2006-021: DRAFT ANNEX TO ISPM 27 – *Fusarium ciricinatum*

Para	Text	Comment	SC's response
G	(General Comment)	*Category : TECHNICAL* **(221) Venezuela (1 Oct 2016 2:51 AM)** El grupo de Venezuela no tiene comentarios por ahora.	Noted
G	(General Comment)	*Category : TECHNICAL* **(220) Zambia (30 Sep 2016 11:14 PM)** Paragraph 144-number of cycles for denaturation, annealing and elongation steps should be placed appropriately. Paragraph 372-Font type Paragraph 390-Reference is complete	Considered, but not incorporated The suggestion is sound, but the presentation of the tables has to stick to the guidelines of the "Draft table template format for PCR reaction conditions" of the instructions to authors.
G	(General Comment)	*Category : TECHNICAL* **Burkina Faso** Lev specificity waters and-Sensitivity ity of these techniques should be fou r ned to allow comparison with other techn ical with diag No. stic (paragraph 232).	Considered but not incorporated The comment is not clear. In addition, the connection with paragraph 232 which corresponds to "Initial denaturation" is not clear.
G	(General Comment)	*Category : SUBSTANTIVE* **(219) Canada (30 Sep 2016 9:23 PM)** Canada supports the Draft Annex to ISPM 27: Fusarium circinatum (2006-021).	Noted
G	(General Comment)	*Category : SUBSTANTIVE* **(217) Guyana (30 Sep 2016 6:40 PM)** We accept the contents of the document.	Noted
G	(General Comment)	*Category : EDITORIAL* **(216) Nepal (30 Sep 2016 3:46 PM)** In the draft, several protocols, recommended for molecular detection and identification, are based on kits being marketed by certain companies. What will happen in case such specific kits are no more available in the market in the future? So, general protocols are required to be recommended which can be used everywhere	Considered, the methods included in the protocol have been selected by the authors based on there best knowledge and experience, and the availability of validation data. However, the methods presented in the protocols may be adjusted to the standards of individual laboratories, or even replaced, provided that they are adequately validated.
G	(General Comment)	*Category : TECHNICAL* **(214) Mali (30 Sep 2016 11:33 AM)** o Les méthodes décrites dans ce protocole	Noted. This comment is beyond the TPDP capacity as it relates to potential

Para	Text	Comment	SC's response
		sont sophistiquées pour les niveaux d'équipement de nos laboratoires. Il serait intéressant que des études évoluent vers le développement de kits d'analyse rapide plus faciles à utiliser par les services d'inspection aux frontières	implementation issues. It will be forwarded to the SC and apropriate bodies.
G	(General Comment)	*Category : TECHNICAL* **(209) Bolivia (30 Sep 2016 4:04 AM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC	Modified Text followed as provided in Instructions to authors. The standard footnote regarding brands will be added as in other diagnostic protocols.
G	(General Comment)	*Category : TECHNICAL* **(179) Peru (29 Sep 2016 6:54 PM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC.	Modified Text followed as provided in Instructions to authors. The standard footnote regarding brands will be added as in other diagnostic protocols.
G	(General Comment)	*Category : TECHNICAL* **(178) Nigeria (29 Sep 2016 4:42 PM)** The diagnostic methods described are sophisticated and beyond the capacity of many national laboratories and inspectors. Scientists should come up with simpler and quicker diagnostic kits ready for use at border points.	Noted The diagnostic protocol includes well described, validated diagnostic methods. The authors wellcome new, quicker diagnostic kits, however, no kits are available or have been developed for the detection or identification of F. circinatum.
G	(General Comment)	*Category : EDITORIAL* **(177) Nigeria (29 Sep 2016 4:24 PM)** Paragraph 3.4.3 Line 134 -144 The normal PCR cycles namely Denaturation ,Elongation and Annealing should be listed according to the sequence of those steps in the PCR machine.	Considered but not incorporated The comment is not clear. In addition, the presentation of the tables has to stick to the guidelines of the "Draft table template format for PCR reaction conditions" of the Instructions to authors.
G	(General Comment)	*Category : TECHNICAL* **(165) Brazil (29 Sep 2016 3:45 PM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text	Modified Text followed as provided in Instructions to authors. The standard footnote regarding brands will be added as in other diagnostic protocols.

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		previously agreed by the SC.	
G	(General Comment)	*Category : SUBSTANTIVE* **(148) China (29 Sep 2016 11:12 AM)** Add the section of tests in this draft.The related molecular detection methods are all cited from the documents, so they should be verified by tests to ensure their accuracy.	Considered, not incorporated They have been tested in a proficency test.
G	(General Comment)	*Category : TECHNICAL* **(108) Argentina (28 Sep 2016 7:25 PM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC	Modified Text followed as provided in Instructions to authors. A standard footnote regarding brands will be added as in other diagnostic protocols.
G	(General Comment)	*Category : TECHNICAL* **(95) Chile (28 Sep 2016 4:55 PM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC.	Modified Text followed as provided in Instructions to authors. A standard footnote regarding brands will be added as in other diagnostic protocols.
G	(General Comment)	*Category : TECHNICAL* **(94) Iraq (28 Sep 2016 10:55 AM)** No comment	Noted
G	(General Comment)	*Category : TECHNICAL* **(93) Burundi (28 Sep 2016 8:53 AM)** The diagnostic methods described in this protocol are sophisticated, compared to the level of equipment of laboratories in African countries. Studies should be continued to come out with quick diagnostic kits ready to be used by inspection services at the border points. Levels of sensitivity and specificity of the proposed methods should be indicated in order to compare these methods with other methods(Paragraphs 60 and 77).	Noted The diagnostic protocol includes well described, validated diagnostic methods. The authors wellcome new, quicker diagnostic kits, however, no kits are available or have been developed for the detection or identification of F. circinatum.
G	(General Comment)	*Category : SUBSTANTIVE* **(92) Barbados (27 Sep 2016 8:48 PM)** There are no issues with this document.	Noted
G	(General Comment)	*Category : TECHNICAL* **(80) Viet Nam (27 Sep 2016 6:12 AM)** 1) The draft should consider adding the synonym name of the fungus at its	Considered but not incorporated

Para	Text	Comment	SC's response
		teleomorphic stage or not, as in June 2014 the Brazilian authors (Ludvig et al., 2014) have identified that teleomorphic stage of the fungus is Gibberella fujikuroi complex (GFC), based on gene sequence analysis, in "first report and characterization of Fusarium circinatum, the causal agent of pitch canker in Brazil" Trop.plant patho.vol.39 Brasilia no.3 May / Jun 2014 (http: / /www.scielo.br/scielo.phb). 2) Due to the morphological characteristics of F. circinatum have no clear distinction to other Fusarium species. The comparison of F. circinatum with other Fusarium species that have similar characteristics and that F. circinatum may therefore be confused with. curved tip of mycelium of microconnidi, monophialide / polyphialide are not mainly characteristics to identify (in table 4). Hence, the identification of the fungus based on morphological characteristics will not fully trust. It should be combined morphological characteristics and gene sequence analysis.	1) G. circinata has never been found in nature. The sequences will identify them as Giberella but that is because of the nomenclature issue. 2) Modified See modified text par. 60 Species Complex (FFSC), diagnosis in both method A and method B will sometimes have to be confirmed by an additional DNA sequence analysis step. (see section 3, Detection and Figure 1).
G	(General Comment)	*Category : TECHNICAL* **(77) Japan (26 Sep 2016 5:02 PM)** In the tables related to PCR, a colum of "Master mix for 20(25)µl final reaction volume (µl) " and concentration of each reagent should be added. There are some examples in the draft diagnostic protocol of Phytophthora ramorum. It is useful to specify concentration and additive volume of each reagent and final volume in the tables.	Considered, not incorporated The suggestion is sound, but the presentation of the tables has to stick to the guidelines of the "Draft table template format for PCR reaction conditions" of the Instructions to authors.
G	(General Comment)	*Category : EDITORIAL* **(76) PPPO (25 Sep 2016 10:47 PM)** PPPO does not have any comments on the draft ISPMs	Noted
G	(General Comment)	*Category : TECHNICAL* **(73) Samoa (22 Sep 2016 4:04 AM)** no further comment	Noted
G	(General Comment)	*Category : TECHNICAL* *Attachment : Herron et al 2015.pdf* **(72) United States of America (21 Sep 2016 9:11 PM)** See US comment in paragraph 109	See comment under paragraph 109
G	(General Comment)	*Category : SUBSTANTIVE* **(52) Thailand (21 Sep 2016 6:00 AM)** agree with this diagnostic protocol.	Noted

Para	Text	Comment	SC's response
G	(General Comment)	*Category : TECHNICAL* **(39) Uruguay (15 Sep 2016 8:05 PM)** We request the TPDP to revise the use of footnotes associated to brand names for consistency and modifications are suggested, as commented during other consultation periods, according footnote text agreed by the SC.	Modified Text followed as provided in Instructions to authors. A standard footnote regarding brands will be added as in other diagnostic protocols.
G	(General Comment)	*Category : SUBSTANTIVE* **(38) Zambia (8 Sep 2016 6:00 AM)** In agreement with the Draft Annex to ISPM 27	Noted
G	(General Comment)	*Category : SUBSTANTIVE* **(16) Tajikistan (22 Aug 2016 12:13 PM)** I support the document as it is and I have no comments	Noted
G	(General Comment)	*Category : TECHNICAL* **(4) COSAVE (11 Aug 2016 10:08 PM)** We request the TPDP to revise the use of Footnotes associated to brand names for consistency and modification are suggested as commented in others consultation periods according text previously agreed by the SC.	Modified Text followed as provided in Instructions to authors. A standard footnote regarding brands will be added as in other diagnostic protocols.
G	(General Comment)	*Category : TECHNICAL* **(1) Sri Lanka (22 Jul 2016 8:40 AM)** The entire content could be accepted	Noted
39	This diagnostic protocol was adopted by the Commission on Phytosanitary Measures in 20--.	*Category : TECHNICAL* **(199) Ghana (29 Sep 2016 11:59 PM)** The diagnostic methods described in this protocol are sophisticated, compared to the level of equipment of laboratories in African countries. We are proposing that more studies should be done in order to come out with quick diagnostic kits to be used by inspection services at the entry points.	Considered,, but not incorporated The diagnostic protocol includes well described, validated diagnostic methods. TPDP wellcomes new, quicker diagnostic kits, however, no kits are availabler or have been developed for the detection or identification of F. circinatum. This comment is beyond the TPDP capacity as it relates to potential implementation issues. It will be forwarded to the SC and apropriate bodies.
42	*Fusarium circinatum* is an ascomycete fungus formerly described as the anamorph of *Gibberella circinata* (Geiser *et al*., 2013) and it is the causal agent of pitch canker disease. The disease almost exclusively affects *Pinus* spp., but has also been described on *Pseudotsuga menziesii* (Douglas fir). The disease affects plantations and nurseries in several countries worldwide and is a serious threat to pine forests	*Category : TECHNICAL* **(149) China (29 Sep 2016 11:15 AM)** Add the host species and geographical distribution of the pest, vector insect species and other contents.The related content is	Considered, but not incorporated The host species as well as general occurrence of the species have been

