tomato apical stunt	true potato seeds	2	13	2
viroid (TASVd)	tomato seeds	1	14	2
	seeds of other solanaceous vegetables (please specify)	0	15	2
	seeds of solanaceous ornamentals (please specify)	0	15	2
	seeds of other hosts (please specify)	0	15	2
Tomato chlorotic	true potato seeds	2	13	2
dwarf viroid (TCDVd)	tomato seeds	3	12	2
	seeds of other solanaceous vegetables (please specify)	1	14	2
	seeds of solanaceous ornamentals (please specify)	1	14	2
	seeds of other hosts (please specify)	1	14	2
Tomato planta macho	true potato seeds	2	13	2
viroid (TPMVd)	tomato seeds	1	14	2
	seeds of other solanaceous vegetables (please specify)	0	15	2
	seeds of solanaceous ornamentals (please specify)	0	15	2
	seeds of other hosts (please specify)	1	14	2



### D. ESTIMATION OF PROBABILITY OF POTATO SPINDLE TUBER VIROID OUTBREAKS IN EUROPE

### Description of the original dataset from FVO reports

Data from the European Union Member States surveys on *Potato spindle tuber viroid* (PSTVd) conducted in the years 2007, 2008, 2009 and 2010 were extracted from the respective reports of the Food and Veterinary Office DG SANCO EC Commission (FVO 2008, 2009, 2010 and 2011).

The original data set in the FVO reports included information on:

- Reporting Member State
- Year of the survey: 2007, 2008, 2009, 2010
- Host plant
- Total production (in crops or ha)
- Number of crops/lots inspected
- Number of inspections
- Number of plants or ha inspected
- Number of crops/lots sampled
- Number of samples taken
- Number of positive samples
- Number of crops/lots with outbreaks
- Comments

No definition or legend for the fields above was available in the reports. We assume that the given data are comparable between the member states and different years, esp. that the ratio of number of outbreaks and inspections respective samples gives rough estimate of the observed rate of infection. Uncertainty remains due to the missing explanation of "crops/lots" in the reported tables.

## Data cleaning

The extracted data set included information on:

- Reporting Member State
- Year of the survey: 2007, 2008, 2009, 2010
- Host plant
- Specifications [further specifications obtained from the "host plant" and "comments" fields of the FVO reports on: host species, type of product/crop (e.g. nursery, nuclear stock, ware potatoes, etc.)]
- Number of crops/lots inspected
- Number of crops/lot sampled
- Number of crops/lot with outbreaks

For each record, missing values of the fields "Number of crops/lots inspected" and "Number of crops/lot sampled" were respectively substituted with values, when available, of the fields "Number of inspections" and "Number of samples taken" of the original data set.

Information were given for 210 combinations of country and host plants. Years without information on the number of crops/lots with outbreaks (missing information) were disregarded from the analysis.

As noted above, no definition or legend were available in the FVO reports to describe the methods used for the estimation of the number of crops/lots inspected, sampled and with outbreaks. Also crops and lots differ very much in size, e.g. varying for crops between industrial plantations and smallholdings and for lots between consignments of thousands and a few plants.



The host plants surveyed were divided for the purpose of this analysis into the following four categories:

- Solanum jasminoides
- Brugmansia spp.
- other ornamentals:, calibrachoa, campanula, celosia, chrysanthemum, cestrum, datura, gloxinia, impatiens, iochroma, lantana, *Lycianthes rantonnetii*, nemathanthus, petunia *Physalis* sp., *Solanum aculeastrum, Solanum capsicastrum, Solanum crispum, Solanum pseudocapsicum,* Streptocarpus, Streptosolen, Surfinia, Verbena, Vinca,
- solanaceous vegetables: tomato, pepper, aubergine and potatoes
- **unknown or mixed species**: including all cases where the number of inspections, samples or outbreaks was provided cumulatively for a a group of species or where information provided (e.g. indicating only the genus as in *Solanum*) did not allow allocation to the other Categories

To investigate whether there is any effect of the industry type on the reduction of PSTVd outbreaks following the implementation of current emergency measures, the reporting Member States were classified based on the volume of production of the ornamental pot plant *S. jasminoides* in two categories:

- High volumes producing countries (HP): Germany and the Netherlands
- Lower volumes producing countries (LP): all other reporting European member states

#### Short description of the dataset

Following numbers were reported from the member states

**Table 26:** Number of inspections, samples and PSTVd outbreaks per reporting EU member state (HP1 and HP2 are countries with high production of *S. jasminoides*, while LP1 to LP24 are countries with lower production of *S. jasminoides* plants)

