



Animal and Plant Health Inspection Service
U.S. DEPARTMENT OF AGRICULTURE

A Qualitative Pest Risk Assessment for Six Pospiviroids (*Columnea latent viroid*, *Pepper chat fruit viroid*, *Potato spindle tuber viroid*, *Tomato apical stunt viroid*, *Tomato chlorotic dwarf viroid*, and *Tomato planta macho viroid*) Associated with Imported Tomato and Pepper Seeds

Version 1

November 1, 2021

Agency Contact

Plant Pest Risk Analysis (PPRA)
Science and Technology (ST)
Plant Protection and Quarantine (PPQ)
Animal and Plant Health Inspection Service (APHIS)
United States Department of Agriculture (USDA)
920 Main Campus Drive, Suite 400
Raleigh, NC 27606

Executive Summary

We assessed the pest risk of six quarantine pospiviroids, *Columnea latent viroid* (CLVd), *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) (Pospiviroidae), entering the United States and territories via the importation of *Solanum lycopersicum* (tomato) or *Capsicum annuum* (pepper) seeds. PPQ requested this assessment to support their effort to establish a long-term policy regarding these pests. We did not consider any production, harvest, post-harvest, shipping, or storage pest mitigation procedures in this assessment, as they will be evaluated during the risk management stage of pest risk analysis.

We determined all six pospiviroids can follow the tomato seed pathway, whereas only PCFVd and PSTVd can follow the pepper seed pathway. Introduction into tomato and pepper fields is more likely to occur from the planting of transplants first produced in greenhouses from infested seed lots.

Generally, we expect the pospiviroids' impacts to be higher in greenhouses due to conducive conditions for disease development and mechanical transmission of the viroids. Outbreaks may also occur in fields located in plant hardiness zones (PHZ) 9 through 14 because of the environmental conditions found there. We identified the following probable impacts to tomato, pepper, or potato production.

- Establishment of any of the viroids may impact the trade of both tomato and pepper seeds. However, only two viroids will have a direct impact on tomato or pepper seed production. TCDVd-infected tomato plants produce significantly fewer seeds and PSTVd can significantly decrease seed germination in certain tomato varieties.
- All six pospiviroids would impact greenhouse production of tomato fruit, causing yield losses as high as 45 percent, depending on the viroid. Pospiviroid outbreaks are more likely to occur in greenhouses than in fields because the high temperatures, high light intensity, and high plant densities along with frequent plant handling are more conducive for disease development and spread.
- PCFVd would impact greenhouse production of pepper fruit. Infected plants have delayed flowering and produce small, deformed fruit; yield losses can reach 50 percent.
- CLVd, PCFVd, PSTVd, TASVd, and TPMVd could impact tomato field production, though likely only in plant hardiness zones 9 to 14. However, U.S. production practices should be less conducive for disease spread than the practices of other countries.
- PSTVd would affect potato production. Infected plants are dwarfed and produce small, deformed tubers; disease severity depends on the potato variety infected and the viroid strain causing the infection. We found no reports of spread from tomato or pepper production to potato fields.

Detailed examination and choice of appropriate phytosanitary measures to mitigate pest risk are addressed separately from this document.

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Introduction

Background

The purpose of this report is to assess the pest risk for six pospiviroids, *Columnea latent viroid* (CLVd), *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), associated with the importation of tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.) seeds from anywhere in the world into the United States and territories¹ (referred to as the pest risk analysis or PRA area).

The likelihood of pest introduction is expressed as a qualitative rating rather than in numerical terms. This methodology is consistent with guidelines provided by the International Plant Protection Convention (IPPC) in the International Standard for Phytosanitary Measures (ISPM) No. 11, “Pest Risk Analysis for Quarantine Pests” (IPPC, 2017a). The use of biological and phytosanitary terms is consistent with ISPM No. 5, “Glossary of Phytosanitary Terms” (IPPC, 2021).

As defined in ISPM No. 11, this document comprises Stage 1 (Initiation) and Stage 2 (Risk Assessment) of risk analysis. Stage 3 (Risk Management) will be covered in a separate document.

Initiating event

APHIS issued Federal Import Order DA-2019-21 to keep CLVd, PCFVd, PSTVd, TASVd, TCDVd, and TPMVd out of the United States; this order requires imported tomato and pepper seeds to be tested and found free of these pests, or to originate from countries free from the viroids (APHIS, 2019). However, Federal Import Orders are temporary emergency measures meant to address an immediate threat. PPQ requested this analysis to assist their efforts in establishing a long-term policy for these quarantine pospiviroids.

Description of the pathway

A pathway is “any means that allows the entry or spread of a pest” (IPPC, 2021). The specific pathway of concern is the importation of seeds of tomato and pepper for planting. At the request of PPQ, we did not consider any production, harvest, or post-harvest pest mitigation procedures as part of this assessment, nor did we consider shipping and storage conditions. Such processes will be considered separately during the risk management stage of pest risk analysis (IPPC, 2017a).

Pests of concern

CLVd, PCFVd, PSTVd, TASVd, TCDVd, and TPMVd (Pospiviroidae) are of quarantine significance to the United States (USDA, 2021). All six pospiviroids have been reported from or detected in tomato and pepper, except for TCDVd and CLVd, which have never been reported from pepper (Antignus et al., 2002; Antignus et al., 2007; Batuman et al., 2013; CABI, 2021; Antignus et al., 2007; Antignus et al., 2002; Batuman et al., 2013; Constable et al., 2019; Marn and Pleško, 2012; Verhoeven et al., 2008a; Verhoeven et al., 2008c; Verhoeven et al., 2020; Verhoeven et al., 2017; Verhoeven et al., 2004).

¹The PRA area includes all 50 states, Guam, the Commonwealth of the Northern Mariana Islands, Puerto Rico, and the U.S. Virgin Islands.

Defining the endangered area

The Endangered Area is defined as the portion of the PRA area where ecological factors favor natural pest establishment and where pest presence would result in economically important losses. As such, the Endangered Area may only constitute a portion of the PRA area. Below, we describe the factors we considered.

Climatic suitability

We considered confirmed reports of pospiviroid outbreaks in field production areas when determining Plant Hardiness Zones (PHZ) at risk within the United States (Table 1) (Takeuchi et al., 2018). However, all the pospiviroids have also been reported in greenhouses (e.g., Ling and Bledsoe, 2009; Ling et al., 2013; Matsushita et al., 2008; Parrella et al., 2010; Parrella and Numitone, 2014; Verhoeven et al., 2011a), which shows these pests may be found in protected environments beyond their natural climatic boundaries.

CLVd has been reported in Mali, Costa Rica, Germany, Denmark, France, and Italy (EPPO, 2019; Nielsen and Nicolaisen, 2010; Spieker, 1996) and has been eradicated from the United Kingdom (Nixon et al., 2010), Belgium (EPPO, 2019), and the Netherlands (Verhoeven et al., 2004). It was first described from asymptomatic *Columnea erythrophaea* in a nursery in Maryland (Owens et al., 1978; Hammond et al., 1989), although there have been no recent reports to confirm its status in the United States. It was later detected in Canada on an asymptomatic *Nematanthus wettsteinii* (Singh et al., 1992); its current status there is unknown. Based on a comparison of CLVd's distribution in fields and greenhouses with a global map of PHZ (Takeuchi et al., 2018), we estimate that it could establish in the United States in PHZ 2 through 13 where hosts are found; however, pospiviroid survival in colder PHZ would depend on greenhouse production.

PCFVd has been reported in the Netherlands, Thailand, and Canada (Verhoeven et al., 2009b; Reanwarakorn et al., 2011; Verhoeven et al., 2011a). PCFVd was also detected in Australia but has since been eradicated (Anonymous, 2013). Based on a comparison of PCFVd's distribution in fields and greenhouses with a global map of PHZ (Takeuchi et al., 2018), we estimate that it could establish in the United States in PHZ 2 through 13 where hosts are found; however, pospiviroid survival in colder PHZ would depend on greenhouse production.

PSTVd has been reported in Egypt, Ghana, Kenya, Nigeria, Uganda (Allam et al., 2004; Batuman et al., 2019; Skelton et al., 2019; Lapido, 1977), Afghanistan, China, Georgia, India, Iran, Japan, Kazakhstan, Pakistan (EFSA, 2011; Qiu et al., 2016; CABI/EPPO, 2014; Owens et al., 1992; Arezou et al., 2008; Tsushima et al., 2019; Nadirova et al., 2015; Sial and Khan, 2018), Austria, Azerbaijan, Belarus, Belgium, Croatia, the Czech Republic, Germany, Italy, Malta, Montenegro, the Netherlands, Poland, Russia, Slovenia, Spain, Turkey (EFSA, 2011; O'Hanlon et al., 2019; CABI/EPPO, 2014; Blotskaya and Berlinchik, 1998; Milanović et al., 2014; Červená et al., 2008; Verhoeven et al., 2009a; Malandraki et al., 2010; Luigi et al., 2011; EPPO, 2013; Luigi et al., 2016; EPPO, 2021; Verhoeven et al., 2008c; EPPO, 2016b; Ryazantsev and Zavriev, 2009; Marn and Pleško, 2012; Bostan et al., 2010; Guner et al., 2012; Monger, 2018); Australia (Mackie et al., 2016), Costa Rica, the Dominican Republic (Badilla et al., 1999; Ling et al., 2014), Peru, and Venezuela (Querci et al., 1995; Singh, 1983). It is under eradication in Greece, Hungary, Switzerland, and the United Kingdom (EPPO, 2021). The EPPO Global Database lists PSTVd as present in Bangladesh and Mexico; however, this report is based on germplasm screening research (Singh et al.,

1999), not on recent detections in the countries. PSTVd has been eradicated from all potato production in Canada (Singh, 2014) and the United States since 2003 (NAPPO PAS, 2004; Sun et al., 2004). Although detected in greenhouse-grown tomatoes in California and North Carolina (Ling et al., 2013; Ling and Sfetcu, 2010), these infestations were eradicated. There is no evidence PSTVd is present in the United States. Based on a comparison of PSTVd's distribution in fields and greenhouses with a global map of PHZ (Takeuchi et al., 2018), we estimate that it could establish in the United States in PHZ 1 through 14 where hosts are found; however, pospiviroid survival in colder PHZ would depend on greenhouse production.

TASVd has been reported in Cote d'Ivoire, Indonesia, Israel, Ghana, Senegal, Tunisia, Austria, Belgium, Croatia, the Czech Republic, Germany, Italy, the Netherlands, Poland, and Slovenia (Antignus et al., 2002; Batuman et al., 2013; Candresse et al., 2007; Marn and Pleško, 2012; Parrella and Numitone, 2014; Verhoeven et al., 2006; Verhoeven et al., 2008a; Verhoeven et al., 2008c). Based on a comparison of TASVd's distribution in fields and greenhouses with a global map of PHZ (Takeuchi et al., 2018), we estimate that it could establish in the United States in PHZ 4 through 14 where hosts are found; however, pospiviroid survival in colder PHZ would depend on greenhouse production.

TCDVd has been reported in Canada, Mexico, India, Israel, France, Portugal, and the United Kingdom (Candresse et al., 2010; Červená et al., 2008; James et al., 2008; Ling et al., 2009; Ling and Zhang, 2009; Singh et al., 2006; Singh et al., 1999). In 2004, TCDVd was reported in the United States in Arizona and later in Colorado (Ling et al., 2009; Verhoeven et al., 2004). However, we are uncertain of the viroid's status in Colorado because the authors of the primary account listed the origin as United States; Colorado was not mentioned. TCDVd was recently reported from greenhouse-grown tomatoes in Hawaii; eradication efforts are underway (Olmedo-Velarde et al., 2019). We found no additional reports of this viroid in Arizona. Based on a comparison of TCDVd's distribution in fields and greenhouses with a global map of PHZ (Takeuchi et al., 2018), we estimate that it could establish in the United States in PHZ 1 through 14 where hosts are found; however, pospiviroid survival in colder PHZ would depend on greenhouse production.

TPMVd has been reported in Mexico (Diener, 1987; Galindo et al., 1982; Orozco Vargas and Galindo Alonso, 1986). It was also reported in a Canadian greenhouse, where it has since been eradicated (Hadidi et al., 2017; Ling and Bledsoe, 2009; Ling and Zhang, 2009; Orozco Vargas and Galindo Alonso, 1986). Based on a comparison of TPMVd's distribution in fields and greenhouses with a global map of Plant Hardiness Zones (PHZ) (Takeuchi et al., 2018), we estimate that it could establish in the United States in PHZ 2 through 13 where hosts are found; however, pospiviroid survival in colder PHZ would depend on greenhouse production.

Severe pospiviroid outbreaks in field-grown tomatoes or peppers appear to be limited to PHZ 9 to 14 (tropical areas, see Table 1). We found two reports of PCFVd in tomato fields in Thailand (Reanwarakorn et al., 2011; Tangkanchanapas et al., 2013). CLVd was found during surveys in tomato fields in Mali and Ghana (Batuman and Gilbertson, 2012), while TASVd was found in Cote d'Ivoire in tomato plants grown in small gardens (Antignus et al., 2007) and in Ghana during field surveys in tomato fields (Batuman et al., 2013; Batuman et al., 2019). PSTVd was reported in tomato fields in Ghana and the Dominican Republic (Batuman et al., 2019; Ling et al., 2014). TPMVd has been reported in tomato fields in Mexico (Galindo et al., 1982; Orozco Vargas and Galindo Alonso, 1986). We found no reports of TCDVd infecting tomato or pepper under field conditions. These outbreaks are consistent with reports that found high

light intensity and temperatures favor disease development and symptom expression (Carbonell et al., 2008; Flores et al., 2011; Hadidi et al., 2017; Singh, 2003). Verhoeven et al. (2010) suggested cool growing conditions in the Netherlands inhibited mechanical transmission and symptom expression in field crops.

Table 1. Locations where viroid outbreaks have been reported in field-grown tomatoes or peppers

| Viroid | References | Countries (Plant Hardiness Zones) |
|------------------------------------|---|---|
| <i>Columnea latent viroid</i> | Batuman and Gilbertson, 2012 | Mali (10 to 12) Ghana (12 to 14) |
| <i>Pepper chat fruit viroid</i> | Reanwarakorn et al., 2011; Tangkanchanapas et al., 2013 | Thailand (10 to 13) |
| <i>Potato spindle tuber viroid</i> | Batuman et al., 2019; Ling et al., 2014 | Ghana (12 to 14) Dominican Republic (11 to 13) |
| <i>Tomato apical stunt viroid</i> | Antignus et al., 2007; Batuman et al., 2013; Batuman et al., 2019 | Cote d'Ivoire (11 to 14) Ghana (12 to 14) |
| <i>Tomato planta macho viroid</i> | Galindo et al., 1982; Orozco Vargas and Galindo Alonso, 1986 | Mexico (9 to 13) |

Hosts

Below, we provide the host range for each pospiviroid. For the purposes of this document, a host is a “species capable, under natural conditions, of sustaining a specific pest or other organism” (IPPC, 2018). The pospiviroids all infect genera within the Solanaceae (Verhoeven, 2010), and all six infect tomato. However, their ability to infect pepper differs. Overall, CLVd and PSTVd have broader host ranges than the other species we assessed.

CLVd's host range includes species in the SOLANACEAE: *Brunfelsia undulata* and tomato; CUCURBITACEAE: *Cucumis sativus*; ASTERACEAE: *Gynura aurantiaca*; and GESNERIACEAE: *Columnea erythrophaea*, *Gloxinia gymnostoma*, *G. nematanthodes*, *G. purpurascens*, and *Nematanthus wettsteinii* (Batuman and Gilbertson, 2012, 2013; Bhuvitarkorn and Reanwarakorn, 2019; Nielsen and Nicolaisen, 2010; Nixon et al., 2010; Spieker, 1996; Steyer et al., 2010; Verhoeven et al., 2004; Hammond et al., 1989; Singh et al., 1992; Owens et al., 1978). CLVd has been detected in pepper seed consignments (Constable et al., 2019; Verhoeven et al., 2020), although no symptoms have been observed in pepper plants; some researchers believe the lack of symptoms explains the lack of reports of natural infection (Batuman and Gilbertson, 2012, 2013; Matsushita and Tsuda, 2015; Verhoeven et al., 2004; Verhoeven et al., 2020).

PCFVd's host range includes species in the SOLANACEAE: pepper and tomato (Reanwarakorn et al., 2011; Verhoeven et al., 2011a).

PSTVd's host range includes species in the SOLANACEAE: *Brugmansia* spp., *B. sanguinea*, *B. suaveolens*, *Brugmansia* × *candida*, *Brugmansia* × *flava*, *Calibrachoa* sp., *Cestrum* spp., *C. fasciculatum* ‘Newellii,’ *C. elegans* ‘Smithii,’ *C. elegans*, *Datura* sp., *Lycianthes rantonnetii*, pepper, *Physalis peruviana*, *Petunia* × *hybrida*, *Solanum tuberosum* (potato), *S. muricatum*, *S. pseudocapsicum*, *S. jasminoides*, *Streptosolen jamesonii*, and tomato; ASTERACEAE: *Argyranthemum frutescens*, *Chrysanthemum* sp., and *Dahlia* ×

hybrid; LAURACEAE: *Persea americana*; and EUPHORBIACEAE: *Hevea brasiliensis* (Chitambar, 2015; Di Serio, 2007; Diener, 1979; Diener and Raymer, 1967; Kumar et al., 2015; Lebas et al., 2005; Lemmetty et al., 2011; Luigi et al., 2016; Matousek et al., 2014; Mertelik et al., 2010; Puchta et al., 1990; Querci et al., 1995; Ramachandran et al., 2000; Shamloul et al., 1997; Singh, 1973; Singh et al., 2003; Tsushima et al., 2011; Verhoeven et al., 2010; Verhoeven et al., 2008a; Verhoeven et al., 2008b; Verhoeven et al., 2009a).

TASVd's host range includes species in the SOLANACEAE: *Cestrum* sp., *Solanum jasminoides*, and tomato (Antignus et al., 2007; Antignus et al., 2002; Batuman et al., 2013; Marn and Pleško, 2012; Verhoeven et al., 2008a; Verhoeven et al., 2008c). The viroid was detected in a 24-year-old pepper seed lot; artificial inoculation of pepper plants using this isolate resulted in asymptomatic infections (Verhoeven et al., 2020; Verhoeven et al., 2017).

TCDVd's host range includes species in the SOLANACEAE: *Petunia × hybrida*, *Solanum melongena*, and tomato (Verhoeven et al., 2004; Matsushita et al., 2008; Gramazio et al., 2019; James et al., 2008), and APOCYNACEAE: *Vinca minor* (Singh and Dilworth, 2009). No natural infections have been reported for pepper and experimentally infected pepper plants were asymptomatic (Matsushita et al., 2009).

TPMVd's host range includes species in the SOLANACEAE: *Capsicum annuum* var. *aviculare* (cayenne pepper), *Jaltomata procumbens*, *Physalis philadelphica*, *P. foetens*, *Solanum nigrescens*, *S. torvum*, and tomato (Verhoeven et al., 2011b; Yanagisawa and Matsushita, 2017; Ling and Bledsoe, 2009; Orozco Vargas and Galindo Alonso, 1986). Only tomato is symptomatic (Orozco Vargas and Galindo Alonso, 1986).

Economically important hosts in PRA area

While other hosts may also be economically important, we focused on tomato, pepper, and potato because these crops are commercially produced in all 50 states (NASS, 2017).

Tomato is the second most commonly grown fruit crop in the world (Jones, 2007), and the United States is a world leader in fresh tomato production. In 2018, U.S. field production of tomato (fresh and for processing) was valued at \$1.9 billion (NASS, 2019); the major production states were California and Florida. That same year, the value of tomato crops grown in protected environments (greenhouses) was \$688 million, with most production located in California, Texas, and New York (NASS, 2019).

Peppers are widely cultivated for fresh, dried, and processing products (Ramchiary and Kole, 2019); most are produced in open fields (Biswas et al., 2017). In 2018, U.S. field production was valued at \$533 million (NASS, 2019); the major production states were California, Florida, and Georgia. Peppers are also produced in greenhouses, primarily in California and Pennsylvania (NASS, 2019; Correll and Thornsburry, 2013).

Between 2017 and 2019, the United States exported an annual average of 119,211 kg of tomato seed and 167,347 kg of pepper seed for a total value of \$65.8 million (FAS, 2019; USDA-FAS, 2021). By number and volume of shipments, most tomato seed exports were to Mexico, Sudan, Pakistan, the Netherlands, and Saudi Arabia, while most pepper seed exports were to Mexico, the Netherlands, Denmark, South Korea, and Guatemala (USDA-FAS, 2021). In 2018, California producers accounted for 17.2 percent of all U.S. exports of seed for sowing (CDFA, 2020), which included tomato and pepper

seed. Overall, domestic tomato and pepper seed production was difficult to estimate, although the total vegetable seed production nationwide was 21.8 million kg in 2019 (USDA NASS, 2021).

In 2017, potatoes for consumption were produced on more than 1.1 million acres, with a total market value of \$4.6 billion; approximately half of the acreage was planted with processing potatoes. The top producing states were Idaho, Washington, and North Dakota (NASS, 2017). From July 2019 to June 2020, U.S. growers exported 20.5 percent of their harvest to international trading partners (Potatoes USA, 2021).

The endangered area

Based on the information provided above, we determined the endangered area includes PHZ 9 to 14 within the United States and territories where tomato, pepper, or potato are field grown. However, the seasonality of host production and the presence of greenhouse production could allow the viroids to cause outbreaks anywhere within the United States.

Potential impacts

We determined whether the pospiviroids would cause unacceptable impacts on the economically important hosts tomato, pepper, and potato grown within the endangered area. We defined unacceptable impacts as causing a yield loss of 10 percent or greater, as increasing U.S. production costs, as impacting an environmentally important plant species, or as impacting international trade. We looked at the impacts on the production of tomato and pepper seed and on the production of tomatoes, peppers, and potatoes for consumption. We also considered the impacts in greenhouse versus field production systems. We determined all six pospiviroids would cause unacceptable impacts, although the impacts depended on the viroid, the commodity, and how the commodity was produced.

Tomato and pepper seed production

We concluded only PSTVd and TCDVd may impact tomato seed production; our uncertainty for both viroids is moderate because our conclusions are based on single reports for each pathogen.

PSTVd infection reduced tomato seed germination from 98 to 53 percent (Simmons et al., 2015), whereas in an earlier study, seeds collected from infected fruits germinated normally (Kryczyński et al., 1988); however, different varieties were examined in each study, which suggests this impact is variety-dependent.

Tomato plants severely infected with TCDVd produced considerably fewer seeds, although seed viability was not affected (Singh and Dilworth, 2009).

We found conflicting information about the effect of TPMVd infection on tomato or pepper seed production. One study indicated infected cayenne pepper plants experienced mild fruit abortion, while infected tomato fruits were unaffected (Orozco Vargas and Galindo Alonso, 1986); however, the authors did not describe how they quantified fruit abortion. A later study reported the anthers of TPMVd-infected tomato plants produced no pollen and that fruit from infected tomato and pepper plants were smaller, although seed production was not affected (Yanagisawa and Matsushita, 2017). Based on this information, we concluded the impact of TPMVd on tomato or pepper seed production was minimal with moderate uncertainty.

We concluded tomato and pepper seed exports would be impacted should one or more of the pospiviroids be introduced to the endangered area. Our uncertainty is moderate because our largest importer of tomato and pepper seed (by volume currently has no phytosanitary requirements for these pests, although 56 other U.S. trading partners considered CLVd, PCFVd, PSTVd, TASVd, TCDVd or TPMVd to be harmful organisms. Between 2017 and 2019, the United States exported more than 100 shipments of tomato and pepper seed to those trading partners (PExD, 2020; USDA-FAS, 2021). Additional impacts related to trade may include higher costs for seed testing, delays in seed movement, seed shortages, loss of high-value seed due to destructive testing and missed windows for planting.

Tomato fruit for consumption

We concluded all six pospiviroids can have a negative impact on the production of tomato fruit for consumption; our uncertainty varies depending on the pospiviroid. These negative impacts would be mainly in greenhouse produced crops, as the majority of pospiviroid outbreaks reported in the literature occur in greenhouses due to favorable conditions for disease severity and spread (Hadidi et al., 2017). Disease severity is worse when plants are grown under conditions of high light intensity and high temperatures (Carbonell et al., 2008; Flores et al., 2011; Hadidi et al., 2017; Singh, 2003); high temperatures also increase mechanical transmission (Verhoeven et al., 2010; Jones, 2007). In addition, greenhouse crops are produced at high plant densities and undergo frequent handling, which increases the opportunity for mechanical transmission (Hadidi et al., 2017); also, the plants may be cropped for 9 months or more (Jones, 2007). This means that even low initial populations of infected plants can lead to a significant outbreak in greenhouse-grown crops.

CLVd causes stunted growth, downturned leaves (epinasty), leaf chlorosis, necrosis of leaf veins and stems, severe leaf distortion, bronzing, “crunchy leaf,” and growth reduction in infected plants (Batuman and Gilbertson, 2013; Nixon et al., 2010; Verhoeven et al., 2004); disease incidences tend to vary widely (Batuman and Gilbertson, 2013; Nixon et al., 2010; Parrella et al., 2010). Growers may experience losses from eradication activities (Nixon et al., 2010) or reduced yield (DAWE, 2021). Our uncertainty is low, although yield losses have only been quantified experimentally.

PCFVd severely reduces plant growth (Verhoeven et al., 2009b). While we found no reports of tomato yields being affected, PCFVd causes symptoms similar to those caused by other viroids that reduce yields (Hadidi et al., 2017; Kryczyński et al., 1988). Because we lack information on its effects on tomato yield, our uncertainty about PCFVd’s pest potential on tomato fruit production is moderate.

PSTVd severely affects the growth of tomato plants, causing stunting, apical leaf proliferation and mottling, and rough, discolored, and downturned leaves (Owens and Verhoeven, 2009; Zitter, 2014). In a limited study with a severe strain of PSTVd, yields were reduced between 40 and 45 percent depending on the variety, mainly because the fruit either aborted or did not ripen; fruits harvested from infected plants were smaller and unevenly colored (Kryczyński et al., 1988). Our uncertainty about PSTVd’s pest potential on tomato fruit production is Low.

TASVd delays fruit set and results in significantly smaller, discolored tomatoes (Antignus et al., 2002; Batuman et al., 2019; Parrella and Numitone, 2014). TASVd has been characterized as devastating to Israeli greenhouse production, where incidences of up to 100 percent and heavy yield losses have been reported (Antignus et al., 2002; Antignus et al., 2007; Verhoeven et al., 2006). Our uncertainty about TASVd’s pest potential on tomato fruit production is negligible.

TCDVd-infected tomato plants show bunching of apical leaves, leaf yellowing and size reduction, shortening of internodes, epinasty, and dwarfism (Candresse et al., 2010; Gramazio et al., 2019; Ling et al., 2009; Matsushita et al., 2008; Singh and Dilworth, 2009; Ling et al., 2009). Diseased plants produced no or small fruits, which are often deformed (Ling et al., 2009; Ling and Zhang, 2009; Singh and Dilworth, 2009). Fifty percent of tomato plants in a Hawaiian greenhouse showed stunting and chlorosis on the margins of leaves and leaflets (Olmedo-Velarde et al., 2019). The infection rate in greenhouse-grown tomato plants was reported in Arizona at 4 percent (Ling et al., 2009), in France at 20 to 25 percent (Candresse et al., 2010), and in Mexico at 5 percent (Ling and Zhang, 2009a), although no data are available on yield losses associated with these infection rates. Our uncertainty is low, although yield loss data have not been quantified.

TPMVd-infected tomato plants are severely stunted and have brittle, chlorotic leaves with necrotic spots at the top of the plants; these plants produce few, marble-sized fruits that are not marketable, especially if plants were infected before blooming (Diener, 1987; Ling and Bledsoe, 2009; Ling and Zhang, 2009; Matsushita and Yanagisawa, 2018; Verhoeven et al., 2011b). This can lead to severe losses, with farmers occasionally suffering complete crop loss (Diener, 1987; Galindo et al., 1989; Galindo et al., 1982). Our uncertainty is negligible.

Although U.S. trading partners consider CLVd, PCFVd, PSTVd, TASVd, TCDVd, or TPMVd to be harmful organisms, we found no evidence these organisms are regulated in fruit for consumption (USDA, 2019).

Pepper fruit for consumption

We concluded only PCFVd would have a negative impact on pepper fruit production; our uncertainty is low. In pepper, PCFVd delays flower formation, reduces fruit size, and deforms fruit, resulting in yield reductions up to 50 percent (Verhoeven et al., 2009b; Verhoeven et al., 2020).

The remaining pospiviroids have little to no impact on pepper fruit production. Pepper plants naturally infected by PSTVd show only a slight distortion or wavy margin on the leaves near the tops of the plants (Lebas et al., 2005). Although a study with peppers artificially inoculated with PSTVd suggested more severe symptoms could occur, yield losses were not quantified (Verhoeven et al., 2009b). There are no reports of CLVd or TASVd naturally causing disease in peppers, although both viroids have been detected in pepper seed lots, which suggests natural infections can occur (Constable et al., 2019; Verhoeven et al., 2020). Experimental inoculations showed infections were asymptomatic or showed only mild symptoms (Batuman and Gilbertson, 2012; Constable et al., 2019; Verhoeven et al., 2017; Verhoeven et al., 2020). Pepper is not a host of TCDVd and cayenne pepper plants naturally infected by TPMVd were asymptomatic (Orozco Vargas and Galindo Alonso, 1986).

Although U.S. trading partners consider CLVd, PCFVd, PSTVd, TASVd, TCDVd, or TPMVd to be harmful organisms, we found no evidence these organisms are regulated in fruit for consumption (USDA, 2019).

Potatoes for consumption

We concluded only PSTVd would have a negative impact on potato production as none of the other viroids infect potato. Our uncertainty is negligible. Disease severity is affected by the potato variety and by the viroid strain infecting it (CABI, 2021; Diener, 1987; Pfannenstiel and Slack, 1980); infected plants are stunted and produce fewer and smaller tubers, and the tubers may develop cracks that make them unmarketable.

Likelihood of introduction

We estimated the pospiviroid's likelihood of introduction into the endangered area via imported tomato and pepper seed. We looked at introduction via two routes: 1) direct sowing of seeds into field production areas, and 2) planting transplants originally produced from seeds sown in greenhouses into field production areas.

The likelihood of introduction is based on the potential for entry and establishment of a pest. We qualitatively assess this using the ratings: Low, Medium, and High. The elements comprising the likelihood of introduction are interdependent; therefore, the model is multiplicative rather than additive. We define the ratings as follows:

High: This outcome is highly likely because the events required occur frequently.

Medium: This outcome can occur; however, the combination of required events occurs only occasionally.

Low: This outcome is less likely because the exact combination of required events seldom occurs or rarely aligns properly in time and space.

We address uncertainty associated with each element as follows:

Negligible: Additional or more reliable evidence is very unlikely to change the rating.

Low: Additional or more reliable evidence probably will not change rating.

Moderate: Additional or more reliable evidence may or may not change rating.

High: Reliable evidence is not available.

We combine the likelihoods of entry and establishment to give an overall likelihood of introduction into the endangered area. In the context of this document, "entry" means *movement of a pest into an area where it is not yet present, or present but not widely distributed and being officially controlled*, whereas "establishment" means *perpetuation, for the foreseeable future, of a pest within an area after entry* (IPPC, 2021). For the purposes of this document, establishment means the pest's ability to perpetuate in field production areas.

Direct introduction into fields

Likelihood of entry

We based the likelihood of entry on interception data from ports of entry. We considered an interception rate of 10 percent or greater as high pest prevalence on the imported commodity, 1 to 10 percent as medium, and less than 1 percent as low. We found that the likelihood of entry varied depending on the viroid and the commodity seed being imported, with all six pospiviroids following the tomato seed pathway but only CLVd, PCFVd, and PSTVd following the pepper seed pathway. Our uncertainty is moderate, as our data are limited to interceptions at U.S. and Australian ports of entry, with sometimes contradictory results.

All six pospiviroids have been intercepted on imported tomato seed lots at U.S. ports of entry, which shows these viroids can enter the endangered area via contaminated tomato seed (Mavrodieva, 2019; USDA, 2021; USDA, 2018); 26 percent of 299 seed lots tested with molecular diagnostic methods were

infected with a quarantine pospiviroid. However, the frequency with which the viroids were intercepted varied. PSTVd was intercepted most often (13.0 percent of tomato seed lots tested), followed by PCFVd (9.7 percent), CLVd (4.1 percent), and TASVd, TCDVd, and TPMVd (all less than 1.0 percent) (Mavrodieva, 2019). The same viroids, except for TPMVd, were intercepted at Australian ports of entry on imported tomato seed (Constable et al., 2019; Dall et al., 2019), but were intercepted in fewer (5.7 percent) of the tomato seed lots tested. As in the United States, PSTVd was intercepted most often, followed by PCFVd, CLVd, TASVd, and TCDVd, although the detections of TASVd and TCDVd were rare; TPMVd was not detected. Based on these data, we determined the likelihood of entry via tomato seeds was high for PSTVd, medium for CLVd and PCFVd, and low for TASVd, TCDVd, and TPMVd.

PSTVd was intercepted on four imported pepper seed shipments at U.S. ports of entry (USDA, 2021), which shows PSTVd can follow this pathway. In Australia, PCFVd was detected in 3.6 percent of imported pepper seed lots tested between 2013 and 2016, PSTVd in 2.5 percent, and CLVd in less than 1.0 percent (Constable et al., 2019; Dall et al., 2019); TASVd, TCDVd, and TPMVd were not intercepted. Based on these data, the likelihood of entry via pepper seeds was medium for PCFVd and PSTVd and low for CLVd.

In 2019, the United States imported tomato seed primarily from China, India, Thailand, Brazil, and Peru, whereas pepper seed was imported primarily from China, Chile, India, Thailand, and Vietnam (USDA-FAS, 2021). PSTVd has been reported in China and India, PCFVd in Thailand, and TCDVd in India (CABI, 2021; Qiu et al., 2016; Singh et al., 1993; Owens et al., 1992; Parakh et al., 2017), so the United States is importing tomato and pepper seed from countries where quarantine pospiviroids are present. We found a single report of PSTVd causing blackening of tomato seeds, which could be detected by visual inspection (Kryczyński et al., 1988); however, we found no such reports for the other pospiviroids. Viroid detection relies on seed sampling and testing with sensitive molecular detection methods such as conventional or real time RT-PCR, followed by sequence analysis of full-length genome sequences (Hadidi et al., 2017; Mavrodieva, 2018; Naktuinbouw, 2017; Verhoeven, 2010; Yanagisawa et al., 2017); viroids cannot be detected serologically with immunostrips or similar methods (Zitter, 2014) because they consist of a single-stranded, small, circular RNA molecule that does not encode proteins (Hadidi et al., 2003, Hadidi et al., 2017; Verhoeven, 2010).

Likelihood of establishment

The likelihood of establishment varied depending on the commodity seed being imported. All six viroids could establish from infected tomato seed, but only PCFVd and PSTVd could establish from infected pepper seed. We explain our reasoning below.

Since seeds are used to establish crops, contaminated seeds would introduce the viroids directly into agricultural production areas (Matsushita et al., 2011; Gramazio et al., 2019). We found two reports mentioning seeds as the source of pospiviroid outbreaks in fields, although the authors provide little data to support this conclusion (Batuman et al., 2019; Batuman and Gilbertson, 2013). Also, no outbreaks have been reported in U.S. tomato or pepper fields despite sowing seed imported from countries where these pests occur (USDA-FAS, 2021; CABI, 2021; Qiu et al., 2016; Singh et al., 1993; Owens et al., 1992; Parakh et al., 2017).

Seed transmission has been reported in tomato for all the pospiviroids discussed in this document, and in pepper for PCFVd and PSTVd (Table 3). Per ISPM 38 (IPPC, 2017b), a seed-borne pest is a pest carried by seeds externally or internally that may or may not be transmitted to plants growing from these seeds

and cause their infestation, whereas a seed-transmitted pest is a seed-borne pest that is transmitted via seeds directly to plants growing from these seeds and causes their infestation. However, there are also conflicting reports regarding seed transmission of pospiviroids (Antignus et al., 2007; Constable et al., 2019; Matsushita and Tsuda, 2016; Singh and Dilworth, 2009; Verhoeven et al., 2009b; Verhoeven et al., 2017; Verhoeven et al., 2020; Yanagisawa and Matsushita, 2017), with those conflicts attributed to viroid strain, host variety, physiological stage of the plants at the time of infection, and the environmental conditions the plants were grown under (Faggioli et al., 2015; Matsushita and Tsuda, 2016). We considered seed transmission rates of 10 percent or greater as high, 1 to 10 percent as medium, and less than 1 percent as low.

Table 2. Seed transmission reported for six pospiviroids in tomato and pepper seeds

| Pospiviroid | Seed transmission rate ^a | |
|--------------------------------------|--|---|
| | Tomato seed | Pepper seed |
| <i>Columnnea latent viroid</i> | 5.3–100 percent (Matsushita and Tsuda, 2016) | Detected only ^b (Constable et al., 2019; Verhoeven et al., 2020) |
| <i>Pepper chat fruit viroid</i> | 0.0–1.4 percent (Yanagisawa and Matsushita, 2017) | 0.0–19 percent (Verhoeven et al., 2009b; Verhoeven et al., 2020; Yanagisawa and Matsushita, 2017) |
| <i>Potato spindle tuber viroid</i> | 0.0–90.2 percent (Matsushita and Tsuda, 2016; Simmons et al., 2015; Faggioli et al., 2015) | 0.0–0.5 percent (Matsushita and Tsuda, 2016; Verhoeven et al., 2020) |
| <i>Tomato apical stunt viroid</i> | 0.0–80.0 percent (Antignus et al., 2007; Matsushita and Tsuda, 2016) | Detected only ^b (Verhoeven et al., 2020; Verhoeven et al., 2017) |
| <i>Tomato chlorotic dwarf viroid</i> | 0.0–80.0 percent (Singh and Dilworth, 2009; Matsushita and Tsuda, 2016; Candresse et al., 2010) | No evidence found |
| <i>Tomato planta macho viroid</i> | 0.0–4.4 percent (Orozco Vargas and Galindo Alonso, 1986; Yanagisawa and Matsushita, 2017) | Not demonstrated ^c (Yanagisawa and Matsushita, 2017) |

^a The seed transmission rate is the rate at which infected seeds germinate and lead to an infected plant.

^b Seed transmission was not demonstrated.

^c No seed transmission was observed in artificially inoculated pepper plants.

The six pospiviroids can all spread via vegetative propagation and mechanical transmission (Antignus et al., 2002; Hadidi et al., 2017; Hammond and Owens, 2006; Manzer and Merriam, 1961; Owens and Verhoeven, 2009; Verhoeven et al., 2010; Verhoeven et al., 2009b; Verhoeven et al., 2004). Local spread via mechanical transmission occurs through crop handling (Matsushita et al., 2008; Verhoeven et al., 2004) and often along rows (Mackie et al., 2002; Matsushita et al., 2008; Verhoeven et al., 2004). Mackie et al (Mackie et al., 2015) demonstrated PSTVd-infected tomato sap remained infective up to 24

hours on surfaces, although infectivity decreased as the sap dried. Also, viroids are obligate parasites, meaning they require a living host to survive (Hadidi et al., 2003; Hammond et al., 1989; Hadidi et al., 2017). U.S. field production practices, which include minimal plant handling and crop rotations of at least 3 years, should therefore limit pospiviroid spread as the opportunity for mechanical transmission would be reduced and viroid inoculum would not carry over from one year to the next (Blancard, 2012; Jones, 2007). This contrasts with field production in other countries where plants are handled similarly to greenhouse crops (Lopez Marin, 2017).

CLVd, TPMVd, and PSTVd may be spread by pollen (Bhuvitarkorn and Reanwarakorn, 2019; Kryczyński et al., 1988; Matsushita and Tsuda, 2016; Matsushita and Yanagisawa, 2018; Yanagisawa and Matsushita, 2018). Also, *Myzus persicae* has been implicated in the spread of TPMVd (Galindo et al., 1986) and aphids have been implicated as vectors for PSTVd, provided a helper virus (*Potato leaf roll virus*) is also present (Van Bogaert et al., 2014). One or more of these vectors is widespread in the United States (CABI, 2021).

Considering all this information, we determined the likelihood of the six pospiviroids establishing in tomato fields via sowing of infected seeds to be low. Our uncertainty is high, as the information linking outbreaks in field production with the sowing of infected seeds is poorly described. Better information on the sources of those outbreaks may change our conclusion. Similarly, we determined the establishment of PCFVd and PSTVd in pepper fields to be low. However, our uncertainty is high because we are extrapolating from our conclusions for tomato seeds; new or better information may change our conclusion.

Likelihood of introduction

We determined all six pospiviroids could be introduced into tomato fields via the tomato seed pathway whereas only PCFVd and PSTVd could be introduced into pepper fields via the pepper seed pathway (Table 4). However, we found no evidence linking the tomato and pepper seed pathway to the introduction of pospiviroids into potato fields. Also, recent reports have questioned the role seed transmission plays in the introduction of pospiviroids into new geographic areas (Verhoeven et al., 2020; Verhoeven et al., 2021); but these studies evaluated pospiviroid-infested tomato and pepper seed lots that had undergone industry-standard post-harvest processes, which we did not consider in our assessment.

Table 3. Ratings for the likelihood of introduction of six pospiviroids into field-grown tomatoes and peppers by the planting of infested tomato or pepper seeds

| Pospiviroid | Likelihood of introduction | |
|--------------------------------------|----------------------------|-----------------|
| | Tomato seed | Pepper seed |
| <i>Columnea latent viroid</i> | Low | -- ^a |
| <i>Pepper chat fruit viroid</i> | Low | Low |
| <i>Potato spindle tuber viroid</i> | Medium | Low |
| <i>Tomato apical stunt viroid</i> | Low | -- ^a |
| <i>Tomato chlorotic dwarf viroid</i> | Low | -- ^a |
| <i>Tomato planta macho viroid</i> | Low | -- ^a |

^a The -- indicates we found no evidence to support likelihood of introduction via this pathway.

Indirect introduction into fields

Likelihood of entry

The likelihood of entry for the pospiviroids remained the same for tomato and peppers seeds imported for planting in U.S. greenhouses. CLVd has a medium likelihood of entry via imported tomato seeds and a low likelihood via imported pepper seeds. PCFVd has a medium likelihood of entry via both imported tomato and pepper seeds. PSTVd has a high likelihood of entry via imported tomato seeds and a medium likelihood via imported pepper seeds. TASVd, TCDVd, and TPMVd all have a low likelihood of entry via imported tomato seeds and no likelihood of entry via pepper seeds because they were not intercepted at ports of entry. Our uncertainty also remained moderate, for the reasons already cited.

Likelihood of establishment

We determined the likelihood of establishment of pospiviroids in field production areas via transplants produced in U.S. greenhouses from infested tomato or pepper seed was higher than the likelihood via direct sowing of infested seed. This suggests greenhouse production of tomato and pepper fruit for consumption is also at risk for pospiviroid outbreaks due to the planting of infested seed. We explain our reasoning below.

We determined pospiviroid outbreaks can occur in greenhouses even when seed transmission rates are low, and the outbreaks can result in plants with asymptomatic infections. As previously stated, all six pospiviroids can spread via vegetative propagation and mechanical transmission with local spread occurring along rows (Antignus et al., 2002; Hadidi et al., 2017; Hammond and Owens, 2006; Mackie et al., 2002; Manzer and Merriam, 1961; Matsushita et al., 2008; Owens and Verhoeven, 2009; Verhoeven et al., 2010; Verhoeven et al., 2009b; Verhoeven et al., 2004). In addition, bumblebees caused secondary spread of TASVd and TCDVd in greenhouses (Antignus et al., 2007; Matsuura et al., 2010). In Italy, an outbreak that affected 20 to 25 percent of tomato plants began from seed that was determined to have a seed transmission rate of 0.8 percent for TCDVd (Candresse et al., 2010). In Australia, an outbreak that affected 3 percent of tomato plants began from seed that was determined to have a seed transmission rate of 0.3 percent for PSTVd (van Brunshot et al., 2014); in that same greenhouse, PSTVd was detected in 0.7 percent of 300 healthy-appearing tomato plants randomly selected for testing. Finally, PSTVd and TCDVd have both been detected in U.S. greenhouses (Ling et al., 2009; Ling and Sfetcu, 2010; Ling et al., 2013), which indicates outbreaks can occur in U.S. greenhouse production systems.

Based on this information, we concluded the likelihood of establishment in U.S. tomato and pepper fields via infested tomato or pepper seeds first planted in greenhouses and then planted in fields as transplants is medium. Our uncertainty is moderate for tomato seed because our conclusion is extrapolated primarily from PSTVd and TCDVd data to all six pospiviroids. Our uncertainty is high for pepper seed because our conclusion is extrapolated from PSTVd and TCDVd behavior in tomato seeds. However, as with direct sowing of seeds, we expect U.S. field production practices to limit pospiviroid spread and survival from one year to the next (Blancard, 2012; Jones, 2007); we also suspect outbreaks would be localized within individual fields where infested, asymptomatic transplants were planted.

Likelihood of introduction

We determined all six pospiviroids could be introduced into field-grown tomatoes via tomato transplants grown in U.S. greenhouses from infested seeds; PCFVd and PSTVd could follow a similar pathway to become introduced into pepper fields (Table 4). We expect these introductions to be

localized within individual fields. As with direct sowing of tomato and pepper seeds, we found no evidence linking the planting of tomato or pepper transplants produced from infected seed lots to the introduction of pospiviroids into potato fields.

Table 4. Ratings for the likelihood of introduction of six pospiviroids into tomato or pepper fields via greenhouse-grown transplants produced from infested tomato or pepper seed

| Pospiviroid | Likelihood of introduction | |
|--------------------------------------|----------------------------|-----------------|
| | Tomato seed | Pepper seed |
| <i>Columnea latent viroid</i> | Medium | -- ^a |
| <i>Pepper chat fruit viroid</i> | Medium | Medium |
| <i>Potato spindle tuber viroid</i> | Medium | Medium |
| <i>Tomato apical stunt viroid</i> | Medium | -- ^a |
| <i>Tomato chlorotic dwarf viroid</i> | Medium | -- ^a |
| <i>Tomato planta macho viroid</i> | Medium | -- ^a |

^a The -- indicates we found no evidence to support likelihood of introduction via this pathway.

Conclusions

We determined all six pospiviroids can follow the tomato seed pathway, whereas only PCFVd and PSTVd can follow the pepper seed pathway. The impacts caused by the pospiviroids will differ depending on the pospiviroid, the commodity, and whether the commodity is produced in a field or greenhouse. Generally, we determined the pospiviroids' impacts would be higher in greenhouses due to conducive conditions for disease development and mechanical transmission of the viroids. However, outbreaks may also occur in fields in plant hardiness zones 9 to 14 because of the environmental conditions found there, although infections may be localized within individual fields. Introduction into tomato and pepper fields is more likely to occur from the planting of transplants first produced in U.S. greenhouses from infested seed lots.

Detailed examination and choice of appropriate phytosanitary measures to mitigate pest risk are addressed separately from this document.

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