Para	Text	Comment	SC's response
	wherever it occurs (especially on *Pinus radiata*) as it results in extensive tree mortality, reduced tree growth and reduced timber quality. *F. circinatum* causes cankers that girdle branches, aerial roots and even trunks of *Pinus* spp. Cankers are often associated with conspicuous resin exudates ("pitch"). Multiple-branch infections may cause severe crown dieback and eventually lead to the death of the tree. This aggressive fungus may also infect *Pinus* spp. seeds and may cause damping off in seedlings in nurseries. The fungus has been found in regions of North, Central and South America, Asia and South Africa and has been officially reported in parts of Southern Europe. Information on its distribution, updated regularly, is available at the European and Mediterranean Plant Protection Organization (EPPO) Global Database (https://gd.eppo.int/) and on the CABI website (http://www.cabi.org/isc/datasheet/25153).	the important supplementary information on quarantine pest identification.	included in the protocol according to the Instructions to authors. The reference to the Cabi datasheet has been included. These data are more detailed and regularly updated, instead of fixed data that would become out of date.
42	*Fusarium circinatum* is an ascomycete fungus formerly described as the anamorph of *Gibberella circinata* mentionner synonyme car dans les certificats phytosanitaires c'est generalement du synonyme don't il s'agit (Geiser *et al.*, 2013) and it is the causal agent of pitch canker disease. The disease almost exclusively affects *Pinus* spp., but has also been described on *Pseudotsuga menziesii* (Douglas fir). The disease affects plantations and nurseries in several countries worldwide and is a serious threat to pine forests wherever it occurs (especially on *Pinus radiata*) as it results in extensive tree mortality, reduced tree growth and reduced timber quality. *F. circinatum* causes cankers that girdle branches, aerial roots and even trunks of *Pinus* spp. Cankers are often associated with conspicuous resin exudates ("pitch"). Multiple-branch infections may cause severe crown dieback and eventually lead to the death of the tree. This aggressive fungus may also infect *Pinus* spp. seeds and may cause damping off in seedlings in nurseries. The fungus has been found in regions of North, Central and South America, Asia and South Africa and has been officially reported in parts of Southern Europe. Information on its distribution, updated regularly, is available at the European and Mediterranean Plant Protection Organization (EPPO) Global Database (https://gd.eppo.int/) and on the CABI website (http://www.cabi.org/isc/datasheet/25153).	*Category : TECHNICAL* **(81) Algeria (27 Sep 2016 10:48 AM)**	Considered, but not incorporated Names of organisms are accoording to the most recent taxonomy and synonyms are provided in section Taxonomic information. Mentioning names used on Phytosanitary certificates is beyond the scope of this protocol.
42	*Fusarium circinatum* is an ascomycete fungus formerly described as the anamorph of *Gibberella circinata* (Geiser *et al.*, 2013) and it is the causal agent of pitch canker disease. The disease almost exclusively affects *Pinus* spp., but has also been described on *Pseudotsuga menziesii* (Douglas fir). The disease affects plantations and nurseries in several countries worldwide and is a serious threat to pine forests wherever it occurs (especially on *Pinus radiata*) as it results in extensive tree mortality, reduced tree growth and reduced timber quality. *F. circinatum* causes	*Category : EDITORIAL* **(70) United States of America (21 Sep 2016 8:54 PM)** I suggest remove the " aggressive" or describe the reason for using aggressive.	Incorporated, "aggressive" deleted.

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	cankers that girdle branches, aerial roots and even trunks of *Pinus* spp. Cankers are often associated with conspicuous resin exudates ("pitch"). Multiple-branch infections may cause severe crown dieback and eventually lead to the death of the tree. This aggressive fungus may also infect *Pinus* spp. seeds and may cause damping off in seedlings in nurseries. The fungus has been found in regions of North, Central and South America, Asia and South Africa and has been officially reported in parts of Southern Europe. Information on its distribution, updated regularly, is available at the European and Mediterranean Plant Protection Organization (EPPO) Global Database (https://gd.eppo.int/) and on the CABI website (http://www.cabi.org/isc/datasheet/25153).		
42	*Fusarium circinatum* is an ascomycete fungus formerly described as the anamorph of *Gibberella circinata* (Geiser *et al.*, 2013) and it is the causal agent of pitch canker disease. The disease almost exclusively affects *Pinus* spp., but has also been described on *Pseudotsuga menziesii* (Douglas fir). The disease affects plantations and nurseries in several countries worldwide and is a serious threat to pine forests wherever it occurs (especially on *Pinus radiata*) as it results in extensive tree mortality, reduced tree growth and reduced timber quality. *F. circinatum* causes cankers that girdle branches, aerial roots and even trunks of *Pinus* spp. Cankers are often associated with conspicuous resin exudates ("pitch"). Multiple-branch infections may cause severe crown dieback and eventually lead to the death of the tree. This aggressive fungus may also infect *Pinus* spp. seeds and may cause damping off in seedlings in nurseries. The fungus has been found in regions of North, Central and South America, Asia and South Africa and has been officially reported in parts of Southern Europe. Information on its distribution, updated regularly, is available at the European and Mediterranean Plant Protection Organization (EPPO) Global Database (https://gd.eppo.int/) and on the CABI website (http://www.cabi.org/isc/datasheet/25153).	*Category : TECHNICAL* **(53) United States of America (21 Sep 2016 8:26 PM)** The work of Cassandra Swett and others has convincingly shown that grasses harbor F. circinatum.	Incorporated Added the sentence "Fusarium circinatum has been found in asymptomatic grasses (Poaceae) near to native stands of pine trees with symptoms of the disease (Swett and Gordon, 2012; Swett et al., 2014."
42	*Fusarium circinatum* is an ascomycete fungus formerly described as the anamorph of *Gibberella circinata* (Geiser *et al.*, 2013) and it is the causal agent of pitch canker disease. The disease almost exclusively affects *Pinus* spp., but has also been described on *Pseudotsuga menziesii* (Douglas fir). *Fusarium circinatum* has been found in asymptomatic grasses (Poaceae) near to native stands of pine trees with symptoms of the disease (Swett and Gordon, 2012; Swett et al., 2014. The disease affects plantations and nurseries in several countries worldwide and is a serious threat to pine forests wherever it occurs (especially on *Pinus radiata*) as it results in	*Category : SUBSTANTIVE* **(17) New Zealand (1 Sep 2016 5:15 AM)** Recent studies have shown that F. circinatum was isolated from the grass family Poaceae collected near to native stands of pine trees with symptoms of the disease in California.	Incorporated: Added the sentence "Fusarium circinatum has been found in asymptomatic grasses (Poaceae) near to native stands of pine trees with symptoms of the disease (Swett and Gordon, 2012; Swett et al., 2014."

Para	Text	Comment	SC's response
	extensive tree mortality, reduced tree growth and reduced timber quality. *F. circinatum* causes cankers that girdle branches, aerial roots and even trunks of *Pinus* spp. Cankers are often associated with conspicuous resin exudates ("pitch"). Multiple-branch infections may cause severe crown dieback and eventually lead to the death of the tree. This aggressive fungus may also infect *Pinus* spp. seeds and may cause damping off in seedlings in nurseries. The fungus has been found in regions of North, Central and South America, Asia and South Africa and has been officially reported in parts of Southern Europe. Information on its distribution, updated regularly, is available at the European and Mediterranean Plant Protection Organization (EPPO) Global Database (https://gd.eppo.int/) and on the CABI website (http://www.cabi.org/isc/datasheet/25153).		
43	*F. circinatum* is predominantly a wound pathogen that enters the host tree through mechanical wounds or the feeding holes of wood-boring insects. If a wound is not deep enough for the pathogen to reach water within host tissues, ambient moisture or very high relative humidity is required for spore germination. Conidia of *F. circinatum* germinate over a wide range of temperatures; slowly at 10 °C and progressively faster, up to an optimum around 20 °C (Inman *et al.*, 2008). In nature *F. circinatum* is known to propagate only asexually, through production of microconidia and macroconidia. Both spore types are borne in a viscous liquid and appear better suited to dispersal by splashing water or attachment to motile organisms than to aerial dispersal. However, microconidia and macroconidia can become airborne and they are presumably the primary propagules recovered by air sampling in areas where pitch canker is found (Correll *et al.*, 1991). The fungus may move from tree to tree by aerial dispersal of the conidiospores or through vectoring by feeding insects (Gordon *et al.*, 2001; Schweigkofler *et al.*, 2004). However, long-range dispersal of the pathogen from affected areas to disease-free areas may be driven by the movement of infected seeds or infected plant material (Storer *et al.*, 1998). Conifer seeds can be colonized by *F. circinatum* internally (where it can remain dormant until seed germination) and externally (Storer *et al.*, 1998). In many pine species, seed contamination may be largely restricted to the seed coat (Dwinell, 1999).	*Category : SUBSTANTIVE* **(150) China (29 Sep 2016 11:16 AM)** Add wood (wood package) in long-distance transmission paths in this paragraph. Wood (wood package) is also the main path for long-distance spread of the pest (Tkacz, Borys M.; Burdsall, Harold H., et al. Pest risk assessment of the importation in United states of unprocessed Pinus and Abies logs from Mexico.Gen. Tech.Rep.FPL-GTR-104. Madison,WI:U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, 1998，P55)。	Incorporated 'logs, lumber, and other unmanufactured wood articles' as has been given by Tkacz et al, 1998 Reference added to lit list
43	*F. circinatum* is predominantly a wound pathogen that enters the host tree through mechanical wounds or the feeding holes of wood-boring insects. If a wound is not deep enough for the pathogen to reach water within host tissues, ambient moisture or very high relative humidity is required for spore germination. Conidia of *F. circinatum* germinate over a wide range of temperatures; slowly at 10 °C and progressively faster, up to an optimum around 20 °C (Inman *et al.*, 2008). In nature	*Category : SUBSTANTIVE* **(120) Philippines (29 Sep 2016 7:26 AM)** for clarity	Modified: The fungus may be spread from tree to tree by aerial dispersal of the conidiospores or through vectoring by feeding insects (Gordon et al., 2001; Schweigkofler et al., 2004).