	In	spections		Samples	PST	Vd Outbreaks
Country	abs.	rel.(%)	abs.	rel.(%)	abs.	rel.(%)
HP01	4909	3.58	4506	15.17	103	19.81
HP02	13831	10.1	16941	57.03	11	2.12
LP01	81	0.06	119	0.4	7	1.35
LP02	14	0.01	543	1.83	4	0.77
LP03	3465	2.53	270	0.91		
LP04	12	0.01	4	0.01		
LP05	1533	1.12	127	0.43	71	13.65
LP06	66	0.05	73	0.25	5	0.96
LP07	55	0.04	111	0.37	1	0.19
LP08	311	0.23	676	2.28	2	0.38
LP09	1867	1.36	602	2.03	74	14.23
LP10	296	0.22	296	1	3	0.58
LP11	69	0.05	53	0.18		
LP12	2386	1.74	324	1.09		
LP13	715	0.52	1652	5.56	69	13.27



r						
LP14	172	0.13	207	0.7		
LP15	100	0.07	100	0.34		
LP16	53	0.04	44	0.15		
LP17	163	0.12	139	0.47	1	0.19
LP18	57133	41.72	806	2.71	2	0.38
LP19	102	0.07	102	0.34	8	1.54
LP20	5723	4.18	569	1.92		
LP21	145	0.11	36	0.12	3	0.58
LP22	2600	1.9	240	0.81	77	14.81
LP23	12818	9.36	266	0.9	78	15
LP24	28318	20.68	900	3.03	1	0.19
Total	136937	100	29706	100	520	100

<b>Table 27:</b>	Number of inspections,	, samples and PSTVd	outbreaks per year from 2007 to 2010
------------------	------------------------	---------------------	--------------------------------------

	Ins	pections		Samples	Outbreaks		
Year	abs.	rel.(%)	abs.	rel.(%)	abs.	rel.(%)	
2007	17988	13.14	6395	21.53	187	35.96	
2008	32501	23.73	6868	23.12	143	27.50	
2009	34108	24.91	8234	27.72	122	23.46	
2010	52340	38.22	8209	27.63	68	13.08	
Total	136937	100	29706	100	520	100	

 Table 28:
 Number of inspections, samples and PSTVd outbreaks per host category

	Inspections			Samples		Outbreaks
Host category	abs.	rel.(%)	abs.	rel.(%)	abs.	rel.(%)
S. jasminoides	2550	1.86	1758	5.92	406	78.08
Brugmansia	1148	0.84	602	2.03	30	5.77
other ornamentals	818	0.60	360	1.21	6	1.15
solanaceous vegetables	67979	49.64	21603	72.72	13	2.50
unknown / mixed	64442	47.06	5383	18.12	65	12.50
Total	136937	100	29706	100	520	100

# Table 29: Number of PSTVd outbreaks per host category and year

		Outbreaks				
Host category	2007	2008	2009	2010	total	
S. jasminoides	138	119	93	56	406	
Brugmansia	5	9	11	5	30	
other ornamentals	0	1	0	5	6	
solanaceous vegetables	0	5	6	2	13	



unknown / mixed	44	9	12	0	65
Total	187	143	122	68	520

## Data analysis

The data analysis was done using the statistical software SAS (Version 9.2), esp. the GENMOD procedure.

#### Methods

Task of the statistical analysis was:

- to calculate the outbreak rates for each European country
- to test if there is a time effect in the outbreak rates
- to test if there are differences between high and low volume producing countries

#### **Calculation of the Rates**

For the data analysis the number of inspections, samples and outbreaks were aggregated per member state, year and host category.

No analysis was done for unknown or mixed host plants. Other ornamentals were also excluded due to insufficient data.

The rates were calculated twice:

- 1. as ratio of the number of outbreaks and the number of inspections (O/I)
- 2. as ratio of the number of outbreaks and the number of samples (O/S)

#### **Modelling Time and Production Effects**

The resulting outbreak rates were modelled using a logistic regression model with two factors for year (2007, 2008, 2009, 2010) and production volume (high, low). The variance corresponds to a binomial variation with the number of inspections or samples as weight. Overdispersion was corrected by the estimated deviance. Effects were tested using the F statistic.

It is assumed that there is no interaction between year and production volume. An autocorrelation between the years is also not included into the model. The year 2007 (with zero outbreaks) was excluded in the analysis of solanaceous vegetables to obtain convergence of the estimators.

It shall be noted that the model estimates average outbreak rates for each year and production volume. The estimates respect the number of inspections or samples per countries and reflect more the situation of countries with higher numbers of inspections or samples. The figure might be interpreted as probability for an outbreak. This implies that zero is not a possible value of the model. Especially are information on total eradication not part of the model.



# **Results**

## Outbreak rates per year and type of production

**Table 30:** Probability of outbreaks of PSTVd in high and low volume producing countries for the years 2007 to 2010 estimated by the outbreak rates calculated by the ratio of number of outbreaks and <u>inspections</u>

host category	Probability of a PSTVd outbreak (%) (estimated from PSTVd outbreaks/inspection)								
production volume	2007 2008 2009 2010								
	S	5. jasminoides							
	30.2	17.7	10.6	8.1					
low volume production	(20-43)	(12-25)	(7-17)	(5-14)					
_	21.4	12.0	7.0	5.3					
high volume production	(13-33)	(6-22)	(3-14)	(3-11)					
		Brugmansia							
	5.7	1.8	2.2	2.6					
low volume production	(2-17)	(1-4)	(1-4)	(1-7)					
	6.9	2.2	2.6	3.2					
high volume production	(2-21)	(1-6)	(1-7)	(1-9)					
	solan	aceous vegetables							
		0.01	0.01	0.00					
low volume production		(0.00-0.1)	(0.00-0.1)	(0.00-0.02)					
<u>^</u>		0.1	0.1	0.02					
high volume production		(0.04-0.3)	(0.05-0.3)	(0.00-0.1)					

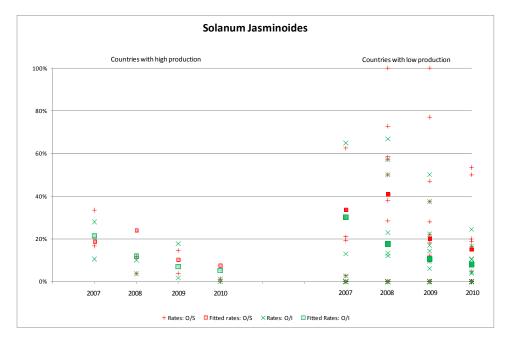
In bracket the 95% confidence intervals

**Table 31:** Probability of outbreaks of PSTVd in high and low volume producing countries for the years 2007 to 2010 estimated by the outbreak rates calculated by the ratio of number of outbreaks and <u>samples</u>

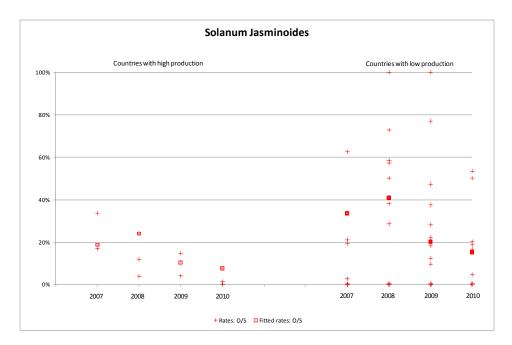
host category	<b>Probability of a PSTVd outbreak (%)</b> (estimated from PSTVd outbreaks/sample)								
production volume	2007	2007 2008 2009 2010							
		S. jasminoides							
	33.5	40.9	20.2	15.1					
low volume production	(24-45)	(28-55)	(13-30)	(9-25)					
_	18.7	24.0	10.3	7.5					
high volume production	(11-30)	(13-39)	(5-20)	(4-15)					
	Brugmansia								
	6.7	5.4	5.8	6.0					
low volume production	(3-17)	(2-13)	(3-11)	(2-16)					
_	3.7	3.0	3.2	3.4					
high volume production	(1-12)	(1-8)	(1-8)	(1-10)					
	sola	anaceous vegetables							
		0.1	0.1	0.03					
low volume production		(0.01-1.5)	(0.01-1.6)	(0.00-0.3)					
_		0.1	0.1	0.03					
high volume production		(0.04-0.3)	(0.05-0.3)	(0.01-0.1)					

In bracket the 95% confidence intervals



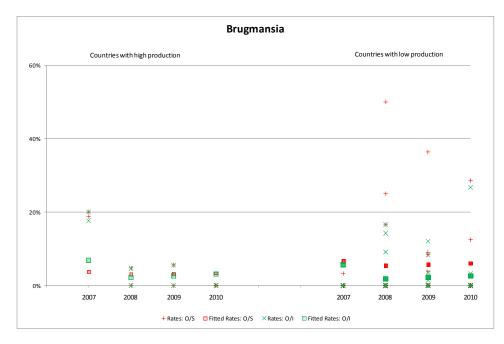


**Figure 7:** Rates of outbreaks of PSTVd on *S. jasminoides* in the years 2007 to 2010 for high volume producing and low volume producing countries calculated by the ratio of recognized PSTVd outbreaks per sample (red) respect the ratio of recognized PSTVd outbreaks per inspections (green).

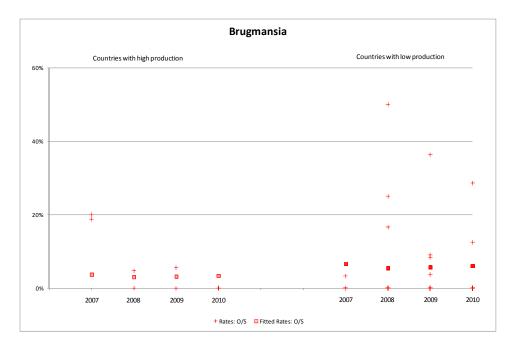


**Figure 8:** Rates of outbreaks of PSTVd on *S. jasminoides* in the years 2007 to 2010 for high volume producing and low volume producing countries calculated by the ratio of recognized PSTVd <u>outbreaks</u> per sample.





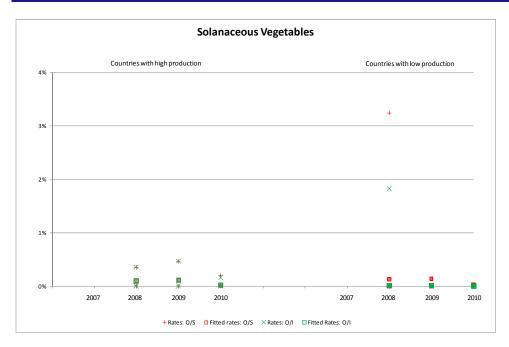
**Figure 9:** Rates of outbreaks of PSTVd on *Brugmansia* spp. in the years 2007 to 2010 for high volume producing and low volume producing countries calculated by the ratio of recognized PSTVd outbreaks per sample (red) respect the ractio of PSTVd recognized outbreaks per inspections (green).



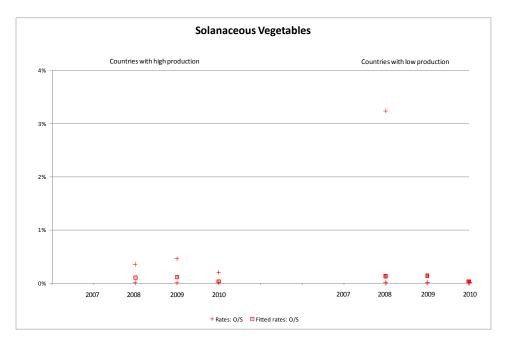
**Figure 10:** Rates of outbreaks of PSTVd on *Brugmansia* spp. in the years 2007 to 2010 for high volume producing and low volume producing countries calculated by the ratio of recognized PSTVd <u>outbreaks per sample</u>.







**Figure 11:** Rates of outbreaks of PSTVd on solanaceous vegetables in the years 2007 to 2010 for countries producing high volumes of *S. jasminoides* plants and low volume producing countries calculated by the ratio of recognized PSTVd outbreaks per sample (red) respect the ratio of PSTVd recognized outbreaks per inspections (green).



**Figure 12:** Rates of outbreaks of PSTVd on solanaceous vegetables in the years 2007 to 2010 for countries producing high volumes of *S. jasminoides* plants and low volume producing countries, calculated by the ratio of recognized PSTVd <u>outbreaks per sample.</u>



## **Test on Time or Production Effects**

Using the outbreak rates calculated by number of PSTVd outbreaks per inspection, the statistical test shows significant results only for *S. jasminoides* for the influence of time. Using the outbreak rates calculated by number of PSTVd outbreaks per sample shows an additional significant effect between countries producing high and low volumes of *S. jasminoides* plants..

For solanaceous vegetables a significant effect is shown for production volume only when using outbreak rates calculated by number of PSTVd outbreaks per number of inspections, but not when using PSTVd outbreaks per number of samples. No effect on the outbreak probability on Brugmansia is significant.

**Table 32:** Table of tests of effects of *S. jasminoides* production level and time in the logistic model for the probability of PSTVd outbreaks <u>per inspection</u> in different host categories

Host category	Deviance	Num DF	Den DF	F value	P-VALUE					
Source										
	S. jasminoides									
S. jasminoides										
production volume				0.28						
(HP vs. LP)	340.1877	1	54		0.5947					
Year (2007 to 2010)	244.8075	3	54	7.01	0.0001					
	Brugmansia									
Brugmansia										
production volume										
(HP vs. LP)	70.5727	1	52	0.30	0.5862					
Year (2007 to 2010)	67.4295	3	52	0.81	0.4892					
		Solanaceous v	vegetables							
Sol. vegetables										
production volume										
(HP vs. LP)	50.7087	1	35	20.54	< 0.0001					
Year (2008 to 2010)	45.8477	2	35	1.86	0.1564					

**Table 33:** Table of tests of effects of *S. jasminoides* production level and time in the logistic model for the probability of PSTVd outbreaks <u>per sample</u> in different host categories

Host category	Deviance	Num DF	Den DF	F value	P-VALUE				
Source									
	S. jasminoides								
S. jasminoides									
production volume									
(HP vs. LP)	353.6451	1	56	6.03	0.0141				
Year (2007 to 2010)	278.5406	3	56	5.03	0.0017				
	Brugmansia								
Brugmansia									
production volume									
(HP vs. LP)	62.5373	1	50	2.05	0.1520				
Year (2007 to 2010)	62.4003	3	50	0.04	0.9906				
		Solanaceous v	vegetables						
Sol. vegetables									
production volume									
(HP vs. LP)	45.8023	1	33	0.15	0.7009				
Year (2008 to 2010)	41.8538	2	33	1.56	0.2108				



# DISCUSSION

We were able to estimate the probability of PSTVd outbreaks per inspection or sample. Because the samples were taken risk-based, these estimates were higher.

There is a significant effect of time for the probability of outbreaks on the host *S. jasminoides*. The estimated rates decrease in the last three years. This was not observed for the other host categories.

Differences between countries producing high and lower volumes of *S. jasminoides* plants were not observed (in the case of PSTVd outbreaks on Brugmansia) or were not consistent between outbreaks per inspection respect to outbreaks per sample (in the cases of PSTVd outbreaks on *S. jasminoides* and on solanaceous vegetables).

## REFERENCES

[SAS 2009] SAS institute Inc. SAS Online Doc, Version 9.2. Cary (NC), 2009.

## DATA USED IN THE ANALYSIS

This report uses the following data:

**Table 1:** Rates of outbreaks of pospiviroids on different hosts in the Years 2007 to 2010 for high producing (HP, grey) and low producing countries (LP) calculated by the ratio of recognized outbreaks per sample

Country Code	Year	Host	No. of Inspections	No. of Samples	No. of Outbreaks	Rate of Outbreat calculated by Inspections	ks Samples
			I	S	0	= O/I	= O/S
		Solanu	ım Jasminoi		0	- 0/1	- 0/5
HP01	2007	solanum jasminoides	208	173	58	27.9%	33.5%
HP01	2008	solanum jasminoides	79	68	8	10.1%	11.8%
HP01	2009	solanum jasminoides	45	55	8	17.8%	14.6%
HP01	2010	solanum jasminoides	164	164	2	1.2%	1.2%
HP02	2007	solanum jasminoides	19	12	2	10.5%	16.7%
HP02	2008	solanum jasminoides	27	27	1	3.7%	3.7%
HP02	2009	solanum jasminoides	60	26	1	1.7%	3.9%
HP02	2010	solanum jasminoides	50	27	0	0.0%	0.0%
LP01	2008	solanum jasminoides		4	4		100.0%
LP01	2009	solanum jasminoides		11	2		18.2%
LP01	2010	solanum jasminoides	6	6	1	16.7%	16.7%
LP02	2008	solanum jasminoides		14	4		28.6%
LP02	2009	solanum jasminoides		11	0	0.0%	0.0%
LP02	2010	solanum jasminoides	1	1	0	0.0%	0.0%
LP05	2008	solanum jasminoides	36	33	24	66.7%	72.7%
LP05	2009	solanum jasminoides	56	17	8	14.3%	47.1%
LP05	2010	solanum jasminoides	76	15	8	10.5%	53.3%
LP06	2007	solanum jasminoides	1	1	0	0.0%	0.0%
LP06	2008	solanum jasminoides	4	4	2	50.0%	50.0%
LP06	2009	solanum jasminoides	5	5	0	0.0%	0.0%
LP07	2010	solanum jasminoides	1	1	0	0.0%	0.0%
LP08	2008	solanum jasminoides		3	0	0.0%	0.0%



LP08	2010	solanum jasminoides	1	1	0	0.0%	0.0%
LP09	2008	solanum jasminoides	249	79	30	12.1%	38.0%
LP09	2009	solanum jasminoides	343	95	21	6.1%	22.1%
LP09	2010	solanum jasminoides	163	91	17	10.4%	18.7%
LP10	2009	solanum jasminoides	21	21	2	9.5%	9.5%
LP10	2010	solanum jasminoides	11	11	0	0.0%	0.0%
LP11	2008	solanum jasminoides	2	1	0	0.0%	0.0%
LP11	2009	solanum jasminoides	2	2	0	0.0%	0.0%
LP12	2007	solanum jasminoides	1	0	0	0.0%	
LP12	2008	solanum jasminoides	3	3	0	0.0%	0.0%
LP12	2010	solanum jasminoides	2	1	0	0.0%	0.0%
LP13	2007	solanum jasminoides		150	29		19.3%
LP13	2009	solanum jasminoides		171	21		12.3%
LP13	2010	solanum jasminoides	91	87	4	4.4%	4.6%
LP14	2009	solanum jasminoides	1	1	0	0.0%	0.0%
LP14	2010	solanum jasminoides	1	1	0	0.0%	0.0%
LP16	2008	solanum jasminoides	1	1	0	0.0%	0.0%
LP16	2009	solanum jasminoides	4	4	0	0.0%	0.0%
LP18	2007	solanum jasminoides	4	3	0	0.0%	0.0%
LP18	2008	solanum jasminoides	2	0	0	0.0%	
LP18	2009	solanum jasminoides	2	1	1	50.0%	100.0%
LP18	2010	solanum jasminoides	2	0	0	0.0%	
LP19	2008	solanum jasminoides	7	7	4	57.1%	57.1%
LP19	2009	solanum jasminoides	8	8	3	37.5%	37.5%
LP19	2010	solanum jasminoides	6	6	0	0.0%	0.0%
LP20	2007	solanum jasminoides	2	0	0	0.0%	
LP20	2008	solanum jasminoides	2	1	0	0.0%	0.0%
LP20	2009	solanum jasminoides	1	1	0	0.0%	0.0%
LP20	2010	solanum jasminoides	4	1	0	0.0%	0.0%
LP21	2008	solanum jasminoides	1	1	0	0.0%	0.0%
LP21	2009	solanum jasminoides	2	1	0	0.0%	0.0%
LP21	2010	solanum jasminoides	2	1	0	0.0%	0.0%
LP22	2007	solanum jasminoides	54	56	35	64.8%	62.5%
LP22	2008	solanum jasminoides	122	48	28	23.0%	58.3%
LP22	2009	solanum jasminoides	45	13	10	22.2%	76.9%
LP22	2010	solanum jasminoides	26	5	1	3.9%	20.0%
LP23	2007	solanum jasminoides	101	62	13	12.9%	21.0%
LP23	2008	solanum jasminoides	105		14	13.3%	
LP23	2009	solanum jasminoides	94	57	16	17.0%	28.1%
LP23	2010	solanum jasminoides	94	46	23	24.5%	50.0%
LP24	2007	solanum jasminoides	37	37	1	2.7%	2.7%
LP24	2008	solanum jasminoides	20	2	0	0.0%	0.0%
LP24	2009	solanum jasminoides	5	1	0	0.0%	0.0%
LP24	2010	solanum jasminoides	68	2	0	0.0%	0.0%



			Brugmansia				
HP01	2007	brugmansia	17	16	3	17.7%	18.8%
HP01	2008	brugmansia	30	28	0	0.0%	0.0%
HP01	2009	brugmansia	18	18	1	5.6%	5.6%
HP01	2010	brugmansia	15	14	0	0.0%	0.0%
HP02	2007	brugmansia	5	5	1	20.0%	20.0%
HP02	2008	brugmansia	85	85	4	4.7%	4.7%
HP02	2009	brugmansia	80	72	0	0.0%	0.0%
HP02	2010	brugmansia	59	43	0	0.0%	0.0%
LP01	2010	brugmansia	0	0	0		
LP02	2008	brugmansia		15	0	0.0%	0.0%
LP02	2009	brugmansia		12	0	0.0%	0.0%
LP02	2010	brugmansia	1	1	0	0.0%	0.0%
LP03	2007	brugmansia	4	1	0	0.0%	0.0%
LP05	2008	brugmansia	1	0	0	0.0%	
LP05	2009	brugmansia	4	1	0	0.0%	0.0%
LP05	2010	brugmansia	8	1	0	0.0%	0.0%
LP06	2007	brugmansia	1	1	0	0.0%	0.0%
LP06	2008	brugmansia	6	6	1	16.7%	16.7%
LP06	2009	brugmansia	12	12	1	8.3%	8.3%
LP07	2007	brugmansia		31	1		3.2%
LP07	2008	brugmansia	2	2	0	0.0%	0.0%
LP07	2009	brugmansia	1	1	0	0.0%	0.0%
LP07	2010	brugmansia	1	1	0	0.0%	0.0%
LP08	2008	brugmansia		4	1		25.0%
LP08	2009	brugmansia		1	0	0.0%	0.0%
LP08	2010	brugmansia	2	2	0	0.0%	0.0%
LP09	2008	brugmansia	235	14	0	0.0%	0.0%
LP09	2009	brugmansia	277	44	4	1.4%	9.1%
LP09	2010	brugmansia	31	8	1	3.2%	12.5%
LP10	2009	brugmansia	27	27	1	3.7%	3.7%
LP10	2010	brugmansia	13	13	0	0.0%	0.0%
LP11	2009	brugmansia	1	1	0	0.0%	0.0%
LP12	2010	brugmansia	1	1	0	0.0%	0.0%
LP13	2009	brugmansia		15	0	0.0%	0.0%
LP13	2010	brugmansia	9	3	0	0.0%	0.0%
LP14	2008	brugmansia	6	6	0	0.0%	0.0%
LP14	2009	brugmansia	12	5	0	0.0%	0.0%
LP14	2010	brugmansia	7	3	0	0.0%	0.0%
LP16	2008	brugmansia	4	4	0	0.0%	0.0%
LP16	2009	brugmansia	4	2	0	0.0%	0.0%
LP18	2007	brugmansia	8	8	0	0.0%	0.0%
LP18	2008	brugmansia	8	5	0	0.0%	0.0%
LP18	2009	brugmansia	6	1	0	0.0%	0.0%
LP18	2010	brugmansia	4	1	0	0.0%	0.0%
LP19	2008	brugmansia	2	2	0	0.0%	0.0%
LP20	2007	brugmansia	1	0	0	0.0%	0.070
LP20	2008	brugmansia	1	0	0	0.0%	
LP20	2009	brugmansia	4	1	0	0.0%	0.0%
LP20	2009	brugmansia	4	1	0	0.0%	0.0%
LP21	2010	brugmansia	8	0	0	0.0%	0.070
LP21	2007	brugmansia	11	2	1	9.1%	50.0%
		Ũ			0		20.070
LP21	2009	brugmansia	· · · · · · · · · · · · · · · · · · ·			U_U%	
LP21 LP21	2009 2010	brugmansia brugmansia	2	0	0	0.0%	



LP22	2008 brugmansia	8	4	0	0.0%	0.0%
LP22	2009 brugmansia	4	4	0	0.0%	0.0%
LP22	2010 brugmansia	4	2	0	0.0%	0.0%
LP23	2008 brugmansia	14		2	14.3%	
LP23	2009 brugmansia	33	11	4	12.1%	36.4%
LP23	2010 brugmansia	15	14	4	26.7%	28.6%
LP24	2007 brugmansia	5	5	0	0.0%	0.0%
LP24	2008 brugmansia	4	2	0	0.0%	0.0%
LP24	2009 brugmansia	3	3	0	0.0%	0.0%
LP24	2010 brugmansia	1	0	0	0.0%	



	Other Ornamentals								
HP01	2010	other ornamentals	124	111	0	0.00%	0.00%		
HP02	2010	other ornamentals	127	127	0	0.00%	0.00%		
LP01	2010	other ornamentals	6	6	0	0.00%	0.00%		
LP02	2010	other ornamentals	6	6	0	0.00%	0.00%		
LP03	2010	other ornamentals	3	0	0	0.00%			
LP07	2010	other ornamentals	5	5	0	0.00%	0.00%		
LP08	2008	other ornamentals	•	1	1		100.00%		
LP09	2010	other ornamentals	35	19	1	2.86%	5.26%		
LP12	2010	other ornamentals	3	1	0	0.00%	0.00%		
LP13	2010	other ornamentals	11	9	4	36.36%	44.44%		
LP14	2010	other ornamentals	6	2	0	0.00%	0.00%		
LP19	2010	other ornamentals	7	7	0	0.00%	0.00%		
LP20	2010	other ornamentals	7	2	0	0.00%	0.00%		
LP21	2010	other ornamentals	3	3	0	0.00%	0.00%		
LP22	2010	other ornamentals	147	3	0	0.00%	0.00%		
LP23	2010	other ornamentals	1	1	0	0.00%	0.00%		
LP24	2010	other ornamentals	327	57	0	0.00%	0.00%		



	Solana	ceous Vegeta	bles			
HP01 2008	solanaceous vegetables	1134	1134	4	0.4%	0.4%
HP01 2009	solanaceous vegetables	1305	1305	6	0.5%	0.5%
HP01 2010	solanaceous vegetables	1300	1002	2	0.2%	0.2%
HP02 2008	solanaceous vegetables	3609	3609	0	0.0%	0.0%
HP02 2009	solanaceous vegetables	3909	3909	0	0.0%	0.0%
HP02 2010	solanaceous vegetables	4793	4618	0	0.0%	0.0%
LP01 2010	solanaceous vegetables	69	69	0	0.0%	0.0%
LP02 2010	solanaceous vegetables	4	257	0	0.0%	0.0%
LP03 2010	solanaceous vegetables	977	33	0	0.0%	0.0%
LP04 2009	solanaceous vegetables	4	4	0	0.0%	0.0%
LP04 2010	solanaceous vegetables	8	0	0	0.0%	
LP05 2010	solanaceous vegetables	241	4	0	0.0%	0.0%
LP07 2010	solanaceous vegetables	1	1	0	0.0%	0.0%
LP08 2010	solanaceous vegetables	97	89	0	0.0%	0.0%
LP09 2010	solanaceous vegetables	141	88	0	0.0%	0.0%
LP10 2010	solanaceous vegetables	224	224	0	0.0%	0.0%
LP11 2008	solanaceous vegetables	30	22	0	0.0%	0.0%
LP11 2009	solanaceous vegetables	29	22	0	0.0%	0.0%
LP12 2008	solanaceous vegetables	766	102	0	0.0%	0.0%
LP12 2009	solanaceous vegetables	728	54	0	0.0%	0.0%
LP12 2010	solanaceous vegetables	460	102	0	0.0%	0.0%
LP13 2010	solanaceous vegetables	604	81	0	0.0%	0.0%
LP14 2010	solanaceous vegetables	58	58	0	0.0%	0.0%
LP15 2008	solanaceous vegetables	37	37	0	0.0%	0.0%
LP15 2009	solanaceous vegetables	27	27	0	0.0%	0.0%
LP15 2010	solanaceous vegetables	28	28	0	0.0%	0.0%
LP16 2009	solanaceous vegetables	20	13	0	0.0%	0.0%
LP16 2010	solanaceous vegetables	20	20	0	0.0%	0.0%
LP17 2008	solanaceous vegetables	55	31	1	1.8%	3.2%
LP17 2009	solanaceous vegetables	53	53	0	0.0%	0.0%
LP17 2010	solanaceous vegetables	55	55	0	0.0%	0.0%
LP18 2010	solanaceous vegetables	15543	160	0	0.0%	0.0%
LP19 2010	solanaceous vegetables	10	10	0	0.0%	0.0%
LP20 2010	solanaceous vegetables	1472	128	0	0.0%	0.0%
LP21 2010	solanaceous vegetables	22	4	0	0.0%	0.0%
LP22 2010	solanaceous vegetables	424	1	0	0.0%	0.0%
LP23 2008	solanaceous vegetables	2972		0	0.0%	0.0%
LP23 2010	solanaceous vegetables	2907	16	0	0.0%	0.0%
LP24 2010	solanaceous vegetables	20345	190	0	0.0%	0.0%



Unkown or mixed hosts							
HP01	2007	unknown / mixed	98	63	4	4.08%	6.35%
HP01	2008	unknown / mixed	131	118	4	3.05%	3.39%
HP01	2009	unknown / mixed	234	230	3	1.28%	1.30%
HP01	2010	unknown / mixed	3	3	0	0.00%	0.00%
HP02	2007	unknown / mixed	249	123	0	0.00%	0.00%
HP02	2008	unknown / mixed	451	202	0	0.00%	0.00%
HP02	2009	unknown / mixed	302	96	2	0.66%	2.08%
HP02	2010	unknown / mixed	6	6	0	0.00%	0.00%
LP01	2008	unknown / mixed		8	0	0.00%	0.00%
LP01	2009	unknown / mixed		15	0	0.00%	0.00%
LP02	2008	unknown / mixed		112	0	0.00%	0.00%
LP02	2009	unknown / mixed	•	112	0	0.00%	0.00%
LP02	2010	unknown / mixed	2	2	0	0.00%	0.00%
LP03	2007	unknown / mixed	568	67	0	0.00%	0.00%
LP03	2008	unknown / mixed	985	75	0	0.00%	0.00%
LP03	2009	unknown / mixed	928	94	0	0.00%	0.00%
LP05	2007	unknown / mixed	35		25	71.43%	
LP05	2008	unknown / mixed	788	41	3	0.38%	7.32%
LP05	2009	unknown / mixed	173	13	3	1.73%	23.08%
LP05	2010	unknown / mixed	115	2	0	0.00%	0.00%
LP06	2007	unknown / mixed	9	16	1	11.11%	6.25%
LP06	2008	unknown / mixed	3	3	0	0.00%	0.00%
LP06	2009	unknown / mixed	25	25	0	0.00%	0.00%
LP07	2007	unknown / mixed		25	0	0.00%	0.00%
LP07	2008	unknown / mixed	10	10	0	0.00%	0.00%
LP07	2009	unknown / mixed	33	33	0	0.00%	0.00%
LP07	2010	unknown / mixed	1	1	0	0.00%	0.00%
LP08	2008	unknown / mixed		303	0	0.00%	0.00%
LP08	2009	unknown / mixed		252	0	0.00%	0.00%
LP08	2010	unknown / mixed	211	20	0	0.00%	0.00%
LP09	2008	unknown / mixed	207	41	0	0.00%	0.00%
LP09	2009	unknown / mixed	186	123	0	0.00%	0.00%
LP11	2007	unknown / mixed	5	5	0	0.00%	0.00%
LP13	2007	unknown / mixed		798	11		1.38%
LP13	2009	unknown / mixed		338	0	0.00%	0.00%
LP14	2008	unknown / mixed	73	73	0	0.00%	0.00%
LP14	2009	unknown / mixed	7	58	0	0.00%	0.00%
LP14	2010	unknown / mixed	1	0	0	0.00%	
LP18	2007	unknown / mixed	11251	290	1	0.01%	0.34%
LP18	2008	unknown / mixed	17266	174	0	0.00%	0.00%
LP18	2009	unknown / mixed	12627	161	0	0.00%	0.00%
LP18	2010	unknown / mixed	410	2	0	0.00%	0.00%
LP19	2010	unknown / mixed	35	35	1	2.86%	2.86%
LP19	2009	unknown / mixed	27	27	0	0.00%	0.00%
LP20	2007	unknown / mixed	1211	181	0	0.00%	0.00%
LP20	2007	unknown / mixed	1426	129	0	0.00%	0.00%
LP20	2009	unknown / mixed	1588	129	0	0.00%	0.00%
LP21	2007	unknown / mixed	1388	124	0	0.00%	0.00%
LP21 LP21	2007	unknown / mixed	48	15	1	2.08%	6.67%
LP21 LP21	2008	unknown / mixed	26	8	1	3.85%	12.50%
	2007	anknown / mincu	20	0	1	5.0570	12.3070



LP22	2008	unknown / mixed	796	29	0	0.00%	0.00%
LP22	2009	unknown / mixed	574	41	1	0.17%	2.44%
LP22	2010	unknown / mixed	15	1	0	0.00%	0.00%
LP23	2009	unknown / mixed	3418	42	2	0.06%	4.76%
LP24	2007	unknown / mixed	189	189	0	0.00%	0.00%
LP24	2008	unknown / mixed	602	89	0	0.00%	0.00%
LP24	2009	unknown / mixed	6703	323	0	0.00%	0.00%
LP24	2010	unknown / mixed	9	0	0	0.00%	



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# **ABBREVIATIONS**

EU	European Union
EPPO	European and Mediterranean Plant Protection Organisation
FAO	Food and Agriculture Organisation
FVO	Food and veterinary office, European Commission Health and Consumers Directorate General
IPM	Integrated pest management
IPPC	International Plant Protection Convention
MS	Member State(s)
NPPO	National Plant Protection Organisation
WTO	World Trade Organisation