2006-022: Draft Annex to ISPM 27:2006 – Potato spindle tuber viroid

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
1.	*G*	Editorial	I support the document as it is and I have no comments		English	Malaysia
2.	*G*	Editorial	I support the document as it is and I have no comments		English	Lao People's Democratic Republic
3.	*G*	Editorial	I support the document as it is and I have no comments		English	Korea, Republic of
4.	*G*	Editorial	I support the document as it is and I have no comments		English	Guyana
5.	*G*	Editorial	I support the document as it is and I have no comments		English	Mexico
6.	*G*	Editorial	I support the document as it is and I have no comments		English	Barbados
7.	*G*	Editorial	References sited in the text should follow a particular order.	For clarity	English	Ghana
8.	*G*	Editorial	I support the document as it is and I have no comments		English	New Zealand
9.	*G*	Editorial	I support the document as it is and I have no comments		English	Nepal
10.	*G*	Editorial	I support the document as it is and I have no comments		English	Congo
11.	*G*	Editorial	I support the document as it is and I have no comments		English	Lesotho
12.	*G*	Substantive		We note that no substantial information on validation is provided in the protocol. We suggest adding a short paragraph on where to find more information on validation. We consider the reference "Recommendations on method validation in phytodiagnostics are provided by EPPO (2010)." provides limited information.	English	United States of America
13.	*G*	Substantive		Several personal communications are used to support technical elements of the protocol. Have they subsequently been published and if so should be referenced. If not, should they be	English	Australia

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				removed from the protocol? Many could be removed without affecting the detail in the protocol.		
14.	*G*	Technical	This Annex is for the detection and identification of PSTVd and not for ~~the~~ other pospiviroids.	A large part of the Annex provides methods for detecting all or almost all pospiviroids. However, the Annex was intended only for the detection of PSTVd. That intention should be stated clearly. Methods for detecting and identifying other viroids haven't been as carefully researched as those for PSTVd. The discussion of the other viroids isn't as well developed. The reviewers probably haven't considered previous drafts as if they were meant for the detection and identification of all pospiviroids. If the Annex was broadened to cover all the viroids mentioned, then there would be problems. For example, the methods of Shamloul 1997, Boonham 2004 and Verhoeven 2004 very probably won't detect all variants of other pospiviroid species, but that isn't discussed in the Annex. Australian experience is that the Verhoeven primers won't detect all PCFVd isolates and the Speiker primers won't detect all CLVd variants. Whereas Botermans et al. 2013 did experiments on 10 different PSTVd isolates, they only tested one variant each of PCFVd, MPVd and TPMVd, only two isolates of IrVd and three isolates each of CEVd and CLVd. So we are less sure about whether the Botermans primers will detect all CEVd, CLVd, PCFVd, MPVd and TPMVd isolates. This problem is partly picked up in the 'Main discussion points' where it says "Whether generic tests should be included in preference to more specific tests". If this issue is not clarified then this Annex may become the de facto Annex for all pospiviroid species, which would be wrong.	English	Australia
15.	*5*	Technical	Viroids are unencapsidated~~, small (239–401 nucleotides),~~ covalently closed circular single-stranded RNA molecules, 239–401 nucleotides long that are replicated by host enzymes ~~capable of autonomous replication in infected hosts~~ (Mulbach and Sanger 1979), Hammond & Owens, 2006). *Potato spindle tuber viroid* (PSTVd; genus *Pospiviroid*) is commonly 359	It is suggested that the words 'small' and 'autonomous' are cut from the sentence. To say viroids are "small" is an understatement as they are extremely small, about 10-7 meters long, and about one tenth the size of the smallest virus	English	Australia

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			nucleotides in length but nucleotide lengths of 341–364 have been reported (Jeffries, 1998; Shamloul *et al*., 1997; Wassenegger *et al*., 1994). Mild and severe strains have been described based on symptoms produced in sensitive tomato cultivars; for example, *Solanum lycopersicum* (tomato) cv. Rutgers (Fernow, 1967).	genome. They are barely visible on an EM. The word 'autonomous' is being used in a special virological way that is confusing for non-virologists – as the ordinary meaning is something like 'not controlled by another'. Viroids are replicated by host RNA polymerase enzymes, so how can this be said to be autonomous in the ordinary sense.		
16.	6	Editorial	The natural host range of PSTVd is relatively narrow. The primary hosts are *Solanum tuberosum* (potato) and stolon- and tuber-forming *Solanum* spp. and *S. lycopersicum* (tomato). PSTVd has been found also in *Capsicum annuum* (pepper), *Persea americana* and *S. muricatum*. Recently, PSTVd has been detected in mainly vegetatively propagated ornamental plant species in the family Solanaceae – namely, *Brugmansia* spp., *Cestrum* spp., *Datura* sp., *Lycianthes rantonetti*, *Petunia* spp., *Physalis peruviana*, *Solanum* spp. and *Streptosolen jamesonii* – but also *Dahlia* × *hybrida* in the family Asteraceae (for natural host details, download the European and Mediterranean Plant Protection Organization (EPPO) Plant Quarantine Data Retrieval System (PQR) database available at http://www.eppo.int/DATABASES/databases.htm). The experimental host range of PSTVd is wide. It primarily infects species in the family Solanaceae but also some species in at least nine other families. Most hosts express few or no disease symptoms (Singh, 1973; Singh *et al*., 2003).	Consistency with other hosts e.g. Solanum tuberosum (potato)	English	EPPO, Estonia, Algeria, Morocco
17.	6	Editorial	The natural host range of PSTVd is relatively narrow. The primary hosts are *Solanum tuberosum* (potato) and stolon- and tuber-forming *Solanum* spp. and *S. lycopersicum* (tomato). PSTVd has been found also in *Capsicum annuum* (pepper), *Persea americana* and *S. muricatum*. Recently, PSTVd has been detected in mainly vegetatively propagated ornamental plant species in the family Solanaceae – namely, *Brugmansia* spp., *Cestrum* spp., *Datura* sp., *Lycianthes rantonetti*, *Petunia* spp., *Physalis peruviana*, *Solanum* spp. and *Streptosolen jamesonii* – but also *Dahlia* × *hybrida* in the family Asteraceae (for natural host details, download the European and Mediterranean Plant Protection Organization (EPPO) Plant Quarantine Data Retrieval System (PQR) database available at http://www.eppo.int/DATABASES/databases.htm). The experimental host range of PSTVd is wide. It primarily infects species in the family Solanaceae but also some species in at least nine other families. Most hosts express few or no disease symptoms (Singh, 1973; Singh *et al*., 2003).	Consistency with other hosts e.g. Solanum tuberosum (potato)	English	European Union
18.	6	Editorial	The natural host range of PSTVd is relatively narrow. The primary hosts are stolon- and tuber-forming Solanum spp. for example *Solanum tuberosum* (potato); ~~and stolon- and tuber-forming *Solanum* spp.~~ and *S. lycopersicum* *(tomato)*. PSTVd has been found also in *Capsicum annuum* (pepper), *Persea americana*(avocado) and *S. muricatum* *(pepino)*. Recently, PSTVd has been	potato is a tuber forming Solanum so the sentence needs reordering. Need to add common names to scientific names (some added, others needed)	English	Australia

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			detected in mainly vegetatively propagated ornamental plant species in the family Solanaceae – namely~~,~~ *Brugmansia* spp., *Cestrum* spp., *Datura* sp., *Lycianthes rantonetti*, *Petunia* spp., *Physalis peruviana*, *Solanum* spp. and *Streptosolen jamesonii* – but also *Dahlia* × *hybrida* in the family Asteraceae (for natural host details, download the European and Mediterranean Plant Protection Organization (EPPO) Plant Quarantine Data Retrieval System (PQR) database available at http://www.eppo.int/DATABASES/databases.htm). The experimental host range of PSTVd is wide. It primarily infects species in the family Solanaceae but also some species in at least nine other families. Most hosts express few or no disease symptoms (Singh, 1973; Singh *et al*., 2003).			
19.	6	Editorial	The natural host range of PSTVd is relatively narrow. The primary hosts are *Solanum tuberosum* (potato) and stolon- and tuber-forming *Solanum* spp. and *S. lycopersicum*. PSTVd has been found also in *S. muricatum,*Capsicum annuum* (pepper)~~,~~ and *Persea americana* ~~and *S. muricatum*~~. Recently, PSTVd has been detected in mainly vegetatively propagated ornamental plant species in the family Solanaceae – namely, *Brugmansia* spp., *Cestrum* spp., *Datura* sp., *Lycianthes rantonetti*, *Petunia* spp., *Physalis peruviana*, *Solanum* spp. and *Streptosolen jamesonii* – but also *Dahlia* × *hybrida* in the family Asteraceae (for natural host details, download the European and Mediterranean Plant Protection Organization (EPPO) Plant Quarantine Data Retrieval System (PQR) database available at http://www.eppo.int/DATABASES/databases.htm). The experimental host range of PSTVd is wide. It primarily infects species in the family Solanaceae but also some species in at least nine other families. Most hosts express few or no disease symptoms (Singh, 1973; Singh *et al*., 2003)	Clarity	English	Ghana
20.	6	Substantive	The natural host range of PSTVd is relatively narrow. The primary hosts are *Solanum tuberosum* (potato) and stolon- and tuber-forming *Solanum* spp. and *S. lycopersicum*. PSTVd has been found also in tomato, *Capsicum annuum* (pepper), *Persea americana* and *S. muricatum*. Recently, PSTVd has been detected in mainly vegetatively propagated ornamental plant species in the family Solanaceae – namely, *Brugmansia* spp., *Cestrum* spp., *Datura* sp., *Lycianthes rantonetti*, *Petunia* spp., *Physalis peruviana*, *Solanum* spp. and *Streptosolen jamesonii* – but also *Dahlia* × *hybrida* in the family Asteraceae (for natural host details, download the European and Mediterranean Plant Protection Organization (EPPO) Plant Quarantine Data Retrieval System (PQR) database available at http://www.eppo.int/DATABASES/databases.htm). The experimental host range of PSTVd is wide. It primarily infects species in the family Solanaceae but also some species in at least nine other families. Most hosts express few or no disease symptoms (Singh, 1973; Singh *et al*., 2003).	China has tested PSTVd in the fruit of tomato.	English	China
21.	6	Technical	The natural host range of PSTVd is relatively narrow. The primary hosts are *Solanum tuberosum* (potato) and stolon- and tuber-forming *Solanum* spp. and *S. lycopersicum*. PSTVd has been found also in *Capsicum annuum* (pepper),	Chrysanthemum has been demonstrated as a host. See Lemmetty, A., Laamanen, J., Soukained, M., & Tegel, J. (2011). Emerging	English	EPPO, Estonia,

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			Persea americana and *S. muricatum*. Recently, PSTVd has been detected in mainly vegetatively propagated ornamental plant species in the family Solanaceae – namely, *Brugmansia* spp., *Cestrum* spp., *Datura* sp., *Lycianthes rantonetti*, *Petunia* spp., *Physalis peruviana*, *Solanum* spp. and *Streptosolen jamesonii* – but also *Dahlia* × *hybrida* and Chrysanthemum sp. in the family Asteraceae (for natural host details, download the European and Mediterranean Plant Protection Organization (EPPO) Plant Quarantine Data Retrieval System (PQR) database available at http://www.eppo.int/DATABASES/databases.htm). The experimental host range of PSTVd is wide. It primarily infects species in the family Solanaceae but also some species in at least nine other families. Most hosts express few or no disease symptoms (Singh, 1973; Singh *et al*., 2003).	virus and viroid pathogen species identified for the first time in horticultural plants in Finland in 1997–2010. Agricultural and Food Science, 20, 29–41.		Algeria, Morocco
22.	6	Technical	The natural host range of PSTVd is relatively narrow. The primary hosts are *Solanum tuberosum* (potato) and stolon- and tuber-forming *Solanum* spp. and *S. lycopersicum*. PSTVd has been found also in *Capsicum annuum* (pepper), *Persea americana* and *S. muricatum*. Recently, PSTVd has been detected in mainly vegetatively propagated ornamental plant species in the family Solanaceae – namely, *Brugmansia* spp., *Cestrum* spp., *Datura* sp., *Lycianthes rantonetti*, *Petunia* spp., *Physalis peruviana*, *Solanum* spp. and *Streptosolen jamesonii* – but also *Dahlia* × *hybrida* and Chrysanthemum sp. in the family Asteraceae (for natural host details, download the European and Mediterranean Plant Protection Organization (EPPO) Plant Quarantine Data Retrieval System (PQR) database available at http://www.eppo.int/DATABASES/databases.htm). The experimental host range of PSTVd is wide. It primarily infects species in the family Solanaceae but also some species in at least nine other families. Most hosts express few or no disease symptoms (Singh, 1973; Singh *et al*., 2003).	Chrysanthemum has been demonstrated as a host. See Lemmetty, A., Laamanen, J., Soukained, M., & Tegel, J. (2011). Emerging virus and viroid pathogen species identified for the first time in horticultural plants in Finland in 1997–2010. Agricultural and Food Science, 20, 29–41.	English	European Union
23.	7	Substantive	PSTVd has been found infecting *S. tuberosum* in Africa (Nigeria), Asia (Afghanistan, China, India), parts of Eastern Europe, North America (EPPO/CABI, 1997), Central America (Badilla *et al*., 1999), and the Middle East (Hadidi *et al*., 2003) ~~and Argentina (Bartolini & Salazar, 2003)~~. However, it has a wider geographical distribution in ornamentals and other hosts.	This pest is not present in Argentina	English	Uruguay
24.	7	Substantive	PSTVd has been found infecting *S. tuberosum* in Africa (Nigeria), Asia (Afghanistan, China, India), parts of Eastern Europe, North America (EPPO/CABI, 1997), Central America (Badilla *et al*., 1999), and the Middle East (Hadidi *et al*., 2003) ~~and Argentina (Bartolini & Salazar, 2003)~~. However, it has a wider geographical distribution in ornamentals and other hosts.	This pest is not present in Argentina	English	COSAVE, Paraguay, Chile, Argentina, Peru, Brazil
25.	7	Substantive	PSTVd has been found infecting *S. tuberosum* in Africa (Nigeria), Asia (Afghanistan, China, India), parts of Eastern Europe(the names of country) , North America (the names of country) (EPPO/CABI, 1997), Central America (Badilla *et al*., 1999), the Middle East (Hadidi *et al*., 2003) and Argentina (Bartolini & Salazar, 2003). However, it has a wider geographical distribution in ornamentals	The expression is clearer.	English	China

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			and other hosts.			
26.	7	Substantive	PSTVd has been found infecting *S. tuberosum* in Africa (Nigeria, Egypt), Asia (Afghanistan, China, India), parts of Eastern Europe, North America (EPPO/CABI, 1997), Central America (Badilla *et al*., 1999), the Middle East (Hadidi *et al*., 2003) and Argentina (Bartolini & Salazar, 2003). However, it has a wider geographical distribution in ornamentals and other hosts.	PSTVd has also been reported in Nigeria References: CABI / EPPO, 1998. distribution map of quarantine pests for Europe(edited by Smith. I. M and Charles, L.M. F.) EPPO, 2009. PQR database. Paris, France: European and Mediterranean Plant Protection organization. CABI / EPPO 2012. Potato spindle tuber viriod.Distribution Maps of Plant Disease. (Edition 2).	English	Ghana
27.	8	Editorial	In potato, the main means of spread of PSTVd is by vegetative propagation. It is also spread by contact, mainly by machinery in the field and by cutting seed potato tubers (Hammond & Owens, 2006). PSTVd is transmitted in true potato seed – up to 100% of the seed may be infected (Fernow *et al*., 1970; Singh, 1970) – and also in pollen (Grasmick & Slack, 1985; Singh *et al*., 1992). De Bokx and Pirone (1981) reported a low rate of transmission of PSTVd by the aphid *Macrosiphum euphorbiae* but not by *Myzus persicae* or *Aulacorthum solani.* However, experimental acquisition and transmission of PSTVd by *Myzus persicae* from plants co-infected by *Potato leafroll virus* (PLRV) have been reported (Salazar *et al*., 1995, 1996; Singh & Kurz, 1997). PSTVd was subsequently shown to be heterologously encapsidated within particles of PLRV (Querci *et al*., 1997), a phenomenon that may have important implications for the epidemiology and spread of PSTVd under field conditions. In tomato, PSTVd is easily spread by contact and has been shown to be transmitted by pollen and seed (Kryczynski *et al.*, 1988; Singh, 1970). It is also possible that infected ornamental species may act as an inoculum source if handled before touching other susceptible plants (Verhoeven *et al*., 2010). No transmission of PSTVd was shown with *Apis mellifera*, *Bombus terrestris*, *Frankliniella occidentalis* or *Thrips tabaci* (Nielsen *et al.,* 2012).	Salazar et al., 1996, is missing in the references.	English	EPPO
28.	8	Editorial	In potato, the main means of spread of PSTVd is by vegetative propagation. It is also spread by contact, mainly by machinery in the field and by cutting seed potato tubers (Hammond & Owens, 2006). PSTVd is transmitted in true potato seed – up to 100% of the seed may be infected (Fernow *et al*., 1970; Singh, 1970) – and also in pollen (Grasmick & Slack, 1985; Singh *et al*., 1992). De Bokx and Pirone (1981) reported a low rate of transmission of PSTVd by the aphid *Macrosiphum euphorbiae* but not by *Myzus persicae* or *Aulacorthum solani.* However, experimental acquisition and transmission of PSTVd by *Myzus persicae* from plants co-infected by *Potato leafroll virus* (PLRV) have been reported (Salazar *et al*., 1995, 1996; Singh & Kurz, 1997). PSTVd was subsequently shown to be heterologously encapsidated within particles of PLRV (Querci *et al*., 1997), a phenomenon that may have important implications for the epidemiology and	Salazar et al., 1996, is missing in the references.	English	Estonia, Algeria, Morocco

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			spread of PSTVd under field conditions. In tomato, PSTVd is easily spread by contact and has been shown to be transmitted by pollen and seed (Kryczynski *et al.*, 1988; Singh, 1970). It is also possible that infected ornamental species may act as an inoculum source if handled before touching other susceptible plants (Verhoeven *et al*., 2010). No transmission of PSTVd was shown with *Apis mellifera*, *Bombus terrestris*, *Frankliniella occidentalis* or *Thrips tabaci* (Nielsen *et al.,* 2012).			
29.	*8*	Editorial	In potato, the main means of spread of PSTVd is by vegetative propagation. It is also spread by contact, mainly by machinery in the field and by cutting seed potato tubers (Hammond & Owens, 2006). PSTVd is transmitted in true potato seed – up to 100% of the seed may be infected (Fernow *et al*., 1970; Singh, 1970) – and also in pollen (Grasmick & Slack, 1985; Singh *et al*., 1992). De Bokx and Pirone (1981) reported a low rate of transmission of PSTVd by the aphid *Macrosiphum euphorbiae* but not by *Myzus persicae* or *Aulacorthum solani.* However, experimental acquisition and transmission of PSTVd by *Myzus persicae* from plants co-infected by *Potato leafroll virus* (PLRV) have been reported (Salazar *et al*., 1995, 1996; Singh & Kurz, 1997). PSTVd was subsequently shown to be heterologously encapsidated within particles of PLRV (Querci *et al*., 1997), a phenomenon that may have important implications for the epidemiology and spread of PSTVd under field conditions. In tomato, PSTVd is easily spread by contact and has been shown to be transmitted by pollen and seed (Kryczynski *et al.*, 1988; Singh, 1970). It is also possible that infected ornamental species may act as an inoculum source if handled before touching other susceptible plants (Verhoeven *et al*., 2010). No transmission of PSTVd was shown with *Apis mellifera*, *Bombus terrestris*, *Frankliniella occidentalis* or *Thrips tabaci* (Nielsen *et al.,* 2012).	Salazar et al., 1996, is missing in the references.	English	European Union
30.	*8*	Editorial	In potato, the main means of spread of PSTVd is by vegetative propagation. It is also spread by contact, mainly by machinery in the field and by cutting seed potato tubers (Hammond & Owens, 2006). PSTVd is transmitted in true potato seed – up to 100% of the seed may be infected (Fernow *et al*., 1970; Singh, 1970) – and also in pollen (Grasmick & Slack, 1985; Singh *et al*., 1992). De Bokx and Pirone (1981) reported a low rate of transmission of PSTVd by the aphid *Macrosiphum euphorbiae* but not by *Myzus persicae* or *Aulacorthum solani.* However, experimental acquisition and transmission of PSTVd by *Myzus persicae* from plants co-infected by *Potato leafroll virus* (PLRV) have been reported (Salazar *et al*., 1995, 1996; Singh & Kurz, 1997). PSTVd was subsequently shown to be heterologously encapsidated within particles of PLRV (Querci *et al*., 1997), a phenomenon that may have important implications for the epidemiology and spread of PSTVd under field conditions.	divide para 8 into two separate paragraphs, first dealing with trasmission in potato, the second with transmission in tomato and capsicum.	English	Australia

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			In tomato, PSTVd is easily spread by contact and has been shown to be transmitted by pollen and seed (Kryczynski *et al.*, 1988; Singh, 1970). It is also possible that infected ornamental species may act as an inoculum source if handled before touching other susceptible plants (Verhoeven *et al*., 2010). No transmission of PSTVd was shown with *Apis mellifera*, *Bombus terrestris*, *Frankliniella occidentalis* or *Thrips tabaci* (Nielsen *et al.,* 2012).			
31.	*8*	Substantive	In potato, the main means of spread of PSTVd is by vegetative propagation. It is also spread by contact, mainly by machinery in the field and by cutting seed potato tubers (Hammond & Owens, 2006). PSTVd is transmitted in true potato seed – up to 100% of the seed may be infected (Fernow *et al*., 1970; Singh, 1970) – and also in pollen (Grasmick & Slack, 1985; Singh *et al*., 1992). De Bokx and Pirone (1981) reported a low rate of transmission of PSTVd by the aphid *Macrosiphum euphorbiae* but not by *Myzus persicae* or *Aulacorthum solani.* However, experimental acquisition and transmission of PSTVd by *Myzus persicae* from plants co-infected by *Potato leafroll virus* (PLRV) have been reported (Salazar *et al*., 1995, 1996; Singh & Kurz, 1997). PSTVd was subsequently shown to be heterologously encapsidated within particles of PLRV (Querci *et al*., 1997), a phenomenon that may have important implications for the epidemiology and spread of PSTVd under field conditions. In tomato, PSTVd is easily spread by contact and has been shown to be transmitted by pollen and seed (Kryczynski *et al.*, 1988; Singh, 1970). It is also possible that infected ornamental species may act as an inoculum source if handled before touching other susceptible plants (Verhoeven *et al*., 2010). No transmission of PSTVd was shown with *Apis mellifera*, *Bombus terrestris*, *Frankliniella occidentalis* or *Thrips tabaci* (Nielsen *et al.,* 2012).	A paper of Salazar et al.,1996 is missing in section 8.References.	English	Thailand
32.	*8*	Substantive	In potato, the main means of spread of PSTVd is by vegetative propagation. It is also spread by contact, mainly by machinery in the field and by cutting seed potato tubers (Hammond & Owens, 2006). PSTVd is transmitted in true potato seed – up to 100% of the seed may be infected (Fernow *et al*., 1970; Singh, 1970) – and also in pollen (Grasmick & Slack, 1985; Singh *et al*., 1992). De Bokx and Pirone (1981) reported a low rate of transmission of PSTVd by the aphid *Macrosiphum euphorbiae* but not by *Myzus persicae* or *Aulacorthum solani.* However, experimental acquisition and transmission of PSTVd by *Myzus persicae* from plants co-infected by *Potato leafroll virus* (PLRV) have been reported (Salazar *et al*., 1995, 1996; Singh & Kurz, 1997). PSTVd was subsequently shown to be heterologously encapsidated within particles of PLRV (Querci *et al*., 1997), a phenomenon that may have important implications for the epidemiology and spread of PSTVd under field conditions. ~~In tomato, PSTVd is easily spread by contact and has been shown to be transmitted by pollen and seed (Kryczynski *et al.*, 1988; Singh, 1970).~~ Transmission of PSTVd through tomato seed is a major pathway of international spread (Singh 1970; Kryczynski et al., 1988; Elliot	Replace the first sentence dealing with tomato. The Annex and paragraph 8 downplay country-to-country transfer of PSTVd in tomato seed. Tomato seed is an important pathway for international spread of PSTVd (see the references listed below). Tomato seed is traded internationally in relatively large volumes. Australian testing over the past 18 months has shown many tomato and capsicum seed shipments are infected by PSTVd, including seed from major suppliers and from several countries. Austria, Czech Republic, Germany, Israel and Italy have also intercepted PSTVd in tomato seed (see EPPO Archives of the EPPO Reporting Service, 2002-2011). Tomato seed has been implicated in several incursions of	English	Australia

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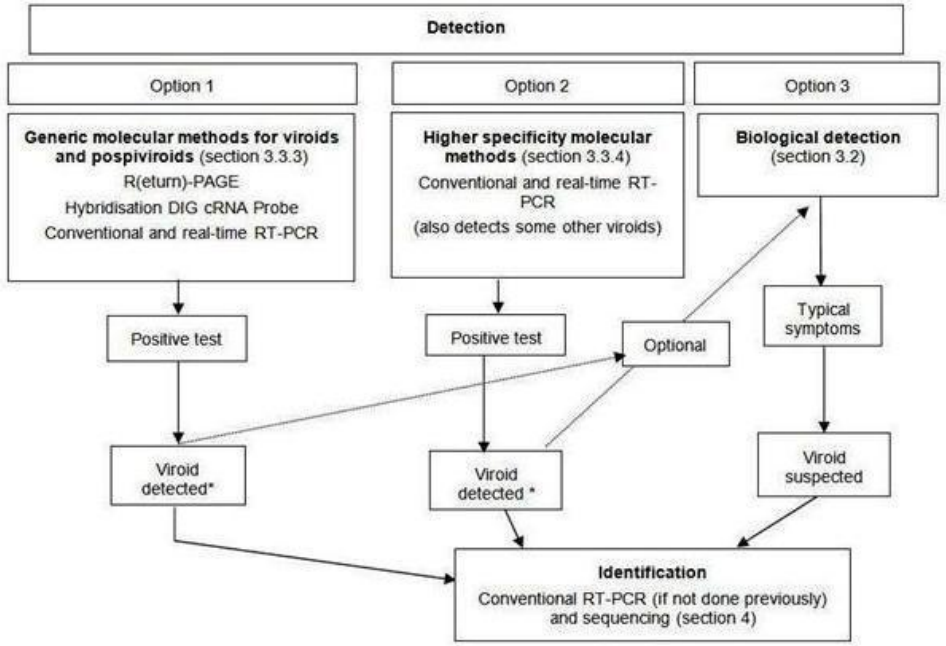
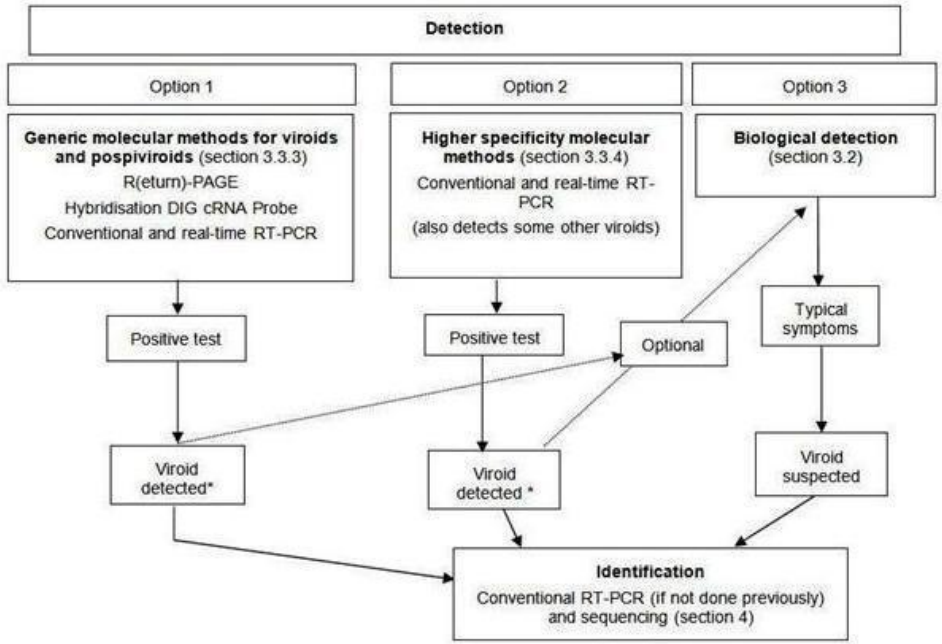
			et al., 2001; EPPO 2002-2011; Mumford et al., 2003; Hailstones et al., 2003; Verhoeven et al., 2004; Verhoeven et al., 2007; Ling et al., 2010; Ling et al., 2013; FERA 2010). PSTVd is probably also spread in infected capsicum seed (Lebas et al. 2005; Owens and Verhoeven, 2009). PSTVd is easily spread by contact within tomato crops on workers hands and on implements (Verhoeven 2010b). PSTVd is also transmitted through tomato pollen (Singh 1970). It is also possible that infected ornamental species may act as an inoculum source if handled before touching other susceptible plants (Verhoeven *et al*., 2010). No transmission of PSTVd was shown with *Apis mellifera*, *Bombus terrestris*, *Frankliniella occidentalis* or *Thrips tabaci* (Nielsen *et al.,* 2012).	PSTVd in Australia. Please see the following references on PSTVd outbreaks in tomato which were probably linked to seed: Elliot et al. (2001) First Report of Potato spindle tuber viroid in Tomato. New Zealand Plant Disease 85, Number 9; Mumford et al. (2003) The first report of Potato spindle tuber viroid (PSTVd) in commercial tomatoes in the UK New Disease Reports 8, 31; Hailstones et al. (2003) Detection and eradication of Potato spindle tuber viroid in tomatoes in commercial production in New South Wales, Australia. Australasian Plant Pathology, 32, 317–318; Verhoeven et al. (2004). Natural infections of tomato by Citrus exorcortis viroid, Columnea latent viroid, Potato spindle tuber viroid and Tomato chlorotic dwarf viroid. Eur. J. Plant Pathol.110:823-831; Verhoeven, et al., (2007) First Report of Potato spindle tuber viroid in Tomato in Belgium. Plant Disease, 91, Number 8; Ling and Sfetcu (2010) First Report of Natural Infection of Greenhouse Tomatoes by Potato spindle tuber viroid in the United States. Plant Disease 94, Number 11; FERA (2010) Emerging viroid threats to UK tomato production. Plant Disease Factsheet; Ling et al. (2013) First Report of Potato spindle tuber viroid Naturally Infecting Greenhouse Tomatoes in North Carolina. Plant Disease 97, Number 1. These references comment on PSTVd in capsicum: Lebas et al. (2005). Distribution of Potato spindle tuber viroid in New Zealand glasshouse crops of capsicum and tomato. Australasian Plant Pathology, 34(2), 129-133; Owens and Verhoeven. (2009) Potato spindle tuber. The Plant Health Instructor. American Phytopathological Society DOI: 10.1094/PHI-I-2009-0804-01. This reference is on mechanical transmission: Verhoeven, et al. (2010b). Mechanical transmission of Potato spindle tuber viroid between plants of Brugmansia suaveolens, Solanum jasminoides and potatoes and		

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
				tomatoes. European Journal of Plant Pathology, 128, 417-421.		
33.	9	Technical	PSTVd is the only viroid known to infect cultivated species of *S. tuberosum* naturally. However, *Mexican papita viroid* infects the wild species *Solanum cardiophyllum* (Martinez-Soriano *et al*., 1996). Experimentally, other viroid species in the genus *Pospiviroid* infect *S. tuberosum* (Verhoeven *et al*., 2004). In addition to PSTVd, other viroids have been found infecting *S. lycopersicum* naturally, including *Citrus exocortis viroid* (CEVd; Mishra *et al*., 1991), *Columnea latent viroid* (CLVd; Verhoeven *et al*., 2004), *Mexican papita viroid* (MPVd; Ling & Bledsoe, 2009), Pepper chat fruit viroid (PCFVd; Verhoeven et al., 2009; Chambers et al. 2013), *Tomato apical stunt viroid* (TASVd; Walter, 1987), *Tomato chlorotic dwarf viroid* (TCDVd; Singh *et al*., 1999) and *Tomato planta macho viroid* (TPMVd; Galindo *et al*., 1982).	If information on other viroids is retained, Pepper chat fruit viroid (PCFVd) should be included as it has been intercepted by Australian quarantine in tomato seed. Reference added to references	English	Australia
34.	12	Editorial	**Synonyms:**potato spindle ~~tuber virus~~ viroid, potato gothic virus, tomato bunchy top viriod	complete the list of synonyms	English	Ghana
35.	14	Editorial	**Common names:**potato spindle tuber, potato spindle tuber viroid	both are in common useage	English	Australia
36.	14	Editorial	**Common names:** potato spindle tuber , bunchy top of tomato	Completed list of common names CABI/ EPPO 2012	English	Ghana
37.	16	Editorial	Symptom appearance and severity depend on PSTVd strain, cultivar and environment. In *S. tuberosum*, infection may be symptomless or ~~produce~~ symptoms produce may rang~~e~~ing from mild to severe (reduction in plant size and uprightness and clockwise phyllotaxy of the foliage when the plants are viewed from above; dark green and rugose leaves). Tubers may be reduced in size, misshapen, spindle or dumbbell shaped, with conspicuous prominent eyes that are evenly distributed (EPPO, 2004). In *S. lycopersicum*, symptoms include stunting, epinasty, rugosity and lateral twisting of new leaflets, leaf chlorosis, reddening, brittleness, necrosis, reduction in fruit size, and fruit not fully ripening (Hailstones *et al*., 2003; ~~Lebas *et al*., 2005~~; Mackie *et al*., 2002; *Lebas et al., 2005*). In *C. annuum*,symptoms are subtle, with leaves near the top of the plant showing a wavy-edged margin (Lebas *et al*., 2005). In ornamental plant species symptoms are absent (Verhoeven, 2010).	For clarity	English	Ghana
38.	16	Technical	Symptom appearance and severity depend on PSTVd strain, cultivar and environment. In *S. tuberosum*, infection may be symptomless or produce symptoms ranging from mild to severe (reduction in plant size and uprightness and clockwise phyllotaxy of the foliage when the plants are viewed from above; dark green and rugose leaves). Tubers may be reduced in size, misshapen, spindle or dumbbell shaped, with conspicuous prominent eyes that are evenly distributed (EPPO, 2004). In *S. lycopersicum*, symptoms include stunting, epinasty, rugosity and lateral twisting of new leaflets, leaf chlorosis, reddening,	Clarification - only a limited number of species was investigated.	English	EPPO, Algeria, Morocco

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			brittleness, necrosis, reduction in fruit size, and fruit not fully ripening (Hailstones *et al*., 2003; Lebas *et al*., 2005; Mackie *et al*., 2002). In *C. annuum*,symptoms are subtle, with leaves near the top of the plant showing a wavy-edged margin (Lebas *et al*., 2005). Up to now all ~~In~~ ornamental plant species investigated did not show symptoms ~~are absent~~ (Verhoeven, 2010).			
39.	*16*	Technical	Symptom appearance and severity depend on PSTVd strain, cultivar and environment. In *S. tuberosum*, infection may be symptomless or produce symptoms ranging from mild to severe (reduction in plant size and uprightness and clockwise phyllotaxy of the foliage when the plants are viewed from above; dark green and rugose leaves). Tubers may be reduced in size, misshapen, spindle or dumbbell shaped, with conspicuous prominent eyes that are evenly distributed (EPPO, 2004). In *S. lycopersicum*, symptoms include stunting, epinasty, rugosity and lateral twisting of new leaflets, leaf chlorosis, reddening, brittleness, necrosis, reduction in fruit size, and fruit not fully ripening (Hailstones *et al*., 2003; Lebas *et al*., 2005; Mackie *et al*., 2002). In *C. annuum*,symptoms are subtle, with leaves near the top of the plant showing a wavy-edged margin (Lebas *et al*., 2005). Up to now all ~~In~~ ornamental plant species investigated did not show symptoms ~~are absent~~ (Verhoeven, 2010).	Clarification - only a limited number of species was investigated.	English	European Union
40.	*17*	Substantive	Because PSTVd may be asymptomatic, tests are required for its detection and identification. Detection of PSTVd can be achieved using the biological and molecular tests shown as options in Figure 1, but for identification, the polymerase chain reaction (PCR) product/amplicon must be sequenced as the tests are not specific for PSTVd and will detect other viroids. Sequencing will also contribute to preventing the reporting of false positives. If pathogenicity is considered to be important, biological indexing may be done. If the identification of PSTVd represents the first finding for a country, the laboratory ~~may wish~~suggest to have the diagnosis confirmed by another laboratory.	The word suggest is more suitable than wish for the meaning of the word wish is subjectivity.	English	China

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
41.	*19*	Technical	 * *Identification may not be needed for every viroid positive sample in certain situations; for example, when dealing with a PSTVd outbreak.* Note: If a viroid is suspected in a sample (i.e. typical symptoms are present) but a test gives a negative result, another of the tests should be carried out for confirmation of the result. **Figure 1.** Minimum requirements for the detection and identification of *Potato spindle tuber viroid*	sary to clarify in Figure 1 that both tic and asymptomatic samples should So that we suggest to add the ext “for symptomatic and asymptomatic after the word “Detection” in the first	English	Uruguay

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
42.	*19*	Technical	 * *Identification may not be needed for every viroid positive sample in certain situations; for example, when dealing with a PSTVd outbreak.* Note: If a viroid is suspected in a sample (i.e. typical symptoms are present) but a test gives a negative result, another of the tests should be carried out for confirmation of the result. **Figure 1.** Minimum requirements for the detection and identification of *Potato spindle tuber viroid*	sary to clarify in Figure 1 that both tic and asymptomatic samples should So that we suggest to add the ext “for symptomatic and asymptomatic after the word “Detection” in the first	English	COSAVE, Paraguay, Chile, Argentina, Peru, Brazil
43.	*20*	Technical	This Annex is for the detection of PSTVd and has not been developed for the detection and identification of other pospiviroids species. Methods needed to detect and identify other viroids are not fully considered, although they are described. Infection by other viroids is discussed as they may be present in host plants and cause similar disease symptoms. The presence of other viroids needs to be considered when choosing a detection and identification method. This	A large part of the Annex provides methods for detecting all or almost all pospiviroids. However, the Annex was intended only for the detection of PSTVd. That intention should be stated clearly. Methods for detecting and identifying other viroids haven’t been as carefully researched as	English	Australia

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			protocol describes non-specific detection methods that will detect all known viroids, pospiviroids and PSTVd (as well as some other closely related viroids). Identification is achieved by sequencing the PCR product.	those for PSTVd. The discussion of the other viroids isn't as well developed. The reviewers probably haven't considered previous drafts as if they were meant for the detection and identification of all pospiviroids. If the Annex was broadened to cover all the viroids mentioned, then there would be problems. For example, the methods of Shamloul 1997, Boonham 2004 and Verhoeven 2004 very probably won't detect all variants of other pospiviroid species, but that isn't discussed in the Annex. Australian experience is that the Verhoeven primers won't detect all PCFVd isolates and the Speiker primers won't detect all CLVd variants. Whereas Botermans et al. 2013 did experiments on 10 different PSTVd isolates, they only tested one variant each of PCFVd, MPVd and TPMVd, only two isolates of IrVd and three isolates each of CEVd and CLVd. So we are less sure about whether the Botermans primers will detect all CEVd, CLVd, PCFVd, MPVd and TPMVd isolates. This problem is partly picked up in the 'Main discussion points' where it says "Whether generic tests should be included in preference to more specific tests". If this issue is not clarified then this Annex may become the de facto Annex for all pospiviroid species, which would be wrong.		
44.	*21*	Substantive	Detection of PSTVd in true (botanical) seed is not dealt with by the Annex as no fully effective seed test method has been published. Several methods are presented in this diagnostic protocol for testing leaf and tuber tissue. As yet, there is no general agreement on the best method for detecting PSTVd in these tissues. In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The protocols described in this standard do not imply that other protocols used by a laboratory are unsuitable, provided that they have been adequately validated. Recommendations on method validation in phytodiagnostics are provided by EPPO (2010).	1st new sentence: There is only one publication provided on seed testing, EUPHRESCO (2010), and it doesn't support effective seed testing and provide a validated method for commercial seed lots. On page 46 of EUPHRESCO (2010) it is stated that "PSTVd infestation/contaminations of seeds are very variable...low concentrations in seeds are not detected." On page 47 of EUPHRESCO (2010) there are some main conclusions and it says: "Variable PSTVd concentrations in individual seeds and lower percentage of seed infections in commercial seed production render it difficult to evaluate a detection threshold and sample size." The	English	Australia

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
				detection threshold and sample size are crucial to effective seed testing, so the methods investigated in the EUPHRESCO project are not satisfactory for traded seed lots. Commercial seed lots cannot be tested without setting a sample size and should not be tested without understanding the detection threshold. Aside from EUPHRESCO (2010), no other scientific publication on testing true botanical seed for PSTVd have been published. As yet there is no fully satisfactory seed testing method. new 2nd/3rd sentence: Methods for detecting PSTVd and pospiviroids are being developed and compared currently. The Annex should indicate that there is not yet general agreement on methods and there is a possibility of changes and improvements in methods in the near future. Australian Department of Agriculture staff have discussed collaborative work on the methods for detecting pospiviroids with the Naktuinbouw laboratory and the Dutch NPPO. A discrepancy in detection between the Australian and Dutch seed testing has been discussed with Dr H. Koenraadt and Dr J Th J Verhoeven. The discrepancy is not simply due to different sample amounts, and it brings into question the methods used for detection. The Botermans et al. (2013) method included in the Annex, was published only very recently, so it is hoped that the authors recognise that research on the methods is continuing and not yet settled.		
45.	*21*	Technical	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The protocols described in this standard do not imply that other protocols used by a laboratory are unsuitable, provided that they have been adequately validated. Recommendations on method validation in phytodiagnostics are provided by EPPO (2010). MIQE guidelines (The Minimum Information for Publication of Quantitative Real-Time PCR Experiments) may be also used for qPCR validation	It is another relevant validation method	English	Uruguay
46.	*21*	Technical	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity	It is another relevant validation method	English	COSAVE, Paraguay,

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			and/or reproducibility achieved. The protocols described in this standard do not imply that other protocols used by a laboratory are unsuitable, provided that they have been adequately validated. Recommendations on method validation in phytodiagnostics are provided by EPPO (2010). MIQE guidelines (The Minimum Information for Publication of Quantitative Real-Time PCR Experiments) may be also used for qPCR validation			Chile, Argentina, Peru, Brazil
47.	*23*	Editorial	General guidance on sampling methodologies is described in ISPM 31:2008~~, *Methodologies for sampling of consignments*~~.	The title of ISPM 31 should be given in the references.	English	EPPO
48.	*23*	Editorial	General guidance on sampling methodologies is described in ISPM 31:2008~~, *Methodologies for sampling of consignments*~~.	The title of ISPM 31 should be given in the references.	English	European Union
49.	*23*	Editorial	General guidance on sampling methodologies is described in ISPM 31:2008~~, *Methodologies for sampling of consignments*~~.	The title of ISPM 31 should be given in the references.	English	Algeria, Morocco
50.	*24*	Editorial	**S. tuberosum *microplants and glasshouse grown* S. tuberosum *plants*** For microplants the whole plant should be used as the sample or the top two-thirds of the plant should be sampled under aseptic conditions so as to enable the rest of the plant to continue growing. Microplants should be four to six weeks old with stems of about 5 cm in length and with well-formed leaves. For glasshouse grown plants a fully expanded leaflet from each plant should be used. Viroid concentration is affected by temperature and light levels, so plants should be grown preferably at a temperature of 18 °C or higher and with a photoperiod of at least 14 h. Microplants or leaves may be bulked; the bulking rate will depend on the test method used. The bulking rate must be validated.	Minor grammatical change	English	Canada
51.	*24*	Editorial	***S. tuberosum*~~S. tuberosum~~ microplants and glasshouse grown ~~*microplants and glasshouse grown*~~ *S. tuberosum*~~S. tuberosum~~plants ~~*plants*~~** For microplants the whole plant should be used as the sample or the top two-thirds of the plant should be sampled under aseptic conditions so as to enable the rest of the plant to continue growing. Microplants should be four to six weeks old with stems of about 5 cm length and with well-formed leaves. For glasshouse grown plants a fully expanded leaflet from each plant should be used. Viroid concentration is affected by temperature and light levels, so plants should be grown preferably at a temperature of 18 °C or higher and with a photoperiod of at least 14 h. Microplants or leaves may be bulked; the bulking rate will depend on the test method used. The bulking rate must be validated.	A scientific name must be italicized or underlined.	English	Thailand
52.	*24*	Editorial	**S. tuberosum *microplants and glasshouse grown* S. tuberosum *plants*** For microplants the whole plant should be used as the sample or the top two-thirds of the plant should be sampled under aseptic conditions so as to enable the rest of	For clarity	English	Ghana

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			the plant to continue growing. Microplants should be four to six weeks old with stems of about 5 cm in length and with well-formed leaves. For glasshouse grown plants a fully expanded leaflet from each plant should be used. Viroid concentration is affected by temperature and light levels, so plants should be grown preferably at a temperature of 18 °C or higher and with a photoperiod of at least 14 h. Microplants or leaves may be bulked; the bulking rate will depend on the test method used. The bulking rate must be validated.			
53.	*24*	Technical	**S. tuberosum *microplants and glasshouse grown* S. tuberosum *plants*** For microplants the whole plant should be used as the sample or the top two-thirds of the plant should be sampled under aseptic conditions so as to enable the rest of the plant to continue growing. Microplants should be four to six weeks old with stems of about 5 cm length and with well-formed leaves. For glasshouse grown plants a fully expanded leaflet from each plant should be used. Viroid concentration islower at low ~~affected by~~ temperature and low light levels, so plants should be grown ~~preferably~~ at a temperature of at least 18 °C ~~or higher~~ and with a photoperiod of at least 14 h. Microplants or leaves may be bulked; the bulking rate will depend on the test method used. The bulking rate must be validated.	Changes to clarify the effect of temperature and light level on viroid concentration.	English	EPPO, Algeria, Morocco
54.	*24*	Technical	**S. tuberosum *microplants and glasshouse grown* S. tuberosum *plants*** For microplants the whole plant should be used as the sample or the top two-thirds of the plant should be sampled under aseptic conditions so as to enable the rest of the plant to continue growing. Microplants should be four to six weeks old with stems of about 5 cm length and with well-formed leaves. For glasshouse grown plants a fully expanded leaflet from each plant should be used. Viroid concentration islower at low ~~affected by~~ temperature and low light levels, so plants should be grown ~~preferably~~ at a temperature of at least 18 °C ~~or higher~~ and with a photoperiod of at least 14 h. Microplants or leaves may be bulked; the bulking rate will depend on the test method used. The bulking rate must be validated.	Changes to clarify the effect of temperature and light level on viroid concentration.	English	European Union
55.	*25*	Editorial	Field grown~~*Field grown*~~ *S. tuberosum*~~S. tuberosum~~plants~~*plants*~~ A fully expanded non senescing terminal leaflet from the top of each plant should be used. Leaves may be bulked together for testing; the bulking rate will depend on the test method used. The bulking rate must be validated.	A scientific name must be italicized or underlined.	English	Thailand
56.	*26*	Editorial	**S. tuberosum *tubers*** PSTVd is systemically distributed in infected *S. tuberosum* tubers, that is, in the “eye”, periderm, cortical zone containing cortical parenchyma and external phloem tissue, xylem ring, perimedullary zone containing internal phloem and phloem parenchyma strands tissue and perimedullary starch-storage parenchyma, and pith (Shamloul *et al*., 1997). It also occurs in almost equal amounts in different parts of both primarily and secondarily infected tubers (Roenhorst *et al*., 2006), that is, in the top and other eyes, heel ends, peel fragments and flesh cores throughout the whole tuber. The highest concentration is found immediately after harvest and hardly decreases during storage at 4 °C for	Error in the writting of "transcription".	English	EPPO

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			up to three months. Six months after harvest and storage at 4 °C, concentrations may decrease by more than 10^4 times. A core from any part of the tuber can be used as a sample. Up to 100 small cores weighing about 50 mg each may be bulked together for extraction if using real-time reverse transcri~~t~~ption (RT)-PCR. Bulking for other methods should be validated.			
57.	*26*	Editorial	**S. tuberosum *tubers*** PSTVd is systemically distributed in infected *S. tuberosum* tubers, that is, in the “eye”, periderm, cortical zone containing cortical parenchyma and external phloem tissue, xylem ring, perimedullary zone containing internal phloem and phloem parenchyma strands tissue and perimedullary starch-storage parenchyma, and pith (Shamloul *et al*., 1997). It also occurs in almost equal amounts in different parts of both primarily and secondarily infected tubers (Roenhorst *et al*., 2006), that is, in the top and other eyes, heel ends, peel fragments and flesh cores throughout the whole tuber. The highest concentration is found immediately after harvest and hardly decreases during storage at 4 °C for up to three months. Six months after harvest and storage at 4 °C, concentrations may decrease by more than 10^4 times. A core from any part of the tuber can be used as a sample. Up to 100 small cores weighing about 50 mg each may be bulked together for extraction if using real-time reverse transcri~~t~~ption (RT)-PCR. Bulking for other methods should be validated.	Error in the writting of "transcription".	English	European Union
58.	*26*	Editorial	**S. tuberosum *tubers*** PSTVd is systemically distributed in infected *S. tuberosum* tubers, that is, in the “eye”, periderm, cortical zone containing cortical parenchyma and external phloem tissue, xylem ring, perimedullary zone containing internal phloem and phloem parenchyma strands tissue and perimedullary starch-storage parenchyma, and pith (Shamloul *et al*., 1997). It also occurs in almost equal amounts in different parts of both primarily and secondarily infected tubers (Roenhorst *et al*., 2006), that is, in the top and other eyes, heel ends, peel fragments and flesh cores throughout the whole tuber. The highest concentration is found immediately after harvest and ~~hardly decreases~~ does not decrease considerably during storage at 4 °C for up to three months. After ~~S~~six months ~~after~~ following harvest and storage at 4 °C, concentrations may decrease by more than 10^4 times. A core from any part of the tuber can be used as a sample. Up to 100 small cores weighing about 50 mg each may be bulked together for extraction if using real-time reverse transcritpion (RT)-PCR. Bulking for other methods should be validated.	Minor changes to enhance sentence structure and readability.	English	Canada
59.	*26*	Editorial	***S. tuberosum*** ~~**S. tuberosum**~~ **tubers** ~~***tubers***~~ PSTVd is systemically distributed in infected *S. tuberosum* tubers, that is, in the “eye”, periderm, cortical zone containing cortical parenchyma and external phloem tissue, xylem ring, perimedullary zone containing internal phloem and phloem parenchyma strands tissue and perimedullary starch-storage parenchyma, and pith (Shamloul *et al*., 1997). It also occurs in almost equal amounts in different parts of both primarily and secondarily infected tubers (Roenhorst *et al*., 2006), that is, in the top and	A scientific name must be italicized or underlined.	English	Thailand

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			other eyes, heel ends, peel fragments and flesh cores throughout the whole tuber. The highest concentration is found immediately after harvest and hardly decreases during storage at 4 °C for up to three months. Six months after harvest and storage at 4 ^{o}C, concentrations may decrease by more than 10^4 times. A core from any part of the tuber can be used as a sample. Up to 100 small cores weighing about 50 mg each may be bulked together for extraction if using real-time reverse transcritpion (RT)-PCR. Bulking for other methods should be validated.			
60.	*26*	Editorial	**S. tuberosum *tubers*** PSTVd is systemically distributed in infected *S. tuberosum* tubers, that is, in the “eye”, periderm, cortical zone containing cortical parenchyma and external phloem tissue, xylem ring, perimedullary zone containing internal phloem and phloem parenchyma strands tissue and perimedullary starch-storage parenchyma, and pith (Shamloul *et al*., 1997). It also occurs in almost equal amounts in different parts of both primarily and secondarily infected tubers (Roenhorst *et al*., 2006), that is, in the top and other eyes, heel ends, peel fragments and flesh cores throughout the whole tuber. The highest concentration is found immediately after harvest and hardly decreases during storage at 4 °C for up to three months. Six months after harvest and storage at 4 ^{o}C, concentrations may decrease by more than 10^4 times. A core from any part of the tuber can be used as a sample. Up to 100 small cores weighing about 50 mg each may be bulked together for extraction if using real-time reverse transcri~~t~~ption (RT)-PCR. Bulking for other methods should be validated.	Error in the writting of "transcription".	English	Algeria
61.	*26*	Substantive	**S. tuberosum *tubers*** PSTVd is systemically distributed in infected *S. tuberosum* tubers, that is, in the “eye”, periderm, cortical zone containing cortical parenchyma and external phloem tissue, xylem ring, perimedullary zone containing internal phloem and phloem parenchyma strands tissue and perimedullary starch-storage parenchyma, and pith (Shamloul *et al*., 1997). It also occurs in almost equal amounts in different parts of both primarily and secondarily infected tubers (Roenhorst *et al*., 2006), that is, in the top and other eyes, heel ends, peel fragments and flesh cores throughout the whole tuber. The highest concentration is found immediately after harvest and hardly decreases during storage at 4 °C for up to three months. Six months after harvest and storage at 4 ^{o}C, concentrations may decrease by more than 10^4 times. A core from any part of the tuber can be used as a sample. Up to 100 small cores weighing about 50 mg each may be bulked together for extraction if using real-time reverse transcritpion (RT)-PCR. Bulking for other methods should be validated.	the spelling of "transcritpion" in last sentence of the paragraph should be corrected to "transcription".	English	Ghana
62.	*26*	Technical	**S. tuberosum *tubers*** PSTVd is systemically distributed in infected *S. tuberosum* tubers, that is, in the “eye”, periderm, cortical zone containing cortical parenchyma and external phloem tissue, xylem ring, perimedullary zone containing internal phloem and phloem parenchyma strands tissue and perimedullary starch-storage	Some labs use the conventional RT -PCR	English	China

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			parenchyma, and pith (Shamloul *et al*., 1997). It also occurs in almost equal amounts in different parts of both primarily and secondarily infected tubers (Roenhorst *et al*., 2006), that is, in the top and other eyes, heel ends, peel fragments and flesh cores throughout the whole tuber. The highest concentration is found immediately after harvest and hardly decreases during storage at 4 °C for up to three months. Six months after harvest and storage at 4 ^{o}C, concentrations may decrease by more than 10^4 times. A core from any part of the tuber can be used as a sample. Up to 100 small cores weighing about 50 mg each may be bulked together for extraction if using real-time reverse transcritpion (RT)-PCR. Bulking for other methods should be validated. Clarify: the sampling number of S. tuberosum tubers (up to 100) is suitable for conventional RT-PCR or not.			
63.	*28*	Technical	***Seed***Viroid concentration may vary greatly between seeds and the level of infection may vary from 100% to less than 5%. This makes it very difficult to recommend a maximum bulking rate. For *S. lycopersicum*, bulking rates of 100–1 000 have been used (EUPHRESCO, 2010) for testing samples of 1 000–3 000 seeds. In some countries bulking rates of 400 seeds are being used for testing samples of 20 000 seeds(H. Koenraadt, Naktuinbouw, the Netherlands, personal communication, 2012). It is better to give the seed sampling number of detection depending on the host commodity in practice.	The natural host range of PSTVd is relatively narrow. It is better to give the seed sampling number of detection depending on the host commodity in practice.	English	China
64.	*28*	Technical	~~***Seed***Viroid concentration may vary greatly between seeds and the level of infection may vary from 100% to less than 5%. This makes it very difficult to recommend a maximum bulking rate. For *S. lycopersicum*, bulking rates of 100–1 000 have been used (EUPHRESCO, 2010) for testing samples of 1 000–3 000 seeds. In some countries bulking rates of 400 seeds are being used for testing samples of 20 000 seeds(H. Koenraadt,~~ Naktuinbouw, the Netherlands, ~~personal communication, 2012).~~	Sentences, paragraphs and sections on testing true seed should be deleted. There is only one publication provided on seed testing, EUPHRESCO (2010), and it doesn't support fully effective seed testing or provide a fully validated method for commercial seed lots. On page 46 of EUPHRESCO (2010) it is stated that "PSTVd infestation/contaminations of seeds are very variable...low concentrations in seeds are not detected." On page 47 of EUPHRESCO (2010) there are some main conclusions and it says: "Variable PSTVd concentrations in individual seeds and lower percentage of seed infections in commercial seed production render it difficult to evaluate a detection threshold and sample size." The detection threshold and sample size are crucial to effective seed testing, so the methods investigated in the EUPHRESCO project are not entirely satisfactory. Commercial seed lots	English	Australia

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
				cannot be tested without setting a sample size and should not be tested without understanding the detection threshold. Aside from EUPHRESCO (2010), no other scientific publication on testing true botanical seed for PSTVd has been published. As yet there is no satisfactory seed testing method. Testing of seed is not mentioned in the introduction to the testing methods given in paragraphs 16 to 21. Seed testing is described cursorily in later sections. Paragraph 28 does not give clear guidance on seed sample sizes. The Annex suggests samples ranging from 1000 to 20,000 seeds. Sample size is critical as commonly there are very few infected seeds in traded seed lots of otherwise healthy seeds. Section 3.2 'Biological detection' does not say if inoculation of plants is an appropriate method for detecting the viroid in seed (it is not). Section 3.3.1 'Tissue maceration' paragraph 39 does not say what length of time to grind or paddle the seed, nor does it give guidance on the outcome. Should the seed be reduced to a powder, completely cracked or macerated? It does not indicate what buffer to use with the paddle or homogenizer. Section '3.3.2 Nucleic acid extraction' indicates three methods for nucleic acid extraction from seed but doesn't say if one method is better than another, and doesn't comment on sensitivity. Section 3.3.3.4 – Australian Department of Agriculure staff were advised by a Dutch scientist that the Botermans et al. 2013 assay could not be validated for tomato seed. It is more difficult to test seed than leaf tissue, but this is not mentioned in the Annex.		
65.	*29*	Substantive	~~Seeds may also be sown in compost in trays and the seedlings tested destructively or non-destructively.~~	see comment at para 28	English	Australia
66.	*29*	Technical	Seeds may also be sown in compost in trays and the seedlings tested destructively or non-destructively.	At what stage of seedling growth is the testing carried out? cotelydon, 1 leaf, 2 leaf stage or what? Why compost?	English	Australia

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
67.	30	Editorial	**3.2 Biological detection, (Pathogenicity)**	Are both words the same?	English	Ghana
68.	31	Substantive	Inoculation of *S. lycopersicum* plants (cvs Rutgers, Moneymaker or Sheyenne)will allow the detection of many viroids, but will not detect certain viroids such as the pospiviroid *Iresine viroid 1* (IrVd-1), and the method is inappropriate for detecting the PSTVd in seed. The method is sensitive, results are repeatable and reproducible, and visual evidence of pathogenicity may be observed. However, some isolates may not be detected because of the absence of symptoms, and if symptoms are produced, they may not be diagnostic for PSTVd. Moreover, biological indexing may require a great deal of greenhouse space, it is labour intensive, and several weeks or more may be needed before the test is completed. No work has been done to compare the sensitivity of this method with other methods described in this protocol.	Section 3.2 'Biological detection' does not say if inoculation of plants is an appropriate method for detecting the viroid in seed (it is not)	English	Australia
69.	31	Technical	Biological detection should only be used if molecular methods are not available. Inoculation of *S. lycopersicum* plants (cvs Rutgers, Moneymaker or Sheyenne)will allow the detection of many viroids, but will not detect certain viroids such as the pospiviroid *Iresine viroid 1* (IrVd-1). The method is sensitive, results are repeatable and reproducible, and visual evidence of pathogenicity may be observed. However, some isolates may not be detected because of the absence of symptoms, and if symptoms are produced, they may not be diagnostic for PSTVd. Moreover, biological indexing may require a great deal of greenhouse space, it is labour intensive, and several weeks or more may be needed before the test is completed. No work has been done to compare the sensitivity of this method with other methods described in this protocol.	new sentence 1: Biological detection (inoculation) is not as sensitive as PCR methods for detection, which is important. Guidance should be given on this to diagnosticians. The paragraph is confusing. The second sentence of paragraph 31 says biological tests are sensitive and repeatable, but the third sentence says some isolates will not be detected and symptoms may not be diagnostic. There are laboratories in several countries that are willing to test samples for PSTVd by PCR, so the molecular methods are available internationally.	English	Australia
70.	32	Technical	Approximately 200–500 mg leaf, root or tuber tissue is ground ina small quantityof0.1 M phosphate inoculation buffer (a1:1 dilution is adequate)containing carborundum (400 mesh). Phosphate buffer (pH 7.4) is made by combining 80.2 ml of 1 M K_2HPO_4 with 19.8 ml of 1 M KH_2PO_4 and adjusting the volume to 1 litre with distilled water.	1. Root material may also be tested (c.f. para 27). 2. Add the expected pH for clarification.	English	EPPO, Algeria, Morocco
71.	32	Technical	Approximately 200–500 mg leaf, root or tuber tissue is ground ina small quantityof0.1 M phosphate inoculation buffer (a1:1 dilution is adequate)containing carborundum (400 mesh). Phosphate buffer (pH 7.4) is made by combining 80.2 ml of 1 M K_2HPO_4 with 19.8 ml of 1 M KH_2PO_4 and adjusting the volume to 1 litre with distilled water.	1. Root material may also be tested (c.f. para 27). 2. Add the expected pH for clarification.	English	European Union
72.	33	Editorial	Young tomato plants with one or two fully expanded leaves are inoculated. Using a gloved finger, a cotton bud, or a cotton swab dipped into the inoculum, the leaf surface is gently rubbed with the inoculum and then the leaves are immediately rinsed with water until the carborundum has been removed. The plants are grown at 25–39° C under a photoperiod of 14 h. If necessary, supplemental illumination	The discription of symptom is overlap with the paragraph 16.	English	China

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			is provided (approximately 650 µE/m^2/s; Grassmick & Slack, 1985). The plants are inspected weekly for symptoms for up to six weeks after inoculation. ~~Symptoms of PSTVd infection include stunting, epinasty, rugosity and lateral twisting of new leaflets, leaf chlorosis, reddening, brittleness and necrosis.~~			
73.	*33*	Technical	Young tomato plants with one or two fully expanded leaves are inoculated. Using a gloved finger, a cotton bud, or a cotton swab dipped into the inoculum, the leaf surface is gently rubbed with the inoculum and then the leaves are immediately rinsed with water until the carborundum has been removed. The plants are grown at 25–30~~9~~° C under a photoperiod of 14 h. If necessary, supplemental illumination is provided (approximately 650 µE/m^2/s; Grassmick & Slack, 1985). The plants are inspected weekly for symptoms for up to six weeks after inoculation. Symptoms of PSTVd infection include stunting, epinasty, rugosity and lateral twisting of new leaflets, leaf chlorosis, reddening, brittleness and necrosis.	39 degrees is very high. Suggest modification to 30 degrees.	English	EPPO, Algeria, Morocco
74.	*33*	Technical	Young tomato plants with one or two fully expanded leaves are inoculated. Using a gloved finger, a cotton bud, or a cotton swab dipped into the inoculum, the leaf surface is gently rubbed with the inoculum and then the leaves are immediately rinsed with water until the carborundum has been removed. The plants are grown at 25–30~~9~~° C under a photoperiod of 14 h. If necessary, supplemental illumination is provided (approximately 650 µE/m^2/s; Grassmick & Slack, 1985). The plants are inspected weekly for symptoms for up to six weeks after inoculation. Symptoms of PSTVd infection include stunting, epinasty, rugosity and lateral twisting of new leaflets, leaf chlorosis, reddening, brittleness and necrosis.	39 degrees is very high. Suggest modification to 30 degrees.	English	European Union
75.	*34*	Technical	A bioassay on tomato will allow detection of many pospiviroids (except IrVd); therefore, RT-PCRshould be carried outon the nucleic acid extracted from symptomatic indicator plants and the PCR product should be sequenced for identification.	For clarification	English	EPPO, Algeria, Morocco
76.	*34*	Technical	A bioassay on tomato will allow detection of many pospiviroids (except IrVd); therefore, RT-PCRshould be carried outon the nucleic acid extracted from symptomatic indicator plants and the PCR product should be sequenced for identification.	For clarification	English	European Union
77.	*36*	Technical	**3.3.1 Sample preparation~~Tissue maceration~~**	More logical title.	English	EPPO, Algeria, Morocco
78.	*36*	Technical	**3.3.1 Sample preparation~~Tissue maceration~~**	More logical title.	English	European Union
79.	*37*	Technical	**Microplants, leaf material and roots** Mortars and pestles or homogenizers (e.g. Homex 6, Bioreba)[1] with extraction bags have been used successfully to grind material. Adding a small quantity of water or lysis ~~extraction~~ buffer or freezing the sample (e.g. in liquid nitrogen) may facilitate homogenization.	If this is the same buffer as used in paragraph 43, then it should be refered to simply as lysis buffer.	English	EPPO

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
80.	*37*	Technical	**Microplants, leaf material and roots** Mortars and pestles or homogenizers (e.g. Homex 6, Bioreba)[1] with extraction bags have been used successfully to grind material. Adding a small quantity of water or lysis ~~extraction~~ buffer or freezing the sample (e.g. in liquid nitrogen) may facilitate homogenization.	If this is the same buffer as used in paragraph 43, then it should be refered to simply as lysis buffer.	English	European Union
81.	*37*	Technical	**Microplants, leaf material and roots** Mortars and pestles or homogenizers (e.g. Homex 6, Bioreba)[1] with extraction bags have been used successfully to grind material. Adding a small quantity of water or lysis extraction buffer (to add composition of lysis extraction buffer)or freezing the sample (e.g. in liquid nitrogen) may facilitate homogenization.	Technical for more harmonisation	English	NEPPO, Algeria, Morocco
82.	*39*	Substantive	**Seeds** For small numbers of seeds (<100) a tissue lyser (e.g. Retsch TissueLyser, Qiagen[2]) may be used. Although mortars and pestles may be used they are probably not practical for routine use, and cross-contamination may be more difficult to control. For larger numbers of seeds, a paddle blender (e.g. MiniMix®, Interscience)[3] or homogenizer (e.g. Homex 6) with a minimum quantity of extraction buffer for the initial homogenization may be used. Alternatively, use liquid nitrogen to freeze the sample, and grind it in a cell mill (this method can also be used for other tissue types).	Is the entire seed potato crushed and used for testing or is a core or vascular bundle taken and used?	English	Jamaica
83.	*39*	Technical	**Seeds** For small numbers of seeds (<100) a tissue lyser (e.g. Retsch TissueLyser, Qiagen[2]) may be used. Although mortars and pestles may be used they are probably not practical for routine use, and cross-contamination may be more difficult to control. For larger numbers of seeds, a paddle blender (e.g. MiniMix®, Interscience)[3] or homogenizer (e.g. Homex 6) with a minimum quantity of extraction buffer for the initial homogenization may be used. Alternatively, use liquid nitrogen to freeze the sample, and grind it in a cell mill (this method can also be used for other tissue types).	Is the extraction buffer the 'lysis buffer' (paragraph 43) or is it the phosphate buffer refered to in paragraph 32?	English	EPPO, Morocco
84.	*39*	Technical	**Seeds** For small numbers of seeds (<100) a tissue lyser (e.g. Retsch TissueLyser, Qiagen[2]) may be used. Although mortars and pestles may be used they are probably not practical for routine use, and cross-contamination may be more difficult to control. For larger numbers of seeds, a paddle blender (e.g. MiniMix®, Interscience)[3] or homogenizer (e.g. Homex 6) with a minimum quantity of extraction buffer for the initial homogenization may be used. Alternatively, use liquid nitrogen to freeze the sample, and grind it in a cell mill (this method can also be used for other tissue types).	Is the extraction buffer the 'lysis buffer' (paragraph 43) or is it the phosphate buffer refered to in paragraph 32?	English	European Union
85.	*39*	Technical	~~**Seeds** For small numbers of seeds (<100) a tissue lyser (e.g. Retsch TissueLyser, Qiagen[2]) may be used. Although mortars and pestles may be used they are probably not practical for routine use, and cross-contamination may be more difficult to control. For larger numbers of seeds, a paddle blender (e.g. MiniMix®, Interscience)[3] or homogenizer (e.g. Homex 6) with a minimum quantity of extraction buffer for the initial homogenization may be used. Alternatively, use liquid nitrogen to freeze the sample, and grind it in a cell mill (this method can also~~	The argument for deleting the para is given at para 28.	English	Australia

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			~~be used for other tissue types).~~			
86.	*40*	Substantive	**3.3.2 Nucleic acid extraction**	For real-time RT-PCR approaches, the use of direct methods of sample preparation prior amplification such as tissue-print or squash even dilution of the extract have been proved to be very reliable, while avoiding RNA purification and the risk of cross-contamination. An additional subheading numbered as 3.3.3 should be included. There is a commercially available kit based on this approach (see www.plantprint.net). For further information, please contact Nuria Duran-Vila (nduran@ivia.es) and/or Mariano Cambra (mcambra@ivia.es). For additional information concerning the usefulness of direct methods of sample preparation please see: De Boer and López (2012). “New grower-friendly methods for plant pathogen monitoring”. Annu. Rev. Phytopathol. 50, 197-218.	English	EPPO
87.	*40*	Substantive	**3.3.2 Nucleic acid extraction**	For real-time RT-PCR approaches, the use of direct methods of sample preparation prior amplification such as tissue-print or squash even dilution of the extract have been proved to be very reliable, while avoiding RNA purification and the risk of cross-contamination. An additional subheading numbered as 3.3.3 should be included. There is a commercially available kit based on this approach (see www.plantprint.net). For further information, please contact Nuria Duran-Vila (nduran@ivia.es) and/or Mariano Cambra (mcambra@ivia.es). For additional information concerning the usefulness of direct methods of sample preparation please see: De Boer and López (2012). “New grower-friendly methods for plant pathogen monitoring”. Annu. Rev. Phytopathol. 50, 197-218.	English	European Union
88.	*41*	Technical	A wide range of nucleic acid extraction methods may be used, from commercial kits to methods published in scientific journals. The following nucleic acid extraction methods have been used successfully for the detection of PSTVd, as indicated for individual methods. Add evaluation of each methods at this paragraph.	It’s more convenient for user to choose some methods according to the condition of the lab.	English	China

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
89.	*42*	Editorial	**Commercial kits** Commercial extraction kits such ~~as RNeasy® (Qiagen)[4] and MasterPure™~~ (Epicentre Biotechnologies)[5]may be usedaccording to the manufacturer's instructions. RNeasy® was evaluated for the extraction of PSTVd RNA from *S. lycopersicum* seed as part of the EUPHRESCO DEP project (EUPHRESCO, 2010).	The brand name should not be present in the standard.	English	China
90.	*42*	Technical	**Commercial kits** Commercial extraction kits such as RNeasy® (Qiagen)[4] and MasterPure™ (Epicentre Biotechnologies)[5]may be usedaccording to the manufacturer's instructions. ~~RNeasy® was evaluated for the extraction of PSTVd RNA from *S. lycopersicum* seed as part of the EUPHRESCO DEP project (EUPHRESCO, 2010).~~	Sentence should be deleted in line with comment at para 28. Section '3.3.2 Nucleic acid extraction' indicates three methods for nucleic acid extraction from seed but doesn't comment on sensitivity nor on other problems with seed testing.	English	Australia
91.	*43*	Technical	**Lysis buffer** A modified extraction lysis buffer described by Mackenzie *et al*. (1997) can be used. It extracts quality RNA from a wide range of plant species. Lysis buffer is not name of method.	It's only a buffer, and not a method.	English	China
92.	*44*	Technical	**EDTA buffer** Plant tissue may be homogenized in a simple extraction buffer (50 mM NaOH, 2.5 mM ethylenediaminetetraacetic acid (EDTA)) and then incubated (at approximately 25° C for 15 min) or centrifuged (at 12 000 *g* at 4 °C for 15 min). The supernatant can then, depending on the level of sensitivity required, be either used directly for RT-PCR (less sensitive) or spotted onto a nitrocellulose membrane and eluted using sterile distilled water (more sensitive) (Singh *et al*., 2006). Although the concentration of viroid is lower for the EDTA method than for the other extraction methods described, this should not be a limiting factor when the method is used with RT-PCR or the digoxigenin (DIG) probe. The method has been used with *S. lycopersicum* and *S. tuberosum* and a range of ornamental plant species. EDTA buffer is not name of method.	It's only a buffer, and not a method.	English	China
93.	*44*	Technical	**EDTA buffer** Plant tissue may be homogenized in a simple extraction buffer (50 mM NaOH, 2.5 mM ethylenediaminetetraacetic acid (EDTA) indicate the w/v)) and then incubated (at approximately 25° C for 15 min) or centrifuged (at 12 000 *g* at 4 °C for 15 min). The supernatant can then, depending on the level of sensitivity required, be either used directly for RT-PCR (less sensitive) or spotted onto a nitrocellulose membrane and eluted using sterile distilled water (more sensitive) (Singh *et al*., 2006). Although the concentration of viroid is lower for the EDTA method than for the other extraction methods described, this should not be a limiting factor when the method is used with RT-PCR or the digoxigenin (DIG) probe. The method has been used with *S. lycopersicum* and *S. tuberosum* and a range of ornamental plant species.	technical	English	NEPPO

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
94.	*46*	Technical	**CTAB** This extraction method using cetyl trimethylammonium bromide (CTAB) (EPPO, 2004) has been used on leaves of a wide range of plant species ~~and tomato seed~~ with real-time RT-PCR.	delete in line with comment at para 28.	English	Australia
95.	*47*	Editorial	**KingFisher (Thermo Scientific[6])** The following automated procedure is based on use of the KingFisher mL Magnetic Particle Processor.With appropriate adjustment of volumes, other KingFisher models may be used. The extraction method has been used on leaves of a wide range of plant species, *S. tuberosum* tubers and *S. lycopersicum* seed. The method has been used with the real-time RT-PCR methods described in this standard. Cycle threshold (Ct) values several cycles higher may be expected using the KingFisher compared with the other extraction methods described in this protocol, but the increased throughput of samples that is achievable makes this a valuable extraction method.To make up the extraction buffer (EB), 200 µl of 8.39% (w/v) tetrasodium pyrophosphate (TNaPP) solution (pH 10.0–10.9) and 100 µl Antifoam B Emulsion (AB) (Sigma)[7] are added to 9.8 ml guanidine lysis buffer (GLB). GLB is comprised of ~~comprises~~ water, 750 ml; absolute ethanol, 250 ml; guanidine-HCl, 764.2 g; disodium EDTA dehydrate, 7.4 g; polyvinylpyrrolidone (PVP), 30.0 g; citric acid monohydrate, 5.25 g; tri-sodium citrate, 0.3 g; and Triton™ X-100, 5 ml. GLB may be stored indefinitely. Store EB at 4° C and ~~discard~~ at the end of the day discard any that has not been used.	Minor changes to enhance sentence structure and readability.	English	Canada
96.	*47*	Technical	**KingFisher (Thermo Scientific[6])** The following automated procedure is based on use of the KingFisher mL Magnetic Particle Processor.With appropriate adjustment of volumes, other KingFisher models may be used. The extraction method has been used on leaves of a wide range of plant species, *S. tuberosum* tubers and *S. lycopersicum* seed. The method has been used with the real-time RT-PCR methods described in this standard. Cycle threshold (Ct) values several cycles higher may be expected using the KingFisher compared with the other extraction methods described in this protocol, but the increased throughput of samples that is achievable makes this a valuable extraction method.To make up the extraction buffer (EB), 200 µl of 8.39% (w/v) tetrasodium pyrophosphate (TNaPP) solution (pH 10.0–10.9) and 100 µl Antifoam B Emulsion (AB) (Sigma)[7] are added to 9.8 ml guanidine lysis buffer (GLB). GLB comprises water, 750 ml; absolute ethanol, 250 ml; guanidine-HCl, 764.2 g; disodium EDTA dehydrate, 7.4 g; polyvinylpyrrolidone (PVP), 30.0 g; citric acid monohydrate, 5.25 g; tri-sodium citrate, 0.3 g; and Triton™ X-100, 5 ml. GLB may be stored indefinitely. Store EB at 4° C and discard at the end of the day any that has not been used.	Add a reference to support the statement that this is a valuable method.	English	EPPO, Algeria, Morocco
97.	*47*	Technical	**KingFisher (Thermo Scientific[6])** The following automated procedure is based on use of the KingFisher mL Magnetic Particle Processor.With appropriate adjustment of volumes, other KingFisher models may be used. The extraction method has been used on leaves of a wide range of plant species, *S. tuberosum* tubers and *S. lycopersicum* seed. The method has been used with the real-time	Add a reference to support the statement that this is a valuable method.	English	European Union

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			RT-PCR methods described in this standard. Cycle threshold (Ct) values several cycles higher may be expected using the KingFisher compared with the other extraction methods described in this protocol, but the increased throughput of samples that is achievable makes this a valuable extraction method.To make up the extraction buffer (EB), 200 µl of 8.39% (w/v) tetrasodium pyrophosphate (TNaPP) solution (pH 10.0–10.9) and 100 µl Antifoam B Emulsion (AB) (Sigma)[7] are added to 9.8 ml guanidine lysis buffer (GLB). GLB comprises water, 750 ml; absolute ethanol, 250 ml; guanidine-HCl, 764.2 g; disodium EDTA dehydrate, 7.4 g; polyvinylpyrrolidone (PVP), 30.0 g; citric acid monohydrate, 5.25 g; tri-sodium citrate, 0.3 g; and Triton™ X-100, 5 ml. GLB may be stored indefinitely. Store EB at 4° C and discard at the end of the day any that has not been used.			
98.	*47*	Technical	**KingFisher (Thermo Scientific[6])** The following automated procedure is based on use of the KingFisher mL Magnetic Particle Processor.With appropriate adjustment of volumes, other KingFisher models may be used. The extraction method has been used on leaves of a wide range of plant species~~,~~and *S. tuberosum* tubers ~~and *S. lycopersicum* seed~~. The method has been used with the real-time RT-PCR methods described in this standard. Cycle threshold (Ct) values several cycles higher may be expected using the KingFisher compared with the other extraction methods described in this protocol, but the increased throughput of samples that is achievable makes this a valuable extraction method.To make up the extraction buffer (EB), 200 µl of 8.39% (w/v) tetrasodium pyrophosphate (TNaPP) solution (pH 10.0–10.9) and 100 µl Antifoam B Emulsion (AB) (Sigma)[7] are added to 9.8 ml guanidine lysis buffer (GLB). GLB comprises water, 750 ml; absolute ethanol, 250 ml; guanidine-HCl, 764.2 g; disodium EDTA dehydrate, 7.4 g; polyvinylpyrrolidone (PVP), 30.0 g; citric acid monohydrate, 5.25 g; tri-sodium citrate, 0.3 g; and Triton™ X-100, 5 ml. GLB may be stored indefinitely. Store EB at 4° C and discard at the end of the day any that has not been used.	delete in line with comment at para 28	English	Australia
99.	*48*	Substantive	For each sample, at least 200 mg leaf or tuber tissue or up to 100 seeds are macerated, and then EB is added immediately at a ratio of 10 ml buffer per 1 g plant tissue or 20 ml buffer per 1 g seed. Maceration is continued until a clear cell lysate with minimal intact tissue debris is obtained.	Is the entire seed potato crushed and used for testing or is a core or vascular bundle taken and used?	English	Jamaica
100.	*49*	Editorial	Approximately 2 ml lysate is decanted into a fresh microcentrifuge tube, which is centrifuged at approximately 5 000 *g* for 1 min. One millilitre of supernatant is removed and placed in the first tube (A) of the KingFisher mL rack, to which 50 µl of vortexed MAP Solution A magnetic beads (Invitek/Thistle Scientific)[8] are added. Tube B has 1 ml GLB added to it; tubes C and D 1 ml of 70% ethanol; and tube E 200 µl water or 1× Tris-EDTA (TE) buffer.	adding a space in number 5 000 g.	English	Thailand
101.	*55*	Substantive	**3.3.3.1 R-PAGE (EPPO, 2004)**	This method requires a positive control. Unless countries has access to a cloned PSTVd DNA,	English	South Africa

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
				this method cannot be implemented. It is also very difficult to to import a cloned copy from other countries.		
102.	*56*	Technical	R-PAGE has been recommended as a detection method for PSTVd infecting *S. tuberosum* leaves (EPPO, 2004), but it is less sensitive than the other molecular methods evaluated. It detects the equivalent of 5–20 mg PSTVd-infected leaf tissue (when mixed with a standard amount of healthy leaf tissue) depending on the laboratory, whereas other methods detect as little as 15.5 µg infected leaf tissue (the lowest weight tested). Results are repeatable and mostly reproducible, with three out of four laboratories detecting PSTVd (Jeffries & James, 2005).	The viroid concentration in infected leafs may differ. Consequently the percentage of infected leaf tissue may only be used for comparison of sensitivity of different methods if the same extract has been used. So either the results are from the same ring test (which should be clarified) or the comparison as presented is not correct.	English	EPPO
103.	*56*	Technical	R-PAGE has been recommended as a detection method for PSTVd infecting *S. tuberosum* leaves (EPPO, 2004), but it is less sensitive than the other molecular methods evaluated. It detects the equivalent of 5–20 mg PSTVd-infected leaf tissue (when mixed with a standard amount of healthy leaf tissue) depending on the laboratory, whereas other methods detect as little as 15.5 µg infected leaf tissue (the lowest weight tested). Results are repeatable and mostly reproducible, with three out of four laboratories detecting PSTVd (Jeffries & James, 2005).	The viroid concentration in infected leafs may differ. Consequently the percentage of infected leaf tissue may only be used for comparison of sensitivity of different methods if the same extract has been used. So either the results are from the same ring test (which should be clarified) or the comparison as presented is not correct.	English	European Union
104.	*62*	Editorial	The probe used is based on a full-length monomer of PSTVd produced by Agdia, Inc.[9] (Cat. No. DLP 08000/0001). This probe should be used according to the manufacturer's instructions, or refer to EPPO (2004) for details of the method. In addition to the Ames buffer (EPPO, 2004), PEG and other extraction buffers may be used for nucleic acid extraction.	A comma is missing.	English	EPPO
105.	*62*	Editorial	The probe used is based on a full-length monomer of PSTVd produced by Agdia, Inc.[9] (Cat. No. DLP 08000/0001). This probe should be used according to the manufacturer's instructions, or refer to EPPO (2004) for details of the method. In addition to the Ames buffer (EPPO, 2004), PEG and other extraction buffers may be used for nucleic acid extraction.	A comma is missing.	English	European Union
106.	*62*	Editorial	The probe used is based on a full-length monomer of PSTVd produced by Agdia, Inc.[9] (Cat. No. DLP 08000/0001). This probe should be used according to the manufacturer's instructions, or refer to EPPO (2004) for details of the method. In addition to the Ames buffer (EPPO, 2004), PEG and other extraction buffers may be used for nucleic acid extraction.	A comma is missing.	English	Algeria
107.	*64*	Substantive	**3.3.3.3 Conventional RT-PCR using the primers of Verhoeven *et al.* (2004)**	This method require a positive control. Unless countries has access to a cloned PSTVd DNA, this method cannot be implemented. It is also very difficult to import cloned copy from other countries.	English	South Africa

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
108.	65	Technical	The primers used in this assay are the Pospi1 and Vid primers of Verhoeven *et al.* (2004). The Pospi1 primers will detect CEVd, CSVd, IrVd-1, MPVd, PCFVd, PSTVd, TASVd, TCDVd and TPMVd. The Vid primers will detect PSTVd, TCDVd, and, additionally CLVd. Using the Pospi1 and Vid primers in two separate reactions will allow detection of all pospiviroids. Sequence mismatch at critical positions of the primer target site may prevent the detection of some isolates (e.g. an isolate of CLVd was not detected using these primers; Steyer *et al*., 2010). *In silico* studies have shown that the following PSTVd isolates may not be detected because of primer/sequence mismatch at critical positions: Pospi1 primers: EU879925, EU273604, EF459697, AJ007489, AY372398, AY372394, FM998551, DQ308555, E00278; Vid primers: EU273604[10]. The Pospi1 primers are much more sensitive than the Vid primers for the detection of PSTVd.	As in paragraph78 on real time RT-PCR, it is suggested to mention that combining Pospi1 primers with primers developed by Spieker (2004) can be used for the specific detection of CLVd. This combination allows the detection of all known isolates even the one cited by Steyer et al (2010)	English	EPPO
109.	65	Technical	The primers used in this assay are the Pospi1 and Vid primers of Verhoeven *et al.* (2004). The Pospi1 primers will detect CEVd, CSVd, IrVd-1, MPVd, PCFVd, PSTVd, TASVd, TCDVd and TPMVd. The Vid primers will detect PSTVd, TCDVd, and, additionally CLVd. Using the Pospi1 and Vid primers in two separate reactions will allow detection of all pospiviroids. Sequence mismatch at critical positions of the primer target site may prevent the detection of some isolates (e.g. an isolate of CLVd was not detected using these primers; Steyer *et al*., 2010). *In silico* studies have shown that the following PSTVd isolates may not be detected because of primer/sequence mismatch at critical positions: Pospi1 primers: EU879925, EU273604, EF459697, AJ007489, AY372398, AY372394, FM998551, DQ308555, E00278; Vid primers: EU273604[10]. The Pospi1 primers are much more sensitive than the Vid primers for the detection of PSTVd.	As in paragraph78 on real time RT-PCR, it is suggested to mention that combining Pospi1 primers with primers developed by Spieker (2004) can be used for the specific detection of CLVd. This combination allows the detection of all known isolates even the one cited by Steyer et al (2010)	English	European Union
110.	73	Editorial	The Qiagen[11] OneStep RT-PCR Kit has been shown to be reliable when used for the detection of PSTVd, CEVd, CLVd, CSVd, TASVd and TCDVd (EUPHRESCO, 2010) and for other pospiviroids listed at the start of this section (T. James, SASA, UK, personal communication, 2010). It is not necessary to use the Q-solution described by EUPHRESCO (2010).	A comma is missing.	English	EPPO
111.	73	Editorial	The Qiagen[11] OneStep RT-PCR Kit has been shown to be reliable when used for the detection of PSTVd, CEVd, CLVd, CSVd, TASVd and TCDVd (EUPHRESCO, 2010) and for other pospiviroids listed at the start of this section (T. James, SASA, UK, personal communication, 2010). It is not necessary to use the Q-solution described by EUPHRESCO (2010).	A comma is missing.	English	European Union
112.	73	Editorial	The Qiagen[11] OneStep RT-PCR Kit has been shown to be reliable when used for the detection of PSTVd, CEVd, CLVd, CSVd, TASVd and TCDVd (EUPHRESCO, 2010) and for other pospiviroids listed at the start of this section (T. James, SASA, UK, personal communication, 2010). It is not necessary to use the Q-solution described by EUPHRESCO (2010).	A comma is missing.	English	Algeria

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
113.	*74*	Technical	Two microlitres of template is added to 23 µl master mix comprising 1.0 µl each of forward and reverse primer (10 µM), 5 µl of 5× Qiagen OneStep RT-PCR buffer, 1.0 µl Qiagen OneStep RT-PCR enzyme mix, 1.0 µl dNTPs (10 mM each dNTP), and 14 µl water. The thermocyling programme may be ~~is~~ as follows: Option 1 50 °C for 30 min; 95 °C for 15 min; 35 cycles of 94 °C for 30 s, 62 °C for 60 s and 72 °C for 60 s; and a final extension step of 72 °C for 7 min. Option 2: 48ºC for 45 min; 94 ºC for 2 min; 15 cycles of 94 ºC for 30 s, 62 ºC for 90 s, 72 ºC for 45 s; 30 cycles of 94 ºC for 30 s, 59 ºC for 90 s, 72 ºC for 45 s; 72 ºC for 7 min, hold at 15ºC. Option 3: 50 ºC for 30 min; 94 ºC for 2 min; 37 cycles of 94 ºC for 30 s, touchdown 61-57 over 1st 5 cycles for 30 s, 72 ºC for 30 s; 72 ºC for 5 min; hold at 15.	The Annex describes a 35 cycle PCR for the Pospi1 primers, but PCRs with greater numbers of cycles may be needed to detect PSTVd and other cycle conditions may be used. Options 2 and 3 are cycles validated by two Australian laboratories.	English	Australia
114.	*77*	Substantive	**3.3.3.4 Real-time RT-PCR using the GenPospi assay (Botermans *et al.*, 2013)**	This method requires a positive control. Unless countries have access to a cloned PSTVd DNA, this method cannot be implemented. It is also difficult to import a cloned copy from other countries.	English	South Africa
115.	*78*	Technical	The GenPospi assay uses TaqMan® real-time RT-PCR to detect all known species of the genus *Pospiviroid*. It consists of two reactions running in parallel: the first (reaction mix 1) targets all pospiviroids except CLVd (Botermans *et al.,* 2013); the second (reaction mix 2) specifically targets CLVd (Monger *et al.*, 2010). To monitor the RNA extraction a *nad5* internal control based on primers developed by Menzel *et al.* (2002) to amplify mRNA from plant mitochondria (the mitochondrial NADH dehydrogenase gene) is included. Method validation on tomato leaves showed that the GenPospi assay detects some isolates of all known pospiviroid~~s~~ species up to a relative infection rate of 0.13% (which equals 770× dilution). The assay was specific as no cross-reactivity was observed with other viroids, viruses or nucleic acid from plant hosts. Repeatability and reproducibility were 100% and the assay appeared robust in an inter-laboratory comparison. The GenPospi assay has been shown to be a suitable tool for large-scale screening of some isolates of ~~for~~ all known pospiviroid~~s~~ species. The assay ~~Although it~~ has been validated for tomato leaves, ~~it can potentially be used for any crop.~~and although it might work with other plant tissue, it is uncertain if the assay can be used for all plant material	The phrase 'all pospiviroids' is not appropriate. Botermans et al. 2013 did experiments on 10 different PSTVd isolates, they only tested one isolate each of PCFVd, MPVd and TPMVd, only two isolates of IrVd and three isolates each of CEVd and CLVd. So we are unsure about whether the Botermans primers will detect all CEVd, CLVd, PCFVd, MPVd and TPMVd isolates. Last sentence: DAFF staff were advised by a Dutch scientist that the Botermans 2013 GenPospi assay could not be validated for tomato seed.	English	Australia
116.	*103*	Substantive	For this method, Botermans *etÂ al*. (2013) interpreted cycle threshold (Ct) values<32 as positive; those between 32 and <37 as ~~doubtful~~inconclusive, requiring confirmation; and those â‰¥37 as negative. However, these values	More appropriate wording	English	United States of America

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			need to be defined in each laboratory.			
117.	*103*	Technical	For this method, Botermans *et al*. (2013) interpreted cycle threshold (Ct) values<32 as positive; those between 32 and <37 as doubtful, requiring confirmation; and those ≥37 as negative. However, these values may exclude low levels of infection in some tissues, and need to be defined in each laboratory.	Work by Australian laboratories suggests that a 32 cycle threshold is too low and may exclude some samples with low levels of infection.	English	Australia
118.	*106*	Editorial	The RT-PCR primers used in this assay are those of Shamloul *et al*. (1997), which are also described by Weidemann and Buchta (1998). The primers will detect MPVd, PSTVd, TCDVd and TPMVd. *In silico* studies have shown that the following PSTVd isolates may not be detected because of primer/sequence mismatch at critical positions: AY372394, DQ308555, EF459698 for the reverse primer[2].	What is the link with footnote 2?	English	EPPO
119.	*106*	Editorial	The RT-PCR primers used in this assay are those of Shamloul *et al*. (1997), which are also described by Weidemann and Buchta (1998). The primers will detect MPVd, PSTVd, TCDVd and TPMVd. *In silico* studies have shown that the following PSTVd isolates may not be detected because of primer/sequence mismatch at critical positions: AY372394, DQ308555, EF459698 for the reverse primer[2].	What is the link with footnote 2?	English	European Union
120.	*106*	Technical	The RT-PCR primers used in this assay are those of Shamloul *et al*. (1997), which are also described by Weidemann and Buchta (1998). The primers will detect MPVd, PSTVd, TCDVd and TPMVd. *In silico* studies have shown that the following PSTVd isolates may not be detected because of primer/sequence mismatch at critical positions: AY372394, DQ308555, EF459698 for the reverse primer[2]. In case DNA was not amplified using conventional RT-PCR, the Vid primers shall be used for pospiviroid detection in this assay.	The RT-PCR primers used in this assay are those of Shamloul et al. (1997). In silico studies have shown that the following PSTVd isolates may not be detected because of primer/sequence mismatch at critical positions: AY372394, DQ308555, EF459698.	English	China
121.	*118*	Substantive	**3.3.4.2 Real-time RT-PCR**	A real-time RT-PCR protocol and complete commercial kit has been developed by Plant Print Diagnostics S.L.. (www.plantprint.net) and validated in Spain by the Reference Laboratory of the Ministry of Agriculture. The protocol should be included as another option. This protocol is specific for PSTVd. For further and specific technical information concerning the protocol, please contact Nuria Duran-Vila (nduran@ivia.es) and/or Mariano Cambra (mcambra@ivia.es) and/or Edson Bertolini (ebertoli@ivia.es) or the company Plant Print Diagnostics (plantprint@wanadoo.es).	English	EPPO
122.	*118*	Substantive	**3.3.4.2 Real-time RT-PCR**	A real-time RT-PCR protocol and complete commercial kit has been developed by Plant Print Diagnostics S.L.. (www.plantprint.net) and	English	European Union

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
				validated in Spain by the Reference Laboratory of the Ministry of Agriculture. The protocol should be included as another option. This protocol is specific for PSTVd. For further and specific technical information concerning the protocol, please contact Nuria Duran-Vila (nduran@ivia.es) and/or Mariano Cambra (mcambra@ivia.es) and/or Edson Bertolini (ebertoli@ivia.es) or the company Plant Print Diagnostics (plantprint@wanadoo.es).		
123.	*132*	Substantive	**3.4 Controls for molecular tests**	A “limit of detection” control should be included as an option (i.e. not mandatory) under positive nucleic acid control to know if the test is done in correct condition allowing the best sensitivity.	English	EPPO
124.	*132*	Substantive	**3.4 Controls for molecular tests**	A “limit of detection” control should be included as an option (i.e. not mandatory) under positive nucleic acid control to know if the test is done in correct condition allowing the best sensitivity.	English	European Union
125.	*132*	Technical	**3.4 Controls for molecular tests**	This is good information but understanding the intent and also taking into consideration the way that this annex is written, it is providing guidance to a lab taking on a new technique. If so, this could even be expanded to include other PCR best practices to eliminate cross contamination, proper storage, etc etc. Overall the Annex touches on good practices here and there, but in some places it assumes the reader is proficient in PCR, other sections it provides very basic guidance (e.g. paragraph 161 recommends searching the web for a sequencing service, very basic procurement practice not really even relevant to PCR). It should be consistent. If we are providing basic guidance, perhaps it deserves this new section.	English	Canada
126.	*143*	Technical	**Negative extraction control** This control is used to monitor contamination during nucleic acid extraction and/or cross-reaction with the host tissue, and it requires nucleic acid extraction and subsequent amplification of uninfected host tissue. Multiple controls are recommended when large numbers of positive samples are expected.	Two types of negative extraction control are known: - water/buffer instead of sample (to control: contamination during extraction) - - healthy sample of the same type (to control: cross-reactions)	English	EPPO, Algeria

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
127.	*143*	Technical	**Negative extraction control** This control is used to monitor contamination during nucleic acid extraction and/or cross-reaction with the host tissue, and it requires nucleic acid extraction and subsequent amplification of uninfected host tissue. Multiple controls are recommended when large numbers of positive samples are expected.	Two types of negative extraction control are known: - water/buffer instead of sample (to control: contamination during extraction) - - healthy sample of the same type (to control: cross-reactions)	English	European Union
128.	*158*	Technical	Identification should be done by sequencing the product obtained from any of the conventional RT-PCR methods described in section 3 (3.3.3.3 and 3.3.4.1). If the PCR product is weak or if the sample is infected by more than one pospiviroid, cloning the PCR product may be effective in allowing sequence to be obtained.	Another circumstance when cloning might be effective.	English	EPPO
129.	*158*	Technical	Identification should be done by sequencing the product obtained from any of the conventional RT-PCR methods described in section 3 (3.3.3.3 and 3.3.4.1). If the PCR product is weak or if the sample is infected by more than one pospiviroid, cloning the PCR product may be effective in allowing sequence to be obtained.	Another circumstance when cloning might be effective.	English	European Union
130.	*158*	Technical	PSTVd should be identified~~Identification should be done~~ by sequencing the~~e~~ PCR product obtained from any of the conventional RT-PCR methods described in section 3 (3.3.3.3 and 3.3.4.1) and by searching the public genetic sequence databases and, if necessary, consulting sequence analysis specialists. If the PCR product is weak, cloning the PCR product may be effective in allowing sequence to be obtained.	The protocol should be for identifying PSTVd and this opening sentence should reflect this purpose. Sequence analysis is a key step for identifying PSTVd. Identification hasn't been sufficiently described in the current draft.	English	Australia
131.	*159*	Technical	For identification of a positive sample detected by real-time PCR, the sample should be retested using conventional RT-PCR to enable the product to be sequenced. However, because of the increased sensitivity of the real-time assay, a product may not be obtained with conventional RT-PCR. Sequencing the real-time amplicon directly will give sequence information that does not allow reliable identification. It will allow the amplicon to be identified as a viroid but will not allow species identification or discrimination from the positive control used. When DNA was not amplified using conventional RT-PCR of a positive sample detected by real-time PCR, the sample should be retested using biological detection. Then detection with conventional RT-PCR to enable the product to be sequenced for identification of a positive sample.	Because of the higher sensitivity of the real-time assay, a product may not be obtained with conventional RT-PCR, a positive sample detected by real-time PCR should be retested with biological detection, that will avoid the neglect for some detection.	English	China
132.	*160*	Editorial	~~4.1~~ **Sequencing and sequence analysis**	There is no section 4.2	English	EPPO
133.	*160*	Editorial	~~4.1~~ **Sequencing and sequence analysis**	There is no section 4.2.	English	European Union
134.	*160*	Editorial	~~4.1~~ **Sequencing and sequence analysis**	There is no section 4.2.	English	Algeria
135.	*161*	Editorial	If facilities are not available for sequencing to be done in-house, ~~or by known~~ commercial companies provide such a service~~, consult the Web for companies offering this service~~. The company will specify their requirements for the	Simplification	English	EPPO, Algeria

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			sequencing of PCR products. Send the purified product (and forward and reverse primers if requested) to the company to carry out the sequencing.			
136.	*161*	Editorial	If facilities are not available for sequencing to be done in-house, ~~or by known~~ commercial companies provide such a service~~, consult the Web for companies offering this service~~. The company will specify their requirements for the sequencing of PCR products. Send the purified product (and forward and reverse primers if requested) to the company to carry out the sequencing.	Simplification	English	European Union
137.	*161*	Technical	If facilities are not available for sequencing to be done in-house or by known commercial companies, consult the Web for companies offering this service. The company will specify their requirements for the sequencing of PCR products. Send the purified product (and forward and reverse primers if requested) to the company to carry out the sequencing. Some companies may also purify the product if requested.	Some companies offer a service of purifying the product.	English	EPPO, Algeria
138.	*161*	Technical	If facilities are not available for sequencing to be done in-house or by known commercial companies, consult the Web for companies offering this service. The company will specify their requirements for the sequencing of PCR products. Send the purified product (and forward and reverse primers if requested) to the company to carry out the sequencing. Some companies may also purify the product if requested.	Some companies offer a service of purifying the product.	English	European Union
139.	*163*	Technical	Export the sequence data output files for the two strands and observe the base calls (A, C, G and Ts) generated by the sequencing instrument's software to detect errors. Sequences extending into a PCR primer site should be truncated to exclude the primer sequence as mismatches between the primer sequence and the binding site will be missed because the primer sequence will be amplified, not the binding site in the viroid genome. Including the primer site may skew the comparison of results. The two independently sequenced DNA strands (forward and reverse primers) should be assembled into a single contig, confirming the base call (identity) of each nucleotide site. Disagreements between the two strands should be coded as ambiguous bases (N) in the edited sequence. The edited consensus sequence (determined by comparing the two strands) can then be compared to a database of pospiviroid sequences. In the case of a mixed infection, the chromatogram will not be readable and the PCR product should be cloned and then sequenced.	To provide guidance on actions in cases of mixed infections.	English	EPPO, Algeria
140.	*163*	Technical	Export the sequence data output files for the two strands and observe the base calls (A, C, G and Ts) generated by the sequencing instrument's software to detect errors. Sequences extending into a PCR primer site should be truncated to exclude the primer sequence as mismatches between the primer sequence and the binding site will be missed because the primer sequence will be amplified, not the binding site in the viroid genome. Including the primer site may skew the comparison of results. The two independently sequenced DNA strands (forward	To provide guidance on actions in cases of mixed infections.	English	European Union

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			and reverse primers) should be assembled into a single contig, confirming the base call (identity) of each nucleotide site. Disagreements between the two strands should be coded as ambiguous bases (N) in the edited sequence. The edited consensus sequence (determined by comparing the two strands) can then be compared to a database of pospiviroid sequences. In the case of a mixed infection, the chromatogram will not be readable and the PCR product should be cloned and then sequenced.			
141.	*163*	Technical	Export the sequence data output files for the two strands and observe the base calls (A, C, G and Ts) generated by the sequencing instrument's software to detect errors. Sequences extending into a PCR primer site should be truncated to exclude the primer sequence as mismatches between the primer sequence and the binding site will be missed because the primer sequence will be amplified, not the binding site in the viroid genome. Including the primer site may skew the comparison of results. Reverse complementary sequences and consensus sequences should be compiled using appropriate software, such as the tools on one of the European Molecular Biology Open Software Suite (EMBOSS) servers (http://emboss.open-bio.org) or using 'Consensus Maker' at the Los Alamos National Laboratory (www.hiv.lanl.gov). The two independently sequenced DNA strands (forward and reverse primers) should be assembled into a single contig, confirming the base call (identity) of each nucleotide site. Disagreements between the two strands should be coded as ambiguous bases (N) in the edited sequence. A complete consensus sequence should be compiled that is at least 150 bases long and that is easily read unambiguously over almost all (95%) of its length. Database searches with shorter sequences or with sequences with many errors may produce uncertain results and PSTVd may not be properly identified. The edited consensus sequence (determined by comparing the two strands) can then be compared to a database of pospiviroid sequences.	new sentence: Assistance on handling sequences and generating consensus sequences is needed for the identification steps to be complete. next new sentence: There needs to be guidance on the length of sequence and the quality of the sequence required for identification. In most cases, a length of 150 bases is sufficient to distinguish PSTVd from TCDVd and other pospiviroids (see comments below). When shorter lengths are obtained it becomes more difficult to identify the viroid. There is a segment of 160 bases, overlapping some of the amplified products, where one TCDVd isolate matches the PSTVd reference sequence with 100% identity. Other TCDVd isolates are 100% identical to PSTVd sequences across a 120 base segment. A Mexican papita viroid isolate is 100% identical over a 70 base segment.	English	Australia
142.	*164*	Technical	The most similar sequences should be identified by searching one of the comprehensive nucleotide databases for significant alignments with the consensus sequence. The search should be done on the GenBank non-redundant nucleotide database at the website of the National Centre for Biotechnology Information (NCBI) or the European Nucleotide Archive at the website of the European Molecular Biology Laboratory (EMBL), by using the Basic Local Alignment Search Tool (BLAST). ~~Consensus nucleotide sequences should be searched against a nucleotide database (e.g. GenBank searched by the Basic Local Alignment Search Tool (BLAST)) to identify the most similar sequences.~~ It may be necessary to use an alignment program (e.g. Clustal or MEGA) to obtain full length sequence alignments, as BLAST may not give uninterrupted alignments of whole genomes.	The ENA at EMBL should be offered as an alternative to GenBank and NCBI, in case there is a problem accessing GenBank. There are small specialist databases that have not been tested. It is important to use the main databases rather than one of the other databases. It is possible that a sequence from a host, rather than a viroid, could be amplified and produce a product of the right size. Alignment against a comprehensive database is more likely to identify such a sequence as a not coming from a viroid (a false positive).	English	Australia

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
143.	*165*	Technical	PSTVd is identified by aligning consensus sequences with sequences of known PSTVd isolates. BLAST search parameters should be used so that at least 100 matching sequences are returned in the search results. If a search is done as described using GenBank or EMBL and BLAST, then sequences from PSTVd isolates should make up the majority of the list of matching sequences in the search results, and should be the most closely related sequences in the list. The list of matching sequences should include PSTVd sequences with at least 94 % identity. TCDVd is a close sister group and care should be taken to distinguish TCDVd and PSTVd sequence matches. At the time of writing, one TCDVd sequence was found to be 100% identical to certain PSTVd sequences over a segment of 160 bases, that partly overlapped some of the PCR products. The BLAST search should be repeated a second time with the same consensus sequence but excluding PSTVd sequences using the 'Organism' search setting. Alignments should be generated with sequences of other pospiviroid species, once PSTVd sequences have been excluded. PSTVd will be identified by comparing percentage identity measures and lengths of alignments made with PSTVd and with other pospiviroid species. PSTVd sequences should produce longer alignments and higher identity scores. If the best matches are of less than 94% identity this may indicate that the consensus sequence is too short or contains errors. It might also indicate that a distinct viroid species has been detected. Careful alignment is required for pospiviroids where a few base pair differences may be the difference between classifying the viroid as a regulated or a non-regulated pest.	As it is currently written, the draft will not always allow the unequivocal identification of PSTVd, because TCDVd sequences are so close to PSTVd sequences. The 90% similarity demarcation point is too low, and "similarity" is not a useful term for measuring nucleotide sequence comparisons in a quantitative way (it is a term used that way for amino acid sequences). Percentage "identity" should be used instead. There are TCDVd isolates that are 90 and 91% identical to PSTVd isolates across their entire length (see AY962324 and EF626530). Australian experience suggests that if matches of 95 % identity or greater are found, then the viroid is PSTVd. The 94% figure gives some leeway if a different isolate is encountered or sequence is poor. The 94% is also the level of identity between the sequence generated using the Pospi1 primers from the Naaldwijk isolate and equivalent sequences from isolates from outside the Naaldwijk cluster (Verhoeven's P. peruviana cluster). The Naaldwijk cluster is the most distinct known PSTVd cluster. Sequences from other pospiviroid species are more than 90% identical to PSTVd sequences across shorter segments. There is a segment of 160 bases where a TCDVd isolate matches the PSTVd reference sequence with 100% identity. Other TCDVd isolates are 100% identical to PSTVd sequences across a 120 base segment. A Mexican papita viroid isolate is 100% identical over a 70 base segment. As more sequences are added to the databases the picture might become more complex and it may be difficult to distinguish PSTVd from TCDVd.	English	Australia
144.	*166*	Editorial	For viroid species identification, the demarcation criteria of the International Committee on Taxonomy of Viruses should be followed (Flores *et al*., 1998, 2005; Owens *et al*., 2011). In most cases the arbitrary level of 90% sequence identity establishes a clear border that separates species from variants. Consequently, a sample is identified as the species with which it shares the greatest similarity if (1) that similarity is >90% and (2) the sample is also <90% similar to other species in	There are two references, each one with several authors, so it should be a plural.	English	EPPO

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			the database. For characterization of a species, however, Flores *et al*. (1998, 2005) also mentions the evaluation of biological properties.			
145.	*166*	Editorial	For viroid species identification, the demarcation criteria of the International Committee on Taxonomy of Viruses should be followed (Flores *et al*., 1998, 2005; Owens *et al*., 2011). In most cases the arbitrary level of 90% sequence identity establishes a clear border that separates species from variants. Consequently, a sample is identified as the species with which it shares the greatest similarity if (1) that similarity is >90% and (2) the sample is also <90% similar to other species in the database. For characterization of a species, however, Flores *et al*. (1998, 2005) also mentions the evaluation of biological properties.	There are two references, each one with several authors, so it should be a plural.	English	European Union
146.	*166*	Editorial	For viroid species identification, the demarcation criteria of the International Committee on Taxonomy of Viruses should be followed (Flores *et al*., 1998, 2005; Owens *et al*., 2011). In most cases the arbitrary level of 90% sequence identity establishes a clear border that separates species from variants. Consequently, a sample is identified as the species with which it shares the greatest similarity if (1) that similarity is >90% and (2) the sample is also <90% similar to other species in the database. For characterization of a species, however, Flores *et al*. (1998, 2005) also mentions the evaluation of biological properties.	There are two references, each one with several authors, so it should be a plural.	English	Algeria
147.	*166*	Technical	For viroid species identification, the demarcation criteria of the International Committee on Taxonomy of Viruses should be followed (Flores *et al*., 1998, 2005; Owens *et al*., 2011). In most cases the arbitrary level of 90% sequence whole genomeidentity establishes a clear border that separates species from variants. Consequently, a sample is identified as the species with which it shares the greatest similarity if (1) that similarity is >90% and (2) the sample is also <90% similar to other species in the database. For characterization of a species, however, Flores *et al*. (1998, 2005) also mentions the evaluation of biological properties.	The whole genome sequence of viroid is short. And 90% sequence of the whole genome is the international practice.	English	China
148.	*166*	Technical	~~For viroid species identification, the demarcation criteria of the International Committee on Taxonomy of Viruses should be followed (Flores *et al*., 1998, 2005; Owens *et al*., 2011). In most cases the arbitrary level of 90% sequence identity establishes a clear border that separates species from variants. Consequently, a sample is identified as the species with which it shares the greatest similarity if (1) that similarity is >90% and (2) the sample is also <90% similar to other species in the database. For characterization of a species, however, Flores *et al*. (1998, 2005) also mentions the evaluation of biological properties.~~	As it is currently written, paragraph 166 will not always allow the unequivocal identification of PSTVd, because TCDVd sequences are so close to PSTVd sequences. The 90% similarity demarcation point is too low, and “similarity” is not a useful term for measuring nucleotide sequence comparisons in a quantitative way (it is a term used that way for amino acid sequences). Percentage “identity” should be used instead. There are TCDVd isolates that are 90 and 91% identical to PSTVd isolates across their entire length (see AY962324 and EF626530). The 94% figure is suggested as that is the level of identity	English	Australia

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
				between the sequence generated using the Pospi1 primers from the Naaldwijk isolate and equivalent sequences from isolates from outside the Naaldwijk cluster (Verhoeven's P. peruviana cluster). The Naaldwijk cluster is the most distinct known PSTVd cluster. Sequences from other pospiviroid species are more than 90% identical to PSTVd sequences across shorter segments. There is a segment of 160 bases where a TCDVd isolate matches the PSTVd reference sequence with 100% identity. Other TCDVd isolates are 100% identical to PSTVd sequences across a 120 base segment. A Mexican papita viroid isolate is 100% identical over a 70 base segment. As more sequences are added to the databases the picture might become more complex and it may be difficult to distinguish PSTVd from TCDVd.		
149.	*180*	Editorial	Department of Primary Industries, Knoxfield Centre, Private Bag 15, Ferntree Gully Delivery Centre, Victoria, Australia (~~e-mail:~~ Dr B. Rodoni, e-mail: brendan.rodoni@dpi.vic.gov.au).	"e-mail" shouldn't be repeated.	English	EPPO
150.	*180*	Editorial	Department of Primary Industries, Knoxfield Centre, Private Bag 15, Ferntree Gully Delivery Centre, Victoria, Australia (~~e-mail:~~ Dr B. Rodoni, e-mail: brendan.rodoni@dpi.vic.gov.au).	"e-mail" shouldn't be repeated.	English	European Union
151.	*180*	Editorial	Department of Environment and Primary Industries, Biosciences Research Division, AgriBio, 5 Ring Road, La Trobe University, Bundoora ~~Knoxfield Centre, Private Bag 15, Ferntree Gully Delivery Centre~~, Victoria 3083, Australia (e-mail: Dr B. Rodoni, e-mail: brendan.rodoni@depi.vic.gov.au).	Department has changed name, moved locations.	English	Australia
152.	*180*	Editorial	Department of Primary Industries, Knoxfield Centre, Private Bag 15, Ferntree Gully Delivery Centre, Victoria, Australia (~~e-mail:~~ Dr B. Rodoni, e-mail: brendan.rodoni@dpi.vic.gov.au).	"e-mail" shouldn't be repeated.	English	Algeria
153.	*182*	Editorial	Conselleria de Agricultura de la Generalitat Valenciana, Centro de Proteccion Vegetal y Biotecnologia, IVIA, 46113 Moncada (Valencia), Spain (Dr N. Duran-Vila, e-mail: nduran@ivia.~~gva.~~es).	Correct email address.	English	EPPO, Algeria
154.	*182*	Editorial	Conselleria de Agricultura de la Generalitat Valenciana, Centro de Proteccion Vegetal y Biotecnologia, IVIA, 46113 Moncada (Valencia), Spain (Dr N. Duran-Vila, e-mail: nduran@ivia.~~gva.~~es).	Correct email address.	English	European Union
155.	*188*	Editorial	**8. References**	Add ISPM 31and ISPM 27 to the reference list (see paragraphs 23 and 168)	English	EPPO, Algeria

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
156.	*188*	Editorial	**8. References**	Add ISPM 31and ISPM 27 to the reference list (see paragraphs 23 and 168)	English	European Union
157.	*188*	Substantive	**8. References** Elliot et al. (2001) First Report of Potato spindle tuber viroid in Tomato. New Zealand Plant Disease 85, Number 9; Mumford et al. (2003) The first report of Potato spindle tuber viroid (PSTVd) in commercial tomatoes in the UK New Disease Reports 8, 31; Hailstones et al. (2003) Detection and eradication of Potato spindle tuber viroid in tomatoes in commercial production in New South Wales, Australia. Australasian Plant Pathology, 32, 317–318; Verhoeven et al. (2004). Natural infections of tomato by Citrus exorcortis viroid, Columnea latent viroid, Potato spindle tuber viroid and Tomato chlorotic dwarf viroid. Eur. J. Plant Pathol.110:823-831; Verhoeven, et al., (2007) First Report of Potato spindle tuber viroid in Tomato in Belgium. Plant Disease, 91, Number 8; Ling and Sfetcu (2010) First Report of Natural Infection of Greenhouse Tomatoes by Potato spindle tuber viroid in the United States. Plant Disease 94, Number 11; FERA (2010) Emerging viroid threats to UK tomato production. Plant Disease Factsheet; Ling et al. (2013) First Report of Potato spindle tuber viroid Naturally Infecting Greenhouse Tomatoes in North Carolina. Plant Disease 97, Number 1. Lebas et al. (2005). Distribution of Potato spindle tuber viroid in New Zealand glasshouse crops of capsicum and tomato. Australasian Plant Pathology, 34(2), 129-133; Owens and Verhoeven. (2009) Potato spindle tuber. The Plant Health Instructor. American Phytopathological Society DOI: 10.1094/PHI-I-2009-0804-01; Verhoeven, et al. (2010b). Mechanical transmission of Potato spindle tuber viroid	references referred to in para 8	English	Australia

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			between plants of Brugmansia suaveoles, Solanum jasminoides and potatoes and tomatoes. European Journal of Plant Pathology, 128, 417-421.			
158.	*193*	Technical	**Botermans, M., van de Vossenberg, B.T.L.H., Verhoeven, J.Th.J., Roenhorst, J.W., Hooftman, M., Dekter, R. & Meekes, E.T.M.** 2013. Development and validation of a real-time RT-PCR assay for generic detection of pospiviroids. *Journal of Virological Methods*, 187: 43–50. Chambers G.A., Seyb A.M., Mackie J., Constable F.E., Rodoni B.C., Letham D., Davis K., Gibbs M.J., 2013. First Report of Pepper chat fruit viroid in Traded Tomato Seed, an Interception by Australian Biosecurity. Plant Disease, 97, 10 1386 http://dx.doi.org/10.1094/PDIS-03-13-0293-PDN	Reference added at para 9	English	Australia
159.	*195*	Substantive	Elliot DR, Alexander BJR, Smales TE, Tang Z, Clover GRG (2001) First report of Potato spindle tuber viroid in tomato in New Zealand. Plant Disease 85: 1027–1027 **EPPO/CABI** (I.M. Smith, D.G. McNamara, P.R. Scott and M. Holderness, eds).1997. *Quarantine pests for Europe*, second edition. Wallingford, UK, CABI.	new reference for added text	English	Australia
160.	*197*	Editorial	**EPPO** (European and Mediterranean Plant Protection Organization).2010. PM 7/98. Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity. *EPPO Bulletin*, 40: 5–22.	A blank is missing after PM.	English	EPPO
161.	*197*	Editorial	**EPPO** (European and Mediterranean Plant Protection Organization).2010. PM 7/98. Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity. *EPPO Bulletin*, 40: 5–22.	A blank is missing after PM.	English	European Union
162.	*197*	Editorial	**EPPO** (European and Mediterranean Plant Protection Organization).2010. PM 7/98. Specific requirements for laboratories preparing accreditation for a plant pest diagnostic activity. *EPPO Bulletin*, 40: 5–22.	A blank is missing after PM.	English	Algeria
163.	*207*	Editorial	**Hammond, R.W. & Owens, R.A.** 2006. Viroids: New and continuing risks for horticultural and agricultural crops. APSnet. Available at http://www.apsnet.org/publications/apsnetfeatures/Pages/Viroids.aspx (last accessed on 20 December 2012). **ISPM 27.** 2006. Diagnostic protocols for regulated pests. Rome, IPPC, FAO. **ISPM 31.** 2008. Methodologies for sampling of consignments. Rome, IPPC, FAO.	ISPM 27 is mentioned in [168]. ISPM 31 is mentioned in [23].	English	EPPO
164.	*207*	Editorial	**Hammond, R.W. & Owens, R.A.** 2006. Viroids: New and continuing risks for horticultural and agricultural crops. APSnet. Available at http://www.apsnet.org/publications/apsnetfeatures/Pages/Viroids.aspx (last	ISPM 27 is mentioned in [168]. ISPM 31 is mentioned in [23].	English	European Union

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			accessed on 20 December 2012). **ISPM 27.** 2006. Diagnostic protocols for regulated pests. Rome, IPPC, FAO. **ISPM 31.** 2008. Methodologies for sampling of consignments. Rome, IPPC, FAO.			
165.	*207*	Editorial	**Hammond, R.W. & Owens, R.A.** 2006. Viroids: New and continuing risks for horticultural and agricultural crops. APSnet. Available at http://www.apsnet.org/publications/apsnetfeatures/Pages/Viroids.aspx (last accessed on 20 December 2012). **ISPM 27.** 2006. Diagnostic protocols for regulated pests. Rome, IPPC, FAO. **ISPM 31.** 2008. Methodologies for sampling of consignments. Rome, IPPC, FAO.	ISPM 27 is mentioned in [168]. ISPM 31 is mentioned in [23].	English	Algeria
166.	*212*	Technical	**Ling, K.S. & Bledsoe, M.E.** 2009. First report of Mexican papita viroid infecting greenhouse tomato in Canada. *Plant Disease*, 93: 839. Ling K-S, Li R, Panthee DR, Gardner RG (2013) First Report of Potato spindle tuber viroid Naturally Infecting Greenhouse Tomatoes in North Carolina. Plant Disease 97 (1):148 Ling K, Sfetcu D, (2010) First report of natural infection of greenhouse tomatoes by potato spindle tuber viroid in the United States. Plant Disease. 94(11):1376. Matthews-Berry S, (2010) Emerging viroid threats to UK tomato production. The Food and Environment Research Agency. Plant Disease Factsheet, pp. 4	reference for inserted text	English	Australia
167.	*216*	Technical	Matthews-Berry S, (2010) Emerging viroid threats to UK tomato production. The Food and Environment Research Agency. Plant Disease Factsheet, pp. 4 **Menzel, W., Jelkmann, W. & Maiss, E.** 2002. Detection of four apple viruses by multiplex RT-PCR assays with co-amplification of plant mRNA as internal control. *Journal of Virological Methods*, 99: 81–92.	reference for inseted text	English	Australia
168.	*218*	Technical	**Monger, W., Tomlinson, J., Boonham, N., Virscek Marn, M., Mavric Plesko, I., Molinero-Demilly, V., Tassus, X., Meekes, E., Toonen, M. & Papayiannis, L.** 2010. Development and inter-laboratory evaluation of real-time PCR assays for the detection of pospiviroids. *Journal of Virological Methods*, 169: 207–210. Mühlbach HP & Sänger HL. 1997. Viroid replication is inhibited by alpha-amanitin.	Reference for new text	English	Australia

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country
			Nature 278: 185–188 Mumford RA, Jarvis B, Skelton A (2004) The first report of Potato spindle tuber viroid (PSTVd) in commercial tomatoes in the UK. Plant Pathology 53: 242–242			
169.	*221*	Technical	**Owens, R.A., Girsova, N.V., Kromina, K.A., Lee, I.M., Mozhaeva, K.A. & Kastalyeva, T.B.** 2009. Russian isolates of *Potato spindle tuber viroid* exhibit low sequence diversity. *Plant Disease*, 93: 752–759. Owens RA, Verhoeven JThJ (2009) Potato spindle tuber. The Plant Health Instructor. http://www.apsnet.org/edcenter/intropp/lessons/viruses/pages/potatospindletuber.aspx Accessed 16 January 2013.	reference for inserted text	English	Australia
170.	*225*	Editorial	**Salazar, L.F., Querci, M., Bartolini, I. & Lazarte, V.** 1995. Aphid transmission of potato spindle tuber viroid assisted by potato leafroll virus. *Fitopatologia*, 30: 56–58. Salazar et al., 1996....	This reference is mentioned in [8].	English	EPPO
171.	*225*	Editorial	**Salazar, L.F., Querci, M., Bartolini, I. & Lazarte, V.** 1995. Aphid transmission of potato spindle tuber viroid assisted by potato leafroll virus. *Fitopatologia*, 30: 56–58. Salazar et al., 1996....	This reference is mentioned in [8].	English	European Union
172.	*225*	Editorial	**Salazar, L.F., Querci, M., Bartolini, I. & Lazarte, V.** 1995. Aphid transmission of potato spindle tuber viroid assisted by potato leafroll virus. *Fitopatologia*, 30: 56–58. Salazar et al., 1996....	This reference is mentioned in [8].	English	Algeria
173.	*237*	Technical	**Verhoeven, J.Th.J., Hüner, L., Virscek Marn, M., Mavric Plesko, I. & Roenhorst, J.W.** 2010. Mechanical transmission of Potato spindle tuber viroid between plants of *Brugmansia suaveolens*, *Solanum jasminoides*, potatoes and tomatoes. *European Journal of Plant Pathology*, 128: 417– 421. Verhoeven JTJ, Jansen CCC, Roenhorst JW, Steyer S, Michelante D (2007a) First report of Potato spindle tuber viroid in tomato in Belgium. Plant Disease 91: 1055–1055	reference for inserted text	English	Australia