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	F. circinatum is known to propagate only asexually, through production of microconidia and macroconidia. Both spore types are borne in a viscous liquid and appear better suited to dispersal by splashing water or attachment to motile organisms than to aerial dispersal. However, microconidia and macroconidia can become airborne and they are presumably the primary propagules recovered by air sampling in areas where pitch canker is found (Correll *et al.*, 1991). The fungus may ~~move from tree to tree~~ infect the trees by aerial dispersal of the conidiospores or through vectoring by feeding insects (Gordon *et al.*, 2001; Schweigkofler *et al.*, 2004). However, long-range dispersal of the pathogen from affected areas to disease-free areas may be driven by the movement of infected seeds or infected plant material (Storer *et al.*, 1998). Conifer seeds can be colonized by *F. circinatum* internally (where it can remain dormant until seed germination) and externally (Storer *et al.*, 1998). In many pine species, seed contamination may be largely restricted to the seed coat (Dwinell, 1999).		
43	*F. circinatum* is predominantly a wound pathogen that enters the host tree through mechanical wounds or the feeding holes of wood-boring insects. If a wound is not deep enough for the pathogen to reach water within host tissues, ambient moisture or very high relative humidity is required for spore germination. Conidia of *F. circinatum* germinate over a wide range of temperatures; slowly at 10 °C and progressively faster, up to an optimum around 20 °C (Inman *et al.*, 2008). In nature *F. circinatum* is known to propagate only asexually, through production of microconidia and macroconidia. Both spore types are borne in a viscous liquid and appear better suited to dispersal by splashing water or attachment to motile organisms than to aerial dispersal. However, microconidia and macroconidia can become airborne and they are presumably the primary propagules recovered by air sampling in areas where pitch canker is found (Correll *et al.*, 1991). The fungus may move from tree to tree by aerial dispersal of the conidiospores or through vectoring by feeding insects (Gordon *et al.*, 2001; Schweigkofler *et al.*, 2004). However, long-range dispersal of the pathogen from affected areas to disease-free areas may be driven by the movement of infected seeds or infected plant material (Storer *et al.*, 1998). Conifer seeds can be colonized by *F. circinatum* internally (where it can remain dormant until seed germination) and externally (Storer *et al.*, 1998). In many pine species, seed contamination may be ~~largely~~ strictly restricted to the seed coat (Dwinell, 1999).	*Category : SUBSTANTIVE* **(82) Algeria (27 Sep 2016 10:58 AM)**	Modified: In many pine species, seed contamination may be restricted to the seed coat (Dwinell, 1999).
43	*F. circinatum* is predominantly a wound pathogen that enters the host tree through mechanical wounds or the feeding holes of wood-boring insects. If a wound is not deep enough for the pathogen to reach water within host tissues, ambient moisture	*Category : EDITORIAL* **(66) United States of America (21 Sep 2016 8:49 PM)** More appropriate term	Comment 1. Incorporated Comment 2: Incorporated:

Para	Text	Comment	SC's response
	or very high relative humidity is required for spore germination. Conidia of *F. circinatum* germinate over a wide range of temperatures; slowly at 10 °C and progressively faster, up to an optimum around 20 °C (Inman *et al.*, 2008). In nature *F. circinatum* is known to propagate only asexually, through production of microconidia and macroconidia. Both spore types are borne in a viscous liquid and appear better suited to dispersal by splashing water or attachment to motile organisms than to aerial dispersal. However, microconidia and macroconidia can become airborne and they are presumably the primary propagules recovered by air sampling in areas where pitch canker is found (Correll *et al.*, 1991). The fungus may move from tree to tree by aerial dispersal of the ~~conidiospores~~ conidia or through ~~vectoring by feeding insects~~ vectors (Gordon *et al.*, 2001; Schweigkofler *et al.*, 2004). However, long-range dispersal of the pathogen from affected areas to disease-free areas may be driven by the movement of infected seeds or infected plant material (Storer *et al.*, 1998). Conifer seeds can be colonized by *F. circinatum* internally (where it can remain dormant until seed germination) and externally (Storer *et al.*, 1998). In many pine species, seed contamination may be largely restricted to the seed coat (Dwinell, 1999).	To simplify. For the second to last sentence: "...and externally" Does this mean on the seed coat? Please clarify.	"and externally on the seed coat".
43	*F. circinatum* is predominantly a wound pathogen that enters the host tree through mechanical wounds or the feeding holes of wood-boring insects. If a wound is not deep enough for the pathogen to reach water within host tissues, ambient moisture or very high relative humidity is required for spore germination. Conidia of *F. circinatum* germinate over a wide range of temperatures; slowly at 10 °C and progressively faster, up to an optimum around 20 °C (Inman *et al.*, 2008). In nature *F. circinatum* is known to propagate only asexually, through production of microconidia and macroconidia. Both spore types are borne in a viscous liquid and appear better suited to dispersal by splashing water or attachment to motile organisms than to aerial dispersal. However, microconidia and macroconidia can become airborne and they are presumably the primary propagules recovered by air sampling in areas where pitch canker is found (Correll *et al.*, 1991). The fungus may move from tree to tree by aerial dispersal of the ~~conidiospores~~ conidia or through vectoring by feeding insects (Gordon *et al.*, 2001; Schweigkofler *et al.*, 2004). However, long-range dispersal of the pathogen from affected areas to disease-free areas may be driven by the movement of infected seeds or infected plant material (Storer *et al.*, 1998). Conifer seeds can be colonized by *F. circinatum* internally (where it can remain dormant until seed germination) and externally (Storer *et al.*, 1998). In many pine species, seed contamination may be largely restricted to the seed coat (Dwinell, 1999).	*Category : EDITORIAL* **(19) New Zealand (1 Sep 2016 5:22 AM)**	Incorporated

Para	Text	Comment	SC's response
43	*F. circinatum* is predominantly a wound pathogen that enters the host tree through mechanical wounds or the feeding holes of wood-boring insects. If a wound is not deep enough for the pathogen to reach water within host tissues, ambient moisture or very high relative humidity is required for spore germination. Conidia of *F. circinatum* germinate over a wide range of temperatures; slowly at 10 °C and progressively faster, up to an optimum around 20 °C (Inman *et al.*, 2008). In nature *F. circinatum* is known to propagate only asexually, through production of microconidia and macroconidia. Both spore types are borne in a viscous liquid and appear better suited to dispersal by splashing water or attachment to motile organisms than to aerial dispersal. However, microconidia and macroconidia can become airborne and they are presumably the primary propagules recovered by air sampling in areas where pitch canker is found (Correll *et al.*, 1991). The fungus may move from tree to tree by aerial dispersal of the conidiospores or through vectoring by feeding insects (Gordon *et al.*, 2001; Schweigkofler *et al.*, 2004). However, long-range dispersal of the pathogen from affected areas to disease-free areas may be driven by the movement of infected seeds or infected plant material (Storer *et al.*, 1998). Conifer seeds can be colonized by *F. circinatum* internally (where it can remain dormant until seed germination) and externally (Storer *et al.*, 1998). In many pine species, seed contamination may be largely restricted to the seed coat (Dwinell, 1999).	*Category : EDITORIAL* **(18) New Zealand (1 Sep 2016 5:20 AM)** The term 'conidia' is widely used and has been used in other sections of this draft protocol. Suggest to use 'conidia' consistently in this document	Incorporated
44	*F. circinatum* ~~is~~ also ~~capable of producing~~ produce perithecia, which contain meiotically derived spores (ascospores). However, while perithecia are readily produced on culture media under laboratory conditions, they have ~~not~~ been rarely observed in nature.	*Category : TECHNICAL* **(84) Algeria (27 Sep 2016 11:09 AM)**	Modified: F. circinatum may also produce perithecia, which contain meiotically derived spores (ascospores). However, perithecia are rarely produced on culture media under laboratory conditions and they have not been observed in nature.
48	*Fusarium moniliforme* var. *subglutinans* Wollenw. & Reinking, 1925	*Category : TECHNICAL* **(67) United States of America (21 Sep 2016 8:50 PM)** Paragraphs 48, 49, 51, and 52 are synonyms, but not synonyms of Fusarium circinatum. They may be names mis-applied to isolates of Fusarium circinatum, but are not currently accepted synonyms.	Incorporated 'synonyms' par 48, 49, 51 and 52 removed Not listed as synonym in Mycobank, neighter in Species Fungorum
49	*Gibberella fujikuroi* var. *subglutinans* (Wollenw. & Reinking) E.T. Edwards, 1933	*Category : EDITORIAL* **(20) New Zealand (1 Sep 2016 5:25**	Incorporated

Para	Text	Comment	SC's response
		AM) Missing authors of the species name.	'synonym' removed (see comment 48)
50	*Fusarium lateritium* f.sp. *pini* Hepting, 1949	*Category : TECHNICAL* **(68) United States of America (21 Sep 2016 8:50 PM)** The reports of F. lateritium f. sp. pini are probably misdeterminations of F. subglutinans.	Modified 'synonym' removed Not listed as synonym in Mycobank, neighter in Species Fungorum
53	*Fusarium subglutinans* f.sp. *pini* J.C. Correll, T.R. Gordon, McCain, J.W. Fox, Koehler, D.L. Wood & M.E. Schultz, 1991	*Category : TECHNICAL* **(69) United States of America (21 Sep 2016 8:51 PM)** 53 and 54 are the current accepted synonyms for Fusarium circinatum. In a taxonomic sense, these are the only two names that should be listed as synonyms for F. circinatum. The other names can be mentioned as names that have been used to refer to the fungus, but are not, actually synonyms.	Modified The synonym Fusarium subglutinans f.sp. pini has been commonly used in litarature
55	**Taxonomic position:** ~~Eukaryota,~~ Fungi, Dikarya, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Hypocreales, Nectriaceae	*Category : SUBSTANTIVE* **(151) China (29 Sep 2016 11:17 AM)**	Considered, but not accepted, Eukaryota is correct.
59	Although they may exhibit different levels of susceptibility to *F. circinatum*, all *Pinus* spp., along with *P. menziesii*, may be potentially affected by the fungus, and the symptoms can be observed at any time of year. In addition, *F. circinatum* can affect plants of different ages, ranging from seedlings to mature trees, and it can be detected on all plant parts (roots, branches, shoots, cones and seeds). *F. circinatum* may also be soil-borne. There are no published methods for the isolation of *F. circinatum* from soil. This protocol describes the identification of *F. circinatum* on symptomatic plant tissue and on seeds. Plants and trees should be inspected for any symptoms typical of pine pitch canker (section 3.1.1) whereas seeds may be analysed by random sampling (section 3.2.2). Diagnostic method A, isolation and culture (section 3.3) and diagnostic method B, molecular tests (section 3.4), may both be used for plant tissue and seeds.	*Category : SUBSTANTIVE* **(152) China (29 Sep 2016 11:18 AM)** Add wood (wood package) in isolation and identification materials.Wood (wood package) is also the main path for long-distance spread of the pest. Vector insects carried by wood (wood package) is likely to spread the pest.（1. Tkacz, Borys M.; Burdsall, Harold H., et al. Pest risk assessment of the importation in United states of unprocessed Pinus and Abies logs from Mexico.Gen. Tech.Rep.FPL-GTR-104. Madison,WI:U.S. Department of Agriculture, Forest Service, Forest Products Laboratory, 1998，P55-56； 2. Gordon, T.R., Storer A.J. and Wood D.L.. The Pitch Canker Epidemic in California[J]. Plant Disease. 2001，85（11）：1128-1139).	Modified, see 43
59	Although they may exhibit different levels of susceptibility to *F. circinatum*, all *Pinus* spp., along with *P. menziesii*, may be potentially affected by the fungus, and the symptoms can be observed at any time of year. In addition, *F. circinatum* can affect plants of different ages, ranging from seedlings to mature trees, and it can be	*Category : TECHNICAL* **(121) Philippines (29 Sep 2016 7:32 AM)** This is to cover both asymptomatic and symptomatic plant tissue and seeds because	Incorporated

Para	Text	Comment	SC's response
	detected on all plant parts (roots, branches, shoots, cones and seeds). *F. circinatum* may also be soil-borne. There are no published methods for the isolation of *F. circinatum* from soil. This protocol describes the identification of *F. circinatum* on asymptomatic and symptomatic plant tissue and ~~on~~ seeds. Plants and trees should be inspected for any symptoms typical of pine pitch canker (section 3.1.1) whereas seeds may be analysed by random sampling (section 3.2.2). Diagnostic method A, isolation and culture (section 3.3) and diagnostic method B, molecular tests (section 3.4), may both be used for plant tissue and seeds.	there are instances that F. circinatum may not manifest symptoms.	
59	Although they may exhibit different levels of susceptibility to *F. circinatum*, all *Pinus* spp., along with *P. menziesii*, may be potentially affected by the fungus, and the symptoms can be observed at any time of year. In addition, *F. circinatum* can affect plants of different ages, ranging from seedlings to mature trees, and it can be detected on all plant parts (roots, branches, shoots, cones and seeds). *F. circinatum* may also be soil-borne. There are no published methods for the isolation of *F. circinatum* from soil. This protocol describes the identification of *F. circinatum* on symptomatic plant tissue and on seeds. Plants and trees should be inspected for any ~~symptoms~~ typical symptoms of pine pitch canker (section 3.1.1) whereas seeds may be analysed by random sampling (section 3.2.2). Diagnostic method A, isolation and culture (section 3.3) and diagnostic method B, molecular tests (section 3.4), may both be used for plant tissue and seeds.	*Category : TRANSLATION* **(86) Algeria (27 Sep 2016 11:17 AM)**	Incorporated
59	~~Although they~~ All pinus spp., along with P.menziesii, may ~~exhibit~~ be affected by the fungus at different levels and the symptoms can be observed at any time of ~~susceptibility to~~ year. In addition, *F. circinatum*~~, all *Pinus* spp., along with *P. menziesii*, may be potentially affected by the fungus, and the symptoms can be observed at any time of year. In addition, *F. circinatum*~~ can affect plants of different ages, ranging from seedlings to mature trees, and it can be detected on all plant parts (roots, branches, shoots, cones and seeds). *F. circinatum* may also be soil-borne. There are no published methods for the isolation of *F. circinatum* from soil. This protocol describes the identification of *F. circinatum* on symptomatic plant tissue and on seeds. Plants and trees should be inspected for any symptoms typical of pine pitch canker (section 3.1.1) whereas seeds may be analysed by random sampling (section 3.2.2). Diagnostic method A, isolation and culture (section 3.3) and diagnostic method B, molecular tests (section 3.4), may both be used for plant tissue and seeds.	*Category : EDITORIAL* **(85) Algeria (27 Sep 2016 11:14 AM)**	Considered, but not incorporated
59	Although they may exhibit different levels of susceptibility to *F. circinatum*, all *Pinus* spp., along with *P. menziesii*, may be potentially affected by the fungus, and the symptoms can be observed at any time of year. In addition, *F. circinatum* can	*Category : TECHNICAL* **(21) New Zealand (1 Sep 2016 5:27 AM)**	Modified

Para	Text	Comment	SC's response
	affect plants of different ages, ranging from seedlings to mature trees, and it can be detected on all plant parts (roots, trunk, branches, shoots, cones and seeds). *F. circinatum* may also be soil-borne. There are no published methods for the isolation of *F. circinatum* from soil. This protocol describes the identification of *F. circinatum* on symptomatic plant tissue and on seeds. Plants and trees should be inspected for any symptoms typical of pine pitch canker (section 3.1.1) whereas seeds may be analysed by random sampling (section 3.2.2). Diagnostic method A, isolation and culture (section 3.3) and diagnostic method B, molecular tests (section 3.4), may both be used for plant tissue and seeds.	Fusarium circinatum can be detected from tree trunks	
60	Because of the high diversity and complexity of the *Fusarium* genus, especially in the *fujikuroi* species complex that *F. circinatum* formerly belonged to, diagnosis in both method A and method B will sometimes have to be ascertained by an additional DNA sequence analysis step. (see Figure 1).	*Category : TECHNICAL* **(200) Ghana (30 Sep 2016 12:02 AM)** The levels of sensitivity and specificity of the proposed method should be indicated in order to compare these methods with other methods.	Modified Because of the high diversity and complexity of the Fusarium genus, and challenges in using morpohological characters to distinguish it from other members of the Fusarium fujikuroi Species Complex (FFSC), it is recommended that diagnosis in both method A and method B is confirmed by an additional DNA sequence analysis step. In particular if the outcome is critical (e.g. post-entry quarantine samples, new host record, new country record), it is recommended that the fungus is isolated and confirmed by sequence analysis. PCR cross-reaction might occur with phylogenetically close Fusarium spp., such as the recently described Fusarium species obtained in Colombia (Herron et al., 2015).
60	Because of the high diversity and complexity of the *Fusarium* genus~~, especially in the *fujikuroi*~~, and challenges in using morpohological characters to distinguish it from other members of the *Fusarium fujikuroi* ~~species complex that~~ Species Complex (FFSC), diagnosis in both method A and method B will sometimes have to be confirmed by an additional DNA sequence analysis step. (see Figure 1). ~~*F. circinatum* formerly belonged to, diagnosis in both method A and method B will sometimes have to be ascertained by an additional DNA sequence analysis step. (see Figure 1).~~	*Category : TECHNICAL* **(54) United States of America (21 Sep 2016 8:34 PM)** F. circinatum is a member of the Fusarium fujikuroi Species Complex sensu Geiser et al. 2013 and O'Donnell et al. 2013.	Modified
63	~~*Root infection.* Symptoms are brown discoloration and disintegration of the cortex and are similar to symptoms caused by other root rot pathogens. Root symptoms~~	*Category : SUBSTANTIVE* **(153) China (29 Sep 2016 11:19 AM)**	Considered, but not incorporated

Para	Text	Comment	SC's response
	~~may lead to above-ground symptoms, which are generally not apparent until the pathogen reaches the crown after it girdles the stem, causing yellowing of the foliage. Resin-soaked tissue may then be observed after removal of the bark on the lower part of the stem.~~Propose to revise this section for the description of main symptoms is not clear.	Propose to revise this section for the description of main symptoms is not clear.	As indicated the symptoms of root infection are not specific for F. circinatum, and may lead to abouve ground symptoms.
65	Symptoms in older trees can be mistaken for those caused by *Sphaeropsis sapinea* (Fr.) Dyco & Sutton (synonym *Diplodia pinea*) (Sutton, 1980) or feeding damage caused by wood-boring insects. Therefore, the diagnosis should be based on testing. The resin bleeding sometimes coats the trunk and lower branches for several metres below the level of the infection. The stem cankers are flat or slightly sunken and may sometimes affect large surfaces of cortical and subcortical tissue of the trunk. Removal of the bark shows subcortical lesions with brown and resin-impregnated tissues (Figure 3).	*Category : SUBSTANTIVE* **(218) Mexico (30 Sep 2016 7:38 PM)** Include comparative image of symptoms, damage or differences with this pathogen	Considered, but not incorporated. The symptoms provided in the protocol are rerstricted to Fusarium circinatum, S. sapinea 9or D. pinea) is an example, there will be also fungal species that might be confusing, and also the feeding insects as mentioned.
65	Symptoms in older trees can be mistaken for those caused by *Sphaeropsis sapinea* (Fr.) Dyco & Sutton (synonym *Diplodia pinea*) (Sutton, 1980) or feeding damage caused by wood-boring insects. Therefore, the diagnosis should be based on testing. The resin bleeding sometimes coats the trunk and lower branches for several metres below the level of the infection. The stem cankers are flat or slightly sunken and may sometimes affect large surfaces of cortical and subcortical tissue of the trunk. Removal of the bark shows subcortical lesions with brown and resin-impregnated tissues (Figure 3).	*Category : EDITORIAL* **(55) United States of America (21 Sep 2016 8:35 PM)** In the second sentence, is a descriptor missing before the word "testing"?	Incorporated: "laboratory testing"
65	Symptoms in older trees can be mistaken for those caused by *Sphaeropsis sapinea* (Fr.) Dyco & B.Sutton (synonym *Diplodia pinea*) (Sutton, 1980) or feeding damage caused by wood-boring insects. Therefore, the diagnosis should be based on testing. The resin bleeding sometimes coats the trunk and lower branches for several metres below the level of the infection. The stem cankers are flat or slightly sunken and may sometimes affect large surfaces of cortical and subcortical tissue of the trunk. Removal of the bark shows subcortical lesions with brown and resin-impregnated tissues (Figure 3).	*Category : EDITORIAL* **(22) New Zealand (1 Sep 2016 5:56 AM)** Typo	Incorporated
67	**3.1.2 ~~Seedlings and~~ seed contamination** **Delete the section for description of seedlings.**	*Category : SUBSTANTIVE* **(154) China (29 Sep 2016 11:20 AM)** It's difficult to distinguish for the symptoms of Fusarium circinatum for seedlings with other diseases.	Considered, but not incorporated: Symptoms are provided and might give a first indication of infection, hower, agreed that these symptoms are not specific for F. circinatum
68	Seeds can be infected (Storer *et al.*, 1998). Infected seedlings usually show damping off symptoms: the needles turn red, brown or chlorotic and die from the base up, or	*Category : TECHNICAL* **(71) United States of America (21 Sep**	Considerd, but not incorporated:

Para	Text	Comment	SC's response
	the seedling dies (Figure 4). In some cases affected seedlings may show brown discoloration on roots and the lower part of stems. However, *F. circinatum* may infect seedlings without apparent symptoms.	**2016 9:03 PM)** What about symptoms on seed, such as discoloration or rotting?	Are there clear references for these suggested symptoms?
69	It is reported ~~in the literature~~ by (Storer et al., (1998), that *F. circinatum* may sometimes be present in a quiescent form that cannot be detected in seeds by ~~isolation (Storer~~ isolation~~*et al.*,~~. Therefore, the absence of ~~1998). Therefore, the absence of~~ *F. circinatum* cannot be ascertained by isolation from seeds. In contrast, non-viable propagules of *F. circinatum* may generate positive results using the molecular tests.	*Category : EDITORIAL* **(87) Algeria (27 Sep 2016 11:21 AM)**	Considered, but not incorporated
72	Whole seedlings should be placed in plastic bags that are then sealed and kept under cool conditions until they are sent to the laboratory. In the laboratory, the samples should be kept in a refrigerator until analysis, which should be preferably within two days of arrival. ~~Asymptomatic seedlings are not covered by this protocol.~~	*Category : SUBSTANTIVE* **(123) Philippines (29 Sep 2016 7:41 AM)** Asymptomatic and symptomatic plant tissue should be covered by this protocol, as explained in item 3	Incorporated
72	Whole seedlings should be placed in plastic bags that are then sealed and kept under cool conditions until they are sent to the laboratory. In the laboratory, the samples should be kept in a refrigerator until analysis, which should be preferably within two days of arrival. Asymptomatic seedlings are not covered by this protocol.	*Category : SUBSTANTIVE* **(122) Philippines (29 Sep 2016 7:37 AM)** since temperature is very critical, indicate range of temperature to keep the samples and how long it can be stored before sending into the laboratory.	Modified …..kept in an ice-box or refrigerator at 4 °C until …. in a refrigerator at 4 °C
72	Whole seedlings should be placed in plastic bags that are then sealed and kept under cool conditions until they are sent to the laboratory. In the laboratory, the samples should be kept in a refrigerator until analysis, which should be preferably within two days of arrival. Asymptomatic seedlings are not covered by this protocol~~.~~. Asymptomatic part(party) must be necessarily taken care in this protocol : in most of the loads in our laboratories, plants are asymptomatic	*Category : SUBSTANTIVE* **(88) Algeria (27 Sep 2016 1:06 PM)**	Modified reference to asymptomatic infections has been added
73	For trunk or branch cankers, the inner bark of the area directly around the visible lesion should be cut repeatedly with a sterile blade until a canker margin is observed. Pieces of tissue, including phloem and xylem, should be removed in order to collect portions of the lesion edge, where the fungus is most active. The pieces of tissue should be wrapped in sheets of paper and placed in a plastic bag that is then sealed. All samples of plant material should be sent to the laboratory ~~as soon as possible after sampling, and refrigerated until transfer.~~ as soon as possible after sampling, and refrigerated until transfer. In the laboratory, the samples must be kept in a refrigerator, to be analysed within two days of arrival.	*Category : SUBSTANTIVE* **(124) Philippines (29 Sep 2016 7:44 AM)** we need to be specific with as soon as possible, a definite number of hours or day should be indicated because this pertains to lab samples.	Modified …..kept in an ice-box or refrigerator at 4 °C until …. in a refrigerator at 4 °C

Para	Text	Comment	SC's response
75	As no symptoms can be observed on seeds, the lot should be sampled randomly. As counting of seeds may be laborious, the sampled seeds may be weighed instead of counted. Depending on the method chosen for the identification, the total number of seeds to be tested per lot in order to detect the pathogen at different levels of infection in the lot may be different and needs to be determined statistically (useful guidance is given in tables 1 and 2 of ISPM 31 (*Methodologies for sampling of consignments*)). Sample size recommended by the International Seed Testing Association (ISTA) is 400 seeds for plating (ISTA, ~~2002)~~2016). However, larger samples (e.g. 1 000 seeds) can easily be processed by biological enrichment before DNA analyses (Ioos *et al.*, 2009).	*Category : TECHNICAL* **(161) EPPO (29 Sep 2016 11:47 AM)** Reference should be updated to latest version of the ISTA Rules (see also para 379).	Incorporated
75	As no symptoms can be observed on seeds, the lot should be sampled randomly. As counting of seeds may be laborious, the sampled seeds may be weighed instead of counted. Depending on the method chosen for the identification, the total number of seeds to be tested per lot in order to detect the pathogen at different levels of infection in the lot may be different and needs to be determined statistically (useful guidance is given in tables 1 and 2 of ISPM 31 (*Methodologies for sampling of consignments*)). Sample size recommended by the International Seed Testing Association (ISTA) is 400 seeds for plating (ISTA, 2002). However, larger samples (e.g. 1 000 seeds) can easily be processed by biological enrichment before DNA analyses (Ioos *et al.*, 2009).	*Category : SUBSTANTIVE* **(126) Philippines (29 Sep 2016 7:51 AM)** Why asymptomatic seeds can be sampled but for plant tissue it should be symptomatic only.	Modified included asymptomatic tissue in paragraph 59
75	As no symptoms can be observed on seeds, the lot should be sampled randomly. As counting of seeds may be laborious, the sampled seeds may be weighed instead of counted. Depending on the method chosen for the identification, the total number of seeds to be tested per lot in order to detect the pathogen at different levels of infection in the lot may be different and needs to be determined statistically (useful guidance is given in tables 1 and 2 of ISPM 31 (*Methodologies for sampling of consignments*)). Sample size recommended by the International Seed Testing Association (ISTA) is 400 seeds for plating (ISTA, ~~2002)~~2016). However, larger samples (e.g. 1 000 seeds) can easily be processed by biological enrichment before DNA analyses (Ioos *et al.*, 2009).	*Category : TECHNICAL* **(89) European Union (27 Sep 2016 1:21 PM)** Reference should be updated to latest version of the ISTA Rules (see also para 379).	Incorporated
75	As no symptoms can be observed on seeds, the lot should be sampled randomly. As counting of seeds may be laborious, the sampled seeds may be weighed instead of counted. Depending on the method chosen for the identification, the total number of seeds to be tested per lot in order to detect the pathogen at different levels of infection in the lot may be different and needs to be determined statistically (useful guidance is given in tables 1 and 2 of ISPM 31 (*Methodologies for sampling of*	*Category : TECHNICAL* **(74) EPPO (25 Sep 2016 1:56 PM)** Reference should be updated to latest version of the ISTA Rules (see also para 379).	Incorporated

Para	Text	Comment	SC's response
	consignments)). Sample size recommended by the International Seed Testing Association (ISTA) is 400 seeds for plating (ISTA, ~~2002)~~2016). However, larger samples (e.g. 1 000 seeds) can easily be processed by biological enrichment before DNA analyses (Ioos *et al.*, 2009).		
76	~~Seeds may be analysed by isolation and culture (section 3.3.2) or by conventional or real-time polymerase chain reaction (PCR) after a biological enrichment step (section 3.4.1.2). These methods have been compared in the framework of a European collaborative study, and performance values have been calculated for each of the methods (Ioos *et al.*, 2013).~~	*Category : SUBSTANTIVE* **(125) Philippines (29 Sep 2016 7:47 AM)** This discussion should be on 3.4 Molecular Test..because this item deals with sampling and might create an impression that it is only on seeds that we can conduct molecular test.	Considered, but not incorporated. This item deals with analysis of samples by isaolation and culture, or PCR, not with sampling
77	**3.3 Diagnostic method A: Isolation and culture**	*Category : TECHNICAL* **(201) Ghana (30 Sep 2016 12:02 AM)** The levels of sensitivity and specificity of the proposed method should be indicated in order to compare these methods with other methods.	This method described is generally used for many years, specific data of levels of sensitivity and specificity could be included if these data are available. one known reference has been made in par. 86, Storer et al.,dealing with dormant (quiescent) propagules of F. circinatum.
81	Plant material should be surface-sterilized for up to 1 min in a 1.5% solution of active sodium hypochlorite or 50% alcohol, and rinsed twice in sterile distilled water (Pérez-Sierra *et al.*, 2007). Selective media, such as dichloran chloramphenicol peptone agar (DCPA) or Komada's medium, are recommended for isolations. Potato dextrose agar supplemented with 0.5 mg/ml streptomycin sulphate salt (775 units/mg solid) (PDAS) can also be used (section 3.3.3).	*Category : TECHNICAL* **(202) Bolivia (30 Sep 2016 12:46 AM)** To clarify	incorporated
81	Plant material should be surface-sterilized for up to 1 min in a 1.5% solution of active sodium hypochlorite or 50% alcohol, and rinsed twice in sterile distilled water (Pérez-Sierra *et al.*, 2007). Selective media, such as dichloran chloramphenicol peptone agar (DCPA) or Komada's medium, are recommended for isolations. Potato dextrose agar supplemented with 0.5 mg/ml streptomycin sulphate salt (775 units/mg solid) (PDAS) can also be used (section 3.3.3).	*Category : TECHNICAL* **(180) Peru (29 Sep 2016 6:57 PM)** To clarify	incorporated
81	Plant material should be surface-sterilized for up to 1 min in a 1.5% solution of active sodium hypochlorite or 50% alcohol, and rinsed twice in sterile distilled water (Pérez-Sierra *et al.*, 2007). Selective media, such as dichloran chloramphenicol peptone agar (DCPA) or Komada's medium, are recommended for isolations. Potato dextrose agar supplemented with 0.5 mg/ml streptomycin sulphate salt (775 units/mg solid) (PDAS) can also be used (section 3.3.3).	*Category : TECHNICAL* **(166) Brazil (29 Sep 2016 3:46 PM)** To clarify	incorporated

Para	Text	Comment	SC's response
81	Plant material should be surface-sterilized for up to 1 min in a 1.5% solution of sodium hypochlorite or 50% alcohol, and rinsed twice in sterile distilled water (Pérez-Sierra *et al.*, 2007). Selective media, such as dichloran chloramphenicol peptone agar (DCPA) or Komada's medium, are recommended for isolations. Potato dextrose agar supplemented with 0.5 mg/ml streptomycin sulphate salt (775 units/mg solid) (PDAS) can also be used (section 3.3.3).	*Category : SUBSTANTIVE* **(127) Philippines (29 Sep 2016 7:53 AM)** to include approach for isolating the pathogen from asymptomatic sample	incorporated
81	Plant material should be surface-sterilized for up to 1 min in a 1.5% solution of active sodium hypochlorite or 50% alcohol, and rinsed twice in sterile distilled water (Pérez-Sierra *et al.*, 2007). Selective media, such as dichloran chloramphenicol peptone agar (DCPA) or Komada's medium, are recommended for isolations. Potato dextrose agar supplemented with 0.5 mg/ml streptomycin sulphate salt (775 units/mg solid) (PDAS) can also be used (section 3.3.3).	*Category : TECHNICAL* **(109) Argentina (28 Sep 2016 7:27 PM)** To clarify	incorporated
81	Plant material should be surface-sterilized for up to 1 min in a 1.5% solution of active sodium hypochlorite or 50% alcohol, and rinsed twice in sterile distilled water (Pérez-Sierra *et al.*, 2007). Selective media, such as dichloran chloramphenicol peptone agar (DCPA) or Komada's medium, are recommended for isolations. Potato dextrose agar supplemented with 0.5 mg/ml streptomycin sulphate salt (775 units/mg solid) (PDAS) can also be used (section 3.3.3).	*Category : TECHNICAL* **(96) Chile (28 Sep 2016 4:57 PM)** To clarify	incorporated
81	Plant material should be surface-sterilized for up to 1 min in a 1.5% solution of active sodium hypochlorite or 50% alcohol, and rinsed twice in sterile distilled water (Pérez-Sierra *et al.*, 2007). Selective media, such as dichloran chloramphenicol peptone agar (DCPA) or Komada's medium, are recommended for isolations. Potato dextrose agar supplemented with 0.5 mg/ml streptomycin sulphate salt (775 units/mg solid) (PDAS) can also be used (section 3.3.3).	*Category : TECHNICAL* **(40) Uruguay (15 Sep 2016 8:07 PM)** To clarify	incorporated
81	Plant material should be surface-sterilized for up to 1 min in a 1.5% solution of active sodium hypochlorite or 50% alcohol, and rinsed twice in sterile distilled water (Pérez-Sierra *et al.*, 2007). Selective media, such as dichloran chloramphenicol peptone agar (DCPA) or Komada's medium, are recommended for isolations. Potato dextrose agar supplemented with 0.5 mg/ml streptomycin sulphate salt (775 units/mg solid) (PDAS) can also be used (section 3.3.3).	*Category : TECHNICAL* **(5) COSAVE (11 Aug 2016 10:13 PM)** To clarify	incorporated
82	Plates are incubated at 22 °C ± 6 °C under near ultraviolet (UV) light or daylight. During incubation, the plates are observed daily and all the *Fusarium* spp. colonies are transferred to potato dextrose agar (PDA) and to ~~spezieller nährstoffarmer~~ Spezieller Nährstoffarmer agar (SNA) and incubated for ten days under the same conditions for morphological identification (section 4.1.1).	*Category : EDITORIAL* **(56) United States of America (21 Sep 2016 8:35 PM)** Correction - global check	incorporated

Para	Text	Comment	SC's response
87	**3.3.3 Culture media**	*Category : TECHNICAL* **(57) United States of America (21 Sep 2016 8:36 PM)** In this section, consider adding Nash-Snyder agar	considered, not incorporated. A common used medium has been provided as an example. Other nedia may be used, depending of the experience of the laboratory.
88	*Dichloran chloramphenicol peptone agar*. DCPA is suitable for isolation of *Fusarium* spp. from plant tissue, including seeds, but not for identification. The medium, slightly modified by Ioos *et al*. (2004) after Andrews and Pitt (1986), contains 15.0 g bacteriological peptone, 1.0 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.2 g chloramphenicol, 2 mg 2,6-dichloro-4-nitroaniline (dichloran) (0.2% w/v in ethanol, 1.0 ml), 0.0005 g crystal violet (0.05% w/v in water, 1.0 ml) and 20.0 g agar technical ~~in~~ grade agar make up toin 1 litre distilled water.	*Category : TECHNICAL* **(23) New Zealand (1 Sep 2016 6:05 AM)** The preparation of this medium was to add distilled water into the solution and make up to 1 litre rather than adding 1 litre water into the solution.	modified
89	*Komada's medium*. This medium is suitable for isolation of *Fusarium* spp. from plant tissue, including seeds, but not for identification. The base medium contains 1.0 g K_2HPO_4, 0.5 g KCl, 0.5 g $MgSO_4 \cdot 7H_2O$, 10 mg Fe-Na-ethylenediaminetetraacetic acid (EDTA), 2.0 g L-asparagine, 20.0 g D-galactose and 15.0 g technical ~~agar in 1.0~~ agar, make up to 1 litre with distilled water. The pH is adjusted to 3.8 ± 0.2 with 10% phosphoric acid. The medium is autoclaved at 121 °C for 15 min and slightly cooled before adding the following filter-sterilized supplements: 1.0 g pentachloronitrobenzene (PNCB) (75% w/w), 0.5 g ox-gall, 1.0 g $Na_2B_4O_7 \cdot 10H_2O$ and 6 ml/litre stock solution streptomycin (5 g streptomycin in 100 ml distilled water) (Komada, 1975).	*Category : TECHNICAL* **(24) New Zealand (1 Sep 2016 6:09 AM)** The preparation of this medium was to add distilled water into the solution and make up to 1 litre rather than adding 1 litre water into the solution.	modified
90	*Potato dextrose agar*. PDA is used to study *Fusarium* spp. colony morphology and pigmentation. The medium contains 15 g dextrose, 20 g agar and the broth from 200 g white potatoes made up to 1.0 litre ~~of~~ with distilled water (Hawksworth *et al.*, 1995). Commercially available preparations of PDA are as suitable as those made in the laboratory. PDA supplemented with 0.5 mg/ml streptomycin sulphate salt (775 units/mg solid) (PDAS) can be used for isolation.	*Category : EDITORIAL* **(25) New Zealand (1 Sep 2016 6:11 AM)** typo	incorporated
93	There are several molecular methods currently available to confirm the identity of *F. circinatum* isolates (identification by sequence analysis) or to detect and/or identify it directly *in planta* (conventional PCR, SYBR Green® real-time PCR or real-time PCR using a hydrolysis probe). These methods are fast, efficient and reliable in detecting *F. circinatum* specifically, without ~~previous~~ agar plating, thus saving a lot of space and time, ~~but they require~~ but requires facilities equipped for molecular ~~biology~~biology detection. In addition, as these techniques target the DNA of the fungus, active and quiescent forms of the pathogen are equally detected.	*Category : EDITORIAL* **(128) Philippines (29 Sep 2016 7:56 AM)** for clarity	modified

Para	Text	Comment	SC's response
93	There are several molecular methods currently available to confirm the identity of *F. circinatum* isolates (identification by sequence analysis) or to detect and/or identify it directly *in planta* (conventional PCR, SYBR Green® real-time PCR or real-time PCR using a hydrolysis probe). These methods are fast, efficient and reliable in detecting *F. circinatum* specifically, without previous agar plating, thus saving a lot of space and time, but they require facilities equipped for molecular biology. In addition, as these techniques target the DNA of the fungus, ~~active ,~~ viable and ~~quiescent forms~~ non-viable cells of the pathogen are equally detected.	*Category : TECHNICAL* **(27) New Zealand (1 Sep 2016 6:16 AM)** Both active and quiescent forms of the pathogen remains a risk; however, non-viable cells do not pose any risk but will be detected by PCR tests with positive results.	incorporated
93	There are several molecular methods currently available to confirm the identity of *F. circinatum* isolates (identification by sequence analysis) or to detect and/or identify it directly *in planta* (conventional PCR, SYBR Green® real-time PCR or real-time PCR using a hydrolysis probe). These methods are fast, efficient and reliable in detecting *F. circinatum* specifically, without previous agar plating, thus saving a lot of space and time, but they require facilities equipped for molecular ~~biology~~biology testing. In addition, as these techniques target the DNA of the fungus, active and quiescent forms of the pathogen are equally detected.	*Category : EDITORIAL* **(26) New Zealand (1 Sep 2016 6:14 AM)** To improve clarity	incorporated
94	The real-time PCR using a hydrolysis probe offers enhanced specificity over the conventional PCR and the SYBR Green® real-time PCR. Positive results obtained following real-time PCR using a hydrolysis probe are ~~final~~conclusive, whereas positive results obtained following conventional PCR or SYBR Green® real-time PCR should be confirmed by sequence analysis.	*Category : EDITORIAL* **(129) Philippines (29 Sep 2016 7:59 AM)** grammar	incorporated
94	~~The real-time PCR using a hydrolysis probe offers enhanced specificity over the conventional PCR and the SYBR Green® real-time PCR. Positive results obtained following real-time PCR using a hydrolysis probe are final, whereas positive results obtained following conventional PCR or SYBR Green® real-time PCR should be confirmed by sequence analysis.~~	*Category : SUBSTANTIVE* **(28) New Zealand (1 Sep 2016 6:17 AM)** MPI PHEL has recorded the real-time PCR using a hydrolysis probe (PCR method developed by Ioos et al., 2009) cross reacted with a few isolates that belongs to different species of Fusarium in a project that compared these PCR tests. Therefore, this paragraph should be deleted and Figure 1 should be revised to include a confirmatory step when there are positive results from the real-time PCR using hydrolysis probe.	Considered, but not incorporated Although it is possible that the real-time PCR using a hydrolysis probe (Ioos et al. 2009) cross-reacts with non target Fusarium species, as it is imaginable for any PCR test regarding its initial target, a published reference, according to the Instructions to authors, is needed to take this comment into consideration. At the time the test was developed and since now, no cross reaction has been described in literature.
95	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity	*Category : TECHNICAL* **(203) Bolivia (30 Sep 2016 12:48 AM)** See general comments	Modified

Para	Text	Comment	SC's response
	and reproducibility achieved. ~~The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable.~~ Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.		Text followed as provided in Instructions to authors
95	~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved.~~ The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. ~~Laboratory procedures presented~~ This information in given for the convenience of users of this protocol and does not constitute and endorsment by the ~~protocols~~ CPM of the chemical, reagent and or equipment named. Equivalent products may be ~~adjusted~~ used if they can be shown to lead the ~~standards of individual laboratories, provided that they are adequately validated~~same results.	*Category : TECHNICAL* **(183) Peru (29 Sep 2016 7:16 PM)** See general comments.	Modified Text followed as provided in Instructions to authors
95	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. ~~The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable.~~ Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : TECHNICAL* **(181) Peru (29 Sep 2016 6:59 PM)** See general comments.	Modified Text followed as provided in Instructions to authors
95	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. ~~The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable.~~ Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : TECHNICAL* **(167) Brazil (29 Sep 2016 3:49 PM)** See general comments	Modified Text followed as provided in Instructions to authors
95	In this diagnostic protocol, methods ~~(including reference to brand names)~~ (TissueLlyser from Qiagen) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : EDITORIAL* **(130) Philippines (29 Sep 2016 8:00 AM)**	Modified Text followed as provided in Instructions to authors

Para	Text	Comment	SC's response
95	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. ~~The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable.~~ Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : TECHNICAL* **(110) Argentina (28 Sep 2016 7:28 PM)** See general comments	Modified Text followed as provided in Instructions to authors
95	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. ~~The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable.~~ Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : TECHNICAL* **(97) Chile (28 Sep 2016 4:59 PM)** See general comments.	Modified Text followed as provided in Instructions to authors
95	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. ~~The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable.~~ Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : TECHNICAL* **(41) Uruguay (15 Sep 2016 8:09 PM)** See general comment	Modified Text followed as provided in Instructions to authors
95	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. ~~The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable.~~ Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	*Category : TECHNICAL* **(2) COSAVE (11 Aug 2016 10:01 PM)** See general comments.	Modified Text followed as provided in Instructions to authors
98	Potentially infected plant tissues are picked from the sample and first cut roughly using a sterile scalpel blade, without a prior surface disinfection step. Small pieces of approximately 0.5–1.0 cm^2 should be first collected first then subsequently cut into smaller pieces (< 2–3 mm^2, each side) into a sterile plastic Petri dish. The amount of tissue required for each reaction is recommended in the manufacturer's Instructionss for the DNA extraction kit being used. The sample is then transferred into a 2 ml microcentrifuge tube corresponding to approximately 200 µl and ground for 2 min with two 3 mm steel or tungsten carbide beads and the quantity of	*Category : EDITORIAL* **(204) Bolivia (30 Sep 2016 12:56 AM)** to clarify	incorporated

Para	Text	Comment	SC's response
	lysis buffer recommended by the manufacturer and provided by the DNA extraction kit, at a frequency of 30 Hz with a bead beater ~~(TissueLlyser~~ (Tissue Lyser from Qiagen[1], or equivalent). The samples may also be ground in a mortar by a pestle with liquid nitrogen, or by using other efficient grinding techniques, such as a FastPrep homogenizer ~~(MP bBiomedicals~~(MP Biomedicals[1]).		
98	Potentially infected plant tissues are picked from the sample and first cut roughly using a sterile scalpel blade, without a prior surface disinfection step. Small pieces of approximately 0.5–1.0 cm^2 should be first collected first then subsequently cut into smaller pieces (< 2–3 mm^2, each side) into a sterile plastic Petri dish. The amount of tissue required for each reaction is recommended in the manufacturer's instructions for the DNA extraction kit being used. The sample is then transferred into a 2 ml microcentrifuge tube corresponding to approximately 200 µl and ground for 2 min with two 3 mm steel or tungsten carbide beads and the quantity of lysis buffer recommended by the manufacturer and provided by the DNA extraction kit, at a frequency of 30 Hz with a bead beater ~~(TissueLlyser~~ (TissueLyser from Qiagen[1], or equivalent). The samples may also be ground in a mortar by a pestle with liquid nitrogen, or by using other efficient grinding techniques, such as a FastPrep homogenizer (MP ~~bBiomedicals~~Biomedicals[1]).	*Category : EDITORIAL* **(182) Peru (29 Sep 2016 7:05 PM)**	incorporated
98	Potentially infected plant tissues are picked from the sample and first cut roughly using a sterile scalpel blade, without a prior surface disinfection step. Small pieces of approximately 0.5–1.0 cm^2 should be first collected first then subsequently cut into smaller pieces (< 2–3 mm^2, each side) into a sterile plastic Petri dish. The amount of tissue required for each reaction is recommended in the manufacturer's instructions for the DNA extraction kit being used. The sample is then transferred into a 2 ml microcentrifuge tube corresponding to approximately 200 µl and ground for 2 min with two 3 mm steel or tungsten carbide beads and the quantity of lysis buffer recommended by the manufacturer and provided by the DNA extraction kit, at a frequency of 30 Hz with a bead beater ~~(TissueLlyser~~ (TissueLyser from Qiagen[1], or equivalent). The samples may also be ground in a mortar by a pestle with liquid nitrogen, or by using other efficient grinding techniques, such as a FastPrep homogenizer (MP ~~bBiomedicals~~Biomedicals[1]).	*Category : EDITORIAL* **(168) Brazil (29 Sep 2016 3:50 PM)**	incorporated
98	Potentially infected plant tissues (symptomatic and asymptomatic) are picked from the sample and first cut roughly using a sterile scalpel blade, without a prior surface disinfection step. Small pieces of approximately 0.5–1.0 cm^2 should be first collected first then subsequently cut into smaller pieces (< 2–3 mm^2, each side) into a sterile plastic Petri dish. The amount of tissue required for each reaction is recommended in the manufacturer's instructions for the DNA extraction kit being	*Category : SUBSTANTIVE* **(131) Philippines (29 Sep 2016 8:02 AM)** for consistency with our comments	incorporated

Para	Text	Comment	SC's response
	used. The sample is then transferred into a 2 ml microcentrifuge tube corresponding to approximately 200 µl and ground for 2 min with two 3 mm steel or tungsten carbide beads and the quantity of lysis buffer recommended by the manufacturer and provided by the DNA extraction kit, at a frequency of 30 Hz with a bead beater (TissueLlyser from Qiagen[1], or equivalent). The samples may also be ground in a mortar by a pestle with liquid nitrogen, or by using other efficient grinding techniques, such as a FastPrep homogenizer (MP bBiomedicals[1]).		
98	Potentially infected plant tissues are picked from the sample and first cut roughly using a sterile scalpel blade, without a prior surface disinfection step. Small pieces of approximately 0.5–1.0 cm^2 should be first collected first then subsequently cut into smaller pieces (< 2–3 mm^2, each side) into a sterile plastic Petri dish. The amount of tissue required for each reaction is recommended in the manufacturer's instructions for the DNA extraction kit being used. The sample is then transferred into a 2 ml microcentrifuge tube corresponding to approximately 200 µl and ground for 2 min with two 3 mm steel or tungsten carbide beads and the quantity of lysis buffer recommended by the manufacturer and provided by the DNA extraction kit, at a frequency of 30 Hz with a bead beater ~~(TissueLlyser~~ (TissueLyser from Qiagen[1], or equivalent). The samples may also be ground in a mortar by a pestle with liquid nitrogen, or by using other efficient grinding techniques, such as a FastPrep homogenizer (MP ~~bBiomedicals~~Biomedicals[1]).	*Category : EDITORIAL* **(111) Argentina (28 Sep 2016 7:29 PM)**	incorporated
98	Potentially infected plant tissues are picked from the sample and first cut roughly using a sterile scalpel blade, without a prior surface disinfection step. Small pieces of approximately 0.5–1.0 cm^2 should be first collected first then subsequently cut into smaller pieces (< 2–3 mm^2, each side) into a sterile plastic Petri dish. The amount of tissue required for each reaction is recommended in the manufacturer's instructions for the DNA extraction kit being used. The sample is then transferred incorporated into a 2 ml microcentrifuge tube corresponding to approximately 200 µl and ground for 2 min with two 3 mm steel or tungsten carbide beads and the quantity of lysis buffer recommended by the manufacturer and provided by the DNA extraction kit, at a frequency of 30 Hz with a bead beater ~~(TissueLlyser~~ (TissueLyser from Qiagen[1], or equivalent). The samples may also be ground in a mortar by a pestle with liquid nitrogen, or by using other efficient grinding techniques, such as a FastPrep homogenizer (MP ~~bBiomedicals~~Biomedicals[1]).	*Category : EDITORIAL* **(98) Chile (28 Sep 2016 5:01 PM)**	incorporated
98	Potentially infected plant tissues are picked from the sample and first cut roughly using a sterile scalpel blade, without a prior surface disinfection step. Small pieces of approximately 0.5–1.0 cm^2 should be first collected first then subsequently cut into smaller pieces (< 2–3 mm^2, each side) into a sterile plastic Petri dish. The	*Category : EDITORIAL* **(42) Uruguay (15 Sep 2016 8:11 PM)** Editorial correction	incorporated

Para	Text	Comment	SC's response
	amount of tissue required for each reaction is recommended in the manufacturer's instructions for the DNA extraction kit being used. The sample is then transferred into a 2 ml microcentrifuge tube corresponding to approximately 200 µl and ground for 2 min with two 3 mm steel or tungsten carbide beads and the quantity of lysis buffer recommended by the manufacturer and provided by the DNA extraction kit, at a frequency of 30 Hz with a bead beater ~~(TissueLlyser~~ (TissueLyser from Qiagen[1], or equivalent). The samples may also be ground in a mortar by a pestle with liquid nitrogen, or by using other efficient grinding techniques, such as a FastPrep homogenizer (MP ~~bBiomedicals~~Biomedicals[1]).		
98	Potentially infected plant tissues are picked from the sample and first cut roughly using a sterile scalpel blade, without a prior surface disinfection step. Small pieces of approximately 0.5–1.0 cm^2 should be first collected ~~first~~ then subsequently cut into smaller pieces (< 2–3 mm^2, each side) into a sterile plastic Petri dish. The amount of tissue required for each reaction is recommended in the manufacturer's instructions for the DNA extraction kit being used. The sample is then transferred into a 2 ml microcentrifuge tube corresponding to approximately 200 µl and ground for 2 min with two 3 mm steel or tungsten carbide beads and the quantity of lysis buffer recommended by the manufacturer and provided by the DNA extraction kit, at a frequency of 30 Hz with a bead beater (TissueLlyser from Qiagen[1], or equivalent). The samples may also be ground in a mortar by a pestle with liquid nitrogen, or by using other efficient grinding techniques, such as a FastPrep homogenizer (MP bBiomedicals[1]).	*Category : EDITORIAL* **(29) New Zealand (4 Sep 2016 10:53 PM)** Typo	incorporated
98	Potentially infected plant tissues are picked from the sample and first cut roughly using a sterile scalpel blade, without a prior surface disinfection step. Small pieces of approximately 0.5–1.0 cm^2 should be first collected first then subsequently cut into smaller pieces (< 2–3 mm^2, each side) into a sterile plastic Petri dish. The amount of tissue required for each reaction is recommended in the manufacturer's instructions for the DNA extraction kit being used. The sample is then transferred into a 2 ml microcentrifuge tube corresponding to approximately 200 µl and ground for 2 min with two 3 mm steel or tungsten carbide beads and the quantity of lysis buffer recommended by the manufacturer and provided by the DNA extraction kit, at a frequency of 30 Hz with a bead beater ~~(TissueLlyser~~ (TissueLyser from Qiagen[1], or equivalent). The samples may also be ground in a mortar by a pestle with liquid nitrogen, or by using other efficient grinding techniques, such as a FastPrep homogenizer (MP ~~bBiomedicals~~Biomedicals[1]).	*Category : EDITORIAL* **(6) COSAVE (11 Aug 2016 10:15 PM)**	incorporated

Para	Text	Comment	SC's response
99	~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved.~~ The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. ~~Laboratory procedures presented in~~ This inforamtion in given for theconvenience of users of this protocol and does not constituteand endorsment by the ~~protocols~~ CPM of the Chemical, reagent and or equipment named. Equivalent products may be ~~adjusted~~ used if they can be shown to lead the ~~standards of individual laboratories, provided that they are adequately validated~~same results.	*Category : TECHNICAL* **(205) Bolivia (30 Sep 2016 3:49 AM)** See general comments	Modified Text followed as provided in Instructions to authors
99	~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.~~	*Category : EDITORIAL* **(145) Philippines (29 Sep 2016 8:36 AM)** redundant, adjusted on 3.4	Modified Text followed as provided in Instructions to authors
99	~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved.~~ The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable~~. Laboratory procedures presented~~ .This information in given for the ~~protocols may be adjusted to~~ convenience of users of this protocol and does not constitute and endorsment by the ~~standards~~ CPM of ~~individual laboratories~~the chemical, ~~provided that~~ reagent and or equipment named. Equivalent products may be used if they ~~are adequately validated~~can be shown to lead the same results.	*Category : TECHNICAL* **(107) Chile (28 Sep 2016 5:17 PM)** see general comments	Modified Text followed as provided in Instructions to authors
99	~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved.~~ The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. ~~Laboratory procedures presented in~~ This information is given for the ~~protocols may be adjusted to~~ convenience of users of this protocol and does not constitute an endorsment by the ~~standards~~ CPM of ~~individual laboratories~~the chemical, ~~provided that~~ reagent and/or equipment named. Equivalent products may be used if they ~~are adequately validated~~can be shown to lead the same results.	*Category : TECHNICAL* **(43) Uruguay (15 Sep 2016 8:16 PM)** See general comment	Modified Text followed as provided in Instructions to authors

Para	Text	Comment	SC's response
99	~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and reproducibility achieved.~~ The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. ~~Laboratory procedures presented~~ This inforamtion in given for the ~~protocols may be adjusted to~~ convenience of users of this protocol and does not constitute and endorsment by the ~~standards~~ CPM of ~~individual laboratories~~the chemical, ~~provided that~~ reagent and or equipment named. Equivalent products may be used if they ~~are adequately validated~~can be shown to lead the same results.	*Category : TECHNICAL* **(3) COSAVE (11 Aug 2016 10:05 PM)** See general comments.	Modified Text followed as provided in Instructions to authors
103	Fungal material is harvested from a pure culture grown for seven days on PDA by scraping the aerial mycelium using a sterile scalpel blade or a sterile needle. A pellet of approximately 2–3 mm diameter may be used directly for DNA extraction. It is recommended that the fungal material prior to extraction, is ground in a mortar by a pestle with extraction buffer or by using other techniques efficient, such as grinding FastPrep[1] homogenizer	*Category : TECHNICAL* **(206) Bolivia (30 Sep 2016 3:54 AM)** To improve the process	incorporated
103	Fungal material is harvested from a pure culture grown for seven days on PDA by scraping the aerial mycelium using a sterile scalpel blade or a sterile needle. A pellet of approximately 2–3 mm diameter may be used directly for DNA extraction. It is recommended that the fungal material prior to extraction, is ground in a mortar by a pestle with extraction buffer or by using other techniques efficient, such as grinding FastPrep homogenizer.	*Category : TECHNICAL* **(169) Brazil (29 Sep 2016 3:53 PM)** to improve the process	incorporated
103	Fungal material is harvested from a pure culture grown for seven days on PDA by scraping the aerial mycelium using a sterile scalpel blade or a sterile needle. A pellet of approximately 2–3 mm diameter may be used directly for DNA extraction. It is recommended that the fungal material prior to extraction, is ground in a mortar by a pestle with extraction buffer or by using other techniques efficient, such as grinding FastPrep[1] homogenizer.	*Category : TECHNICAL* **(112) Argentina (28 Sep 2016 7:35 PM)** to improve the process	incorporated
103	Fungal material is harvested from a pure culture grown for seven days on PDA by scraping the aerial mycelium using a sterile scalpel blade or a sterile needle. A pellet of approximately 2–3 mm diameter may be used directly for DNA extraction.It is recommended that the fungal material prior to extraction, is ground in a mortar by a pestle with extraction buffer or by using other techniques efficient, such as grinding FastPrep[1] homogenizer.	*Category : TECHNICAL* **(99) Chile (28 Sep 2016 5:05 PM)** to improve the process	incorporated

Para	Text	Comment	SC's response
103	Fungal material is harvested from a pure culture grown for seven days on PDA by scraping the aerial mycelium using a sterile scalpel blade or a sterile needle. A pellet of approximately 2–3 mm diameter may be used directly for DNA extraction. It is recommended that the fungal material prior to extraction is ground in a mortar by a pestle with extraction buffer or by using other efficient technique, such as grinding with FastPrep1 homogenizer.	*Category : TECHNICAL* **(44) Uruguay (15 Sep 2016 8:20 PM)** Text added to improve the process	incorporated
103	Fungal material is harvested from a pure culture grown for seven days on PDA by scraping the aerial mycelium using a sterile scalpel blade or a sterile needle. A pellet of approximately 2–3 mm diameter may be used directly for DNA extraction~~.~~. It is recommended that the fungal material prior to extraction, is ground in a mortar by a pestle with extraction buffer or by using other techniques efficient, such as grinding FastPrep[1] homogenizer.	*Category : TECHNICAL* **(7) COSAVE (11 Aug 2016 10:20 PM)** to improve the process	incorporated
105	Total DNA from plant tissue, seeds or fungal culture should be extracted preferably following the extraction protocol described by Ioos *et al.* (2009) using a commercial plant DNA extraction kit ~~such as the NucleoSpin~~ (NucleoSpin Plant II kit ~~(Macherey-Nagel~~by Macherey-Nagel[2]), which proved to be efficient. Total DNA is extracted following the manufacturer's instructions with slight modifications. First, the chemical lysis incubation step (with lysis buffer) is extended to 20 min. After this incubation, the sample is centrifuged for 5 min at approximately 11 000 *g* to compact the debris and only the supernatant is recovered to be further processed following the manufacturer's instructions. Total DNA is finally eluted with 100 µl of the elution buffer provided in the kit and stored frozen until analysis. Total DNA or a 1:10 dilution, depending on the presence of inhibiting compounds, is used as a template for conventional or real-time PCR.	*Category : EDITORIAL* **(132) Philippines (29 Sep 2016 8:05 AM)**	considered, but not incorporated "Such as" should be maintained here since there may be alternative DNA extraction kits performing as well.
105	Total DNA from plant tissue, seeds or fungal culture should be extracted preferably following the extraction protocol described by Ioos *et al.* (2009) using a commercial plant DNA extraction kit such as the NucleoSpin Plant II kit (Macherey-Nagel[2]), which proved to be ~~efficient~~efficient or an other kit. Total DNA is extracted following the manufacturer's instructions with slight modifications. First, the chemical lysis incubation step (with lysis buffer) is extended to 20 min. After this incubation, the sample is centrifuged for 5 min at approximately 11 000 *g* to compact the debris and only the supernatant is recovered to be further processed following the manufacturer's instructions. Total DNA is finally eluted with 100 µl of the elution buffer provided in the kit and stored frozen until analysis. Total DNA or a 1:10 dilution, depending on the	*Category : SUBSTANTIVE* **(91) Algeria (27 Sep 2016 3:27 PM)**	considered, but not incorporated NucleoSpin Plant II kit (Macherey-Nagel2) has been given as an example (such as….)

Para	Text	Comment	SC's response
	presence of inhibiting compounds, is used as a template for conventional or real-time PCR.		
105	Total DNA from plant tissue, seeds or fungal culture should be extracted preferably following the extraction protocol described by Ioos *et al.* (2009) using a commercial plant DNA extraction kit such as the NucleoSpin Plant II kit (Macherey-Nagel[2]), which proved to be efficient. Total DNA is extracted following the manufacturer's instructions with slight modifications. First, the chemical lysis incubation step (with lysis buffer) is extended to 20 min. After this incubation, the sample is centrifuged for 5 min at approximately 11 000 *g* to compact the debris and only the supernatant is recovered to be further processed following the manufacturer's instructions. Total DNA is finally eluted with 100 µl of the elution buffer provided in the kit and stored frozen until analysis. Total DNA or a 1:10 dilution, depending on the presence of inhibiting compounds, is used as a template for conventional or real-time PCR.	*Category : TECHNICAL* **(58) United States of America (21 Sep 2016 8:37 PM)** Qiagen plant mini kits should also work well.	considered, but not incorporated NucleoSpin Plant II kit (Macherey-Nagel2) has been given as an example (such as….)
106	~~See footnote [1]~~	*Category : EDITORIAL* **(146) Philippines (29 Sep 2016 8:37 AM)** insignificant	incorporated
108	**3.4.3 Detection of *Fusarium circinatum* by conventional PCR**	*Category : SUBSTANTIVