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

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country	SC response
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	Accepted. Citations in text now in chronological order
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	English	United States of America	Where available validation data added New table added (Table 1) with validation data

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				on validation. We consider the reference "Recommendations on method validation in phytodiagnostics are provided by EPPO (2010)." provides limited information.			
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 removed from the protocol? Many could be removed without affecting the detail in the protocol.	English	Australia	Accepted. Non essential personal communications removed.
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	English	Australia	Accepted. In most cases the tests are not specific to PSTVd so it is impossible to avoid indicating other viroids in the protocol, but ultimately the protocol is specific to PSTVd. If any misuse of the protocol should occur by any party that is their problem This protocol addresses PSTVd detection. The PSTVd aspect has been strengthened in the text (line 20)

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				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			

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				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.			
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 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).	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 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'.	English	Australia	Accepted. Text changed to as requested except that the Mulbach reference was not added, since it is not necessary.

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				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	Accepted .Text changed to as requested
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	Accepted. Text changed to as requested

Comm.no.	Para. no.	Comment type	Comment	Explanation	Language	Country	SC response
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 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).	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	Partially accepted. Text added "stolon- and tuber-forming Solanum spp. for example" However, we do not agree there should be a wholesale introduction of common names since common names may vary by country. OK for potato and tomato since these are subsequently used in text.
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	Not accepted. Best left in alphabetical order
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*,	China has tested PSTVd in the fruit of tomato.	English	China	Not accepted since *S. lycopersicum* is tomato.

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			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).				
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), *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	EPPO, Estonia, Algeria, Morocco	Accepted. Chrysanthemum sp added
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	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	Accepted. Chrysanthemum sp added

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			hosts express few or no disease symptoms (Singh, 1973; Singh *et al*., 2003).				
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	Accepted Reference to specific countries removed. Reference now made to CABI data sheet http://www.cabi.org/isc/datasheet/43659
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	Accepted Reference to specific countries removed. Reference now made to CABI data sheet http://www.cabi.org/isc/datasheet/43659
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 and other hosts.	The expression is clearer.	English	China	Reference to specific countries removed Reference now made to CABI data sheet http://www.cabi.org/isc/datasheet/43659
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	Reference to specific countries removed Reference now made to CABI data sheet http://www.cabi.org/isc/datasheet/43659

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	Agreed. The Salazar 1996 citation has been deleted.
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 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	Estonia, Algeria, Morocco	Agreed. The Salazar 1996 citation has been deleted
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	Salazar et al., 1996, is missing in the	English	European	Agreed. The Salazar 1996 citation has

			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).	references.		Union	been deleted
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. 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).	divide para 8 into two separate paragraphs, first dealing with trasmission in potato, the second with transmission in tomato and capsicum.	English	Australia	Agreed. Divided into 2 paragraphs
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	A paper of Salazar et al.,1996 is missing in section 8.References.	English	Thailand	The Salazar 1996 citation has been

			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).				deleted
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 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	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.	English	Australia	Partially accepted. Text modified to "Transmission via tomato seeds has been shown to contribute to international spread (Van Brunschot *et al*., 2014). It has been speculated that PSTVd is also spread in infected capsicum seed (Lebas *et al*., 2005). In addition, infected ornamental species may act as an inoculum source if handled before touching other susceptible plants and has been shown to be a pathway for international spread (Navarro *et al*., 2009; Verhoeven *et al.,* 2010)." References not accepted. See response to comment 157

			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).	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 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 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	Partially agreed.The following has been added *"Pepper chat fruit viroid* (PCFVd; Reanwarakorn *et al*., 2011," Reference of Chamber et al is not correct as it only describes the detection on tomato seeds. Reference of Verhoeven et al only reports on tomato as an experimental host of PCFVd.
34.	*12*	Editorial	**Synonyms:**potato spindle ~~tuber virus~~ viroid, potato gothic virus, tomato bunchy top viriod	complete the list of synonyms	English	Ghana	Potato spindle tuber virus has been kept and tomato bunchy top added
35.	*14*	Editorial	**Common names:**potato spindle tuber, potato spindle tuber viroid	both are in common useage	English	Australia	Reject. Not needed since potato spindle tuber viroid is the species name

36.	*14*	Editorial	**Common names:** potato spindle tuber , bunchy top of tomato	Completed list of common names CABI/ EPPO 2012	English	Ghana	Accepted Tomato bunchy top added
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 rangeing 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	Accepted. Changes made.
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, 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	EPPO, Algeria, Morocco	Accepted. Changes made.
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	Clarification - only a limited number of species was investigated.	English	European Union	Accepted. Changes made.

			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).				
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	Modified. It now reads “the laboratory may have the diagnosis” .
41.	*19*	Technical	Detection Option 1 — Generic molecular methods for viroids and pospiviroids (section 3.3.3) R(eturn)-PAGE Hybridisation DIG cRNA Probe Conventional and real-time RT-PCR → Positive test → Viroid detected* Option 2 — Higher specificity molecular methods (section 3.3.4) Conventional and real-time RT-PCR (also detects some other viroids) → Positive test → Viroid detected * Option 3 — Biological detection (section 3.2) → Typical symptoms → Viroid suspected Optional Identification — Conventional RT-PCR (if not done previously) and sequencing (section 4) ** 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.	ıat ınd the	English	Uruguay	Accepted This does remove any doubt that you can use it for symptomatic tissue since in text it could be interpreted as just for asymptomatic.

			Figure 1. Minimum requirements for the detection and identification of *Potato spindle tuber viroid*				
42.	19	Technical	Detection Option 1 **Generic molecular methods for viroids and pospiviroids** (section 3.3.3) R(eturn)-PAGE Hybridisation DIG cRNA Probe Conventional and real-time RT-PCR Option 2 **Higher specificity molecular methods** (section 3.3.4) Conventional and real-time RT-PCR (also detects some other viroids) Option 3 **Biological detection** (section 3.2) Positive test Positive test Optional Typical symptoms Viroid detected* Viroid detected * Viroid suspected **Identification** Conventional RT-PCR (if not done previously) and sequencing (section 4) ** 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*	hat and the	English	COSAVE, Paraguay, Chile, Argentina, Peru, Brazil	Accepted This does remove any doubt that you can use it for symptomatic tissue since in text it could be interpreted as just for asymptomatic.
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	A large part of the Annex provides methods for detecting all or almost all pospiviroids. However, the Annex was	English	Australia	Partially accepted. The following text has been added "This Annex is for the detection of PSTVd and has not been developed for the detection and identification of other pospiviroid

			choosing a detection and identification method. This 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.	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			species."

				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	English	Australia	Not accepted Testing seed for any pathogen always has high uncertainty. The methods we present is base line. Better to have a method than none at all. The following text has been added: "Protocols for the detection of PSTVd in leaf, tuber and botanical (true) seed tissue are described. However reliable detection in seed is particularly challenging and no fully effective seed testing method is currently available."

				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 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	Not accepted: This publication is concerned with quantitative real-time PCR for publication not quantitative analysis. Not quantitative analysis performed in this protocol.
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 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	COSAVE, Paraguay, Chile, Argentina, Peru, Brazil	Not accepted: This publication is concerned with quantitative real-time PCR for publication. Not quantitative analysis
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	Not accepted:. This is the way it is in the PPV protocol
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	Not accepted:. This is the way it is in the PPV protocol
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	Not accepted:. This is the way it is in the PPV protocol
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	Accepted

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	Accepted
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 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.	For clarity	English	Ghana	Accepted
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	Accepted
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	Changes to clarify the effect of temperature and light level on	English	European Union	Accepted

			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.	viroid concentration.			
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	Accepted
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 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	EPPO	Accepted. However, this sentence has now been deleted
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	Error in the writting of "transcription".	English	European Union	Accepted. However, this sentence has now been deleted

			extraction if using real-time reverse transcritption (RT)-PCR. Bulking for other methods should be validated.				
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	Accepted, but sentence has been further modified to improve readability
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 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 transcritpion (RT)-PCR. Bulking for other methods should be validated.	A scientific name must be italicized or underlined.	English	Thailand	Accepted
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	Error in the writting of "transcription".	English	Algeria	As Above. OK

			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.				
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 °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	Accepted. However, this sentence has now been deleted
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 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 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.	Some labs use the conventional RT -PCR	English	China	Accepted. However, this sentence has now been deleted

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	Accepted. This information is now included in a table (Table 1)
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	English	Australia	Not accepted. However modification to the text has been made.

				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 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	Not accepted, but sentence modified to "Potato seeds may be sown in growing medium (e.g.compost) in trays and the seedlings/plants tested non-destructively using the same procedure as described for glasshouse grown plants (EPPO, 2006)."
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	See 65 above (EPPO 2006)
67.	*30*	Editorial	**3.2 Biological detection, (Pathogenicity)**	Are both words the same?	English	Ghana	Not accepted. Biological term more usual when referring to detection
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	Not accepted, but text modified to "No work has been done to compare the sensitivity of this method with other methods described in this protocol but if it less sensitive than the molecular methods it might be less suitable for testing seed"
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	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	English	Australia	Partially accepted. Sensitive and repeatable removed from text. Suggested text addition not added because it is up to the labs to make the choice

			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.	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.			
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	Accepted. Text changed to include root and pH 7.4
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	Accepted. Text changed to include root and pH 7.4
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 is provided (approximately 650 ìE/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.~~	The discription of symptom is overlap with the paragraph 16.	English	China	Not accepted. Symptoms in test plants are something different than symptoms in plants to be tested.
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	39 degrees is very high. Suggest modification to 30 degrees.	English	EPPO, Algeria, Morocco	Not accepted. This was the temperature in the original paper. However text modified to reflect temperatures and the diurnal temperature fluctuation from the

			photoperiod of 14 h. If necessary, supplemental illumination is provided (approximately 650 ìE/m²/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.				original paper.
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 25–309° C under a photoperiod of 14 h. If necessary, supplemental illumination is provided (approximately 650 ìE/m²/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	Not accepted. This was the temperature in the original paper. However text modified to reflect temperatures and the diurnal temperature fluctuation from the original paper.
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	Accepted. Text modified to “Inoculation of *S. lycopersicum* plants (cvs Rutgers, Moneymaker or Sheyenne) will allow the detection of many but not all viroids (e.g. tomato is not a host of the pospiviroid *Iresine viroid 1* (IrVd-1)”
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	Accepted. Text modified to “Inoculation of *S. lycopersicum* plants (cvs Rutgers, Moneymaker or Sheyenne) will allow the detection of many but not all viroids (e.g. tomato is not a host of the pospiviroid *Iresine viroid 1* (IrVd-1)”
77.	36	Technical	**3.3.1 Sample preparationTissue maceration**	More logical title.	English	EPPO, Algeria, Morocco	Accepted. Changed to “Sample preparation”
78.	36	Technical	**3.3.1 Sample preparationTissue maceration**	More logical title.	English	European Union	Accepted. Changed to “Sample preparation”
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	If this is the same buffer as used in paragraph 43, then it should be refered to	English	EPPO	Accepted. Changed to lysis buffer

			facilitate homogenization.	simply as lysis buffer.			
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	Accepted. Changed to “lysis buffer (composition depends on the method used for nucleic acid extraction)
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	Accepted. Changed to “lysis buffer (composition depends on the method used for nucleic acid extraction)
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	Not accepted. This is true potato seed not seed potatoes
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	Accepted. Text changed to lysis buffer
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	Accepted. Text changed to lysis buffer
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~~	The argument for deleting the para is given at para 28.	English	Australia	Not accepted (see response at comment no 44)

			~~(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).~~				
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”.	English	EPPO	Accepted. Tissue print now included

				Annu. Rev. Phytopathol. 50, 197-218.			
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.	English	European Union	Accepted. Tissue print now included

				Phytopathol. 50, 197-218.			
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	Accepted. Table 1 includes the nucleic acid extraction method used for a validated assay
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	Not accepted.. Brand names can be used
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	Not accepted. However modification to the text has been made: "RNeasy® was evaluated for the extraction of PSTVd RNA from different matrices as part of the EUPHRESCO DEP project (EUPHRESCO, 2010). "
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	Accepted Changed to The following nucleic acid extraction kits, buffers and procedures. And "**Method described by Macke nzie *et al.* (1997)**"….
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	It's only a buffer, and not a method.	English	China	Accepted. As response to comment no 91 . and changed to "Method using EDTA buffer"

			range of ornamental plant species. EDTA buffer is not name of method.				
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	Accepted: 1:4 w/v added.
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	**Not accepted, but text modified to “**This CTAB (cetyl trimethylammonium bromide) method has been used with (real-time) RT-PCR for a wide range of plant species and tissue types (e.g. leaves and tomato seed; EUPHRESCO, 2010)”.
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 ìl of 8.39% (w/v) tetrasodium pyrophosphate (TNaPP) solution (pH 10.0–10.9) and 100 ìl 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	Accepted but other changes also made. Heading changed to Magnetic bead extraction method 1 to reflect the adding of a Method 2 and some changes made to text to improve structure and readability.

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 ìl of 8.39% (w/v) tetrasodium pyrophosphate (TNaPP) solution (pH 10.0–10.9) and 100 ìl 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	Accepted. Roenhorst et al., 2005 reference added but other changes also made. Heading changed to Magnetic bead extraction method 1 to reflect the adding of a Method 2 and some changes made to text to improve structure and readability.
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 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 ìl of 8.39% (w/v) tetrasodium pyrophosphate (TNaPP) solution (pH 10.0–10.9) and 100 ìl 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	European Union	As response to comment no 96
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	delete in line with comment at para 28	English	Australia	Not accepted. See response to comment no 44. but text modified. See response to comment no 96.

			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 ìl of 8.39% (w/v) tetrasodium pyrophosphate (TNaPP) solution (pH 10.0–10.9) and 100 ìl 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.				
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	Not accepted Confusion between seeds and seed potatoes
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	Accepted. Space added.
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, this method cannot be implemented. It is also very difficult to to import a cloned copy from other countries.	English	South Africa	This is a general problem, because you need a positive control for any (molecular) detection test. So it is not an issue to be solved in this protocol. [If the problems relate to the quarantine status of PSTVd, maybe TCDVd can be used instead if it is not listed as a quarantine pest.
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	English	EPPO	Accepted. To make it clearer the other methods in the ring test are now mentioned in line 56

				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.			
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	Accepted. Response as for comment no 102
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	Accepted
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	Accepted
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	Accepted
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	English	South Africa	Accepted. Response as for comment no

				access to a cloned PSTVd DNA, this method cannot be implemented. It is also very difficult to import cloned copy from other countries.			101
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	Partially accepted. The text has been modified to " However sequence mismatch at critical positions of the primer target site may prevent the detection of some pospiviroid isolates (e.g. an isolate of CLVd was not detected using these primers; Steyer *et al.*, 2010) and use of additional primers to detect these isolates will be required. "
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	Partially accepted. See response to comment no 108
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	Accepted. Comma added
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	A comma is missing.	English	European	Accepted. Comma added

			of this section (T. James, SASA, UK, personal communication, 2010). It is not necessary to use the Q-solution described by EUPHRESCO (2010).			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	Accepted. Comma added
113.	74	Technical	Two microlitres of template is added to 23 ìl master mix comprising 1.0 ìl each of forward and reverse primer (10 µM), 5 ìl of 5× Qiagen OneStep RT-PCR buffer, 1.0 ìl Qiagen OneStep RT-PCR enzyme mix, 1.0 ìl dNTPs (10 mM each dNTP), and 14 ìl 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	Not accepted. No validation data provided that it actually improves the performance of the test over the original programme.
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	Accepted. Response as for comment 101
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	The phrase 'all pospiviroids' is not appropriate. Botermans et al. 2013 did experiments on 10 different PSTVd isolates, they only	English	Australia	Accepted. Text changed to "Method validation (see Table 1) on tomato leaves showed that the GenPospi assay detected isolates from all the known pospivirod species"

			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	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.			"The GenPospi assay has been shown to be a suitable tool for large-scale screening for pospiviroid species. The assay will need to be validated for matrices other than tomato leaves."
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 need to be defined in each laboratory.	More appropriate wording	English	United States of America	Accepted. Text modified to "and <37 as inconclusive, requiring confirmation; and those ≥37 as negative. However, these values may exclude low levels of infection in some tissues, and will 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	Accepted. However increasing the cycling numbers will not solve this problem. Because of exhaustion of the chemicals, increasing the cycle numbers will not increase the sensitivity. Each laboratory that uses the method should define its own cycle threshold. Moreover, it is inherent to all methods that low levels of the pathogen are missed at some point.
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	What is the link with footnote 2?	English	EPPO	Accepted. Footnote to be deleted

			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].				
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	Accepted. Footnote to be deleted
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	Accepted. The following added "If RNA was not amplified using these primers the Vid primers may be used."
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-	English	EPPO	Accepted. This real time method now added. However further investigation has shown that the method is not specific for PSTVd since TCDVd may also be detected.

				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).			
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 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	European Union	Accepted. This real time method now added. However further investigation has shown that the method is not specific for PSTVd since TCDVd may also be detected.
123.	*132*	Substantive	**3.4 Controls for molecular tests**	A “limit of detection” control should be included as an option	English	EPPO	Accepted. At line 134 the following text added “A limit of detection control (not

				(i.e. not mandatory) under positive nucleic acid control to know if the test is done in correct condition allowing the best sensitivity.			mandatory) may also be used.”
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	Accepted. Response as for comment no 123
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	English	Canada	Not accepted. This procedure is for use by experienced personnel.

				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.			
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	Accepted." /or added."
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	Accepted. /or added.
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	Accepted. Text added "or if the sample is infected by more than one pospiviroid"
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	Accepted. Text added "or if the sample is infected by more than one pospiviroid
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	The protocol should be for identifying PSTVd and this opening sentence should reflect this	English	Australia	Accepted. Text now reads "PSTVd should be identified by sequencing the product obtained from the conventional RT-PCR methods using the Shamloul or

			is weak, cloning the PCR product may be effective in allowing sequence to be obtained.	purpose. Sequence analysis is a key step for identifying PSTVd. Identification hasn't been sufficiently described in the current draft.			Vid primers described in section 3 (3.3.4.1 and 3.3.3.3 respectively) and by searching the public genetic sequence databases and, if necessary consulting sequence anaalysis specialists. If the PCR product is weak or if the sample is infected by more than one pospiviroid,"
31.	*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	**Accepted. Additional text added** **"**Alternatively, samples may be inoculated on tomato plants to increase the concentration of the viroid to levels that may be detectable by conventional RT-PCR. However, this approach has not been evaluated and if results are inconclusive then resampling and testing may be required."
132.	*160*	Editorial	~~**4.1**~~ **Sequencing and sequence analysis**	There is no section 4.2	English	EPPO	Accepted. 4.1 deleted
133.	*160*	Editorial	~~**4.1**~~ **Sequencing and sequence analysis**	There is no section 4.2.	English	European Union	Accepted. 4.1 deleted
134.	*160*	Editorial	~~**4.1**~~ **Sequencing and sequence analysis**	There is no section 4.2.	English	Algeria	Accepted. 4.1 deleted
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 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	EPPO, Algeria	Accepted. Text modified to"If facilities are not available for sequencing to be done in-house, use a commercial company"
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	Accepted. Text modified to"If facilities are not available for sequencing to be done in-house, use a commercial company"

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	Accepted. Text added Some companies may also purify the pro duct if requested.
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	Accepted. Text added Some companies may also purify the pro duct if requested.
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 b e readable and the PCR product should be cloned and then sequenced.	To provide guidance on actions in cases of mixed infections.	English	EPPO, Algeria	Accepted. Text added "In the case of a mixed infection, the chromatogram may not be readable and the PCR product should be cloned and sequenced."
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 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 b e readable and the PCR product should be cloned and then sequenced.	To provide guidance on actions in cases of mixed infections.	English	European Union	Accepted. Text added "In the case of a mixed infection, the chromatogram may not be readable and the PCR product should be cloned and 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	new sentence: Assistance on	English	Australia	Partially accepted. Text modified

			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.	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.			considerably (see line 163 in Annex) First insertion suggest that you can use open software to get consensus sequences. However, this software does not use electropherograms (trace files) in their analysis. Therefore, the consensus sequences are of less quality than those produced by using the assemblers that use trace data (e.g. *.abi, *.ab1, *.scf files), such as Geneious, CLC workbench and Lasergene). Unfortunately these are not free available from the internet. Second insertion disagrees with the ICTV species demarcation criteria: Full length sequences are required for species identification. Moreover, if only 150 nts would be required, it should be specified and validated which 150 nts should be used. Additionally In the case full genome sequences have to be used for identification, another problem occurs, i.e. the removal of the primer sequences from the consensus sequence. Because this results in a gap in the consensus sequence, blasting will be a lot more difficult, as in fact you have to blast with a split genome. Therefore, our suggestion is to keep the primer sequences in the consensus sequence. Since these primers are located in the most conserved regions of the viroid genome, this is not likely to influence the identification. A-overhangs build in by the polymerase during

							elongation have to be removed when observed. For identification, it is advisable to use an edited consensus sequence starting at position 1 of the viroid genome for comparison to a database of pospiviroid sequences Furthermore, ambiguous bases should not be indicated by N but by the appropriate code according to IUPAC ambiguity codes
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	Partially accepted. Text modified considerably (see line 164 in Annex). See also response to comment no 141
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,	As it is currently written, the draft will not always allow the unequivocal	English	Australia	Partially accepted. Text modified considerably (see line 165 in Annex).

			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.	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			See also response to comment no 141

				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.			
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 the database. For characterization of a species, however, Flores *et al.* (1998, 2005) also mention~~s~~ the evaluation of biological properties.	There are two references, each one with several authors, so it should be a plural.	English	EPPO	Accepted but text modified to "PSTVd identification should be done using the full genome sequence or the full genome sequence excluding the nucleotides at the primer positions. According to the International Committee on Taxonomy of Viruses (ICTV) the main criterion for species identification is more than 90% sequence identity (Owens *et al.*, 2011). However, if the sequence obtained shows

							identities near 90% additional parameters should be included such as biological properties. The ICTV Viroid Study Group is currently discussing the viroid classification and the criteria for species demarcation”
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 mention~~s~~ the evaluation of biological properties.	There are two references, each one with several authors, so it should be a plural.	English	European Union	See response to Comment no. 144
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 mention~~s~~ the evaluation of biological properties.	There are two references, each one with several authors, so it should be a plural.	English	Algeria	See response to Comment no. 144
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	See response to Comment no. 144
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,~~	As it is currently written, paragraph 166 will not always allow the unequivocal identification of PSTVd, because TCDVd sequences are so close to PSTVd	English	Australia	Not accepted. Each percentage of identity is arbitrarily assigned. Therefore, we should stick to the demarcation criteria of the ICTV. See response to comment 144

			~~2005) also mentions the evaluation of biological properties.~~	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 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	Accepted and changed
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	English	EPPO, Algeria	

				paragraphs 23 and 168)			
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	references referred to in para 8	English	Australia	Not accepted. We had been instructed to reduce the number of references. Instead of these references we propose that a more appropriate reference is **van Brunschot, S.L., Verhoeven, J.Th.J., Persley, D.M., Geering, A.D.W., Drenth, A. & Thomas, J.E.** 2014. An outbreak of Potato spindle tuber viroid in tomato is linked to imported seed. *European Journal of Plant Pathology* doi:10.1007/s10658-014-0379-8 used

			Instructor. American Phytopathological Society DOI: 10.1094/PHI-I-2009-0804-01; Verhoeven, et al. (2010b). Mechanical transmission of Potato spindle tuber viroid 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	Not accepted. Reference of Chamber et al is not correct as it only describes the detection on tomato seeds. A more appropriate reference is Reanwarakorn K, Klinkong S & Porsoongnurn J. 2011. First report of natural infection of Pepper chat fruit viroid in tomato plants in Thailand. New Disease Reports (2011) 24, 6. [doi:10.5197/j.2044-0588.2011.024.006]
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	Not accepted. See response to comment no. 32
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	Accepted
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	Accepted
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	Accepted
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	ISPM 27 is mentioned in [168]. ISPM 31 is mentioned in [23].	English	EPPO	Accepted. Now included in references

			(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.				
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 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	European Union	Accepted
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	Accepted
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	Not accepted. See response to comment no 157
167.	*216*	Technical	Matthews-Berry S, (2010) Emerging viroid threats to UK tomato production. The Food and Environment Research Agency. Plant Disease	reference for inseted text	English	Australia	Not accepted. See response to comment

			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.				no 157
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. 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	Reference for new text	English	Australia	Not accepted. Mühlbach reference not required. For Mumford reference see response to comment no 157
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	Not accepted. See response to comment no 157
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	The Salazar 1996 citation has been removed from the text so reference not required
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	The Salazar 1996 citation has been removed from the text so reference not required
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	The Salazar 1996 citation has been removed from the text so reference not required

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	Not accepted. See response to comment no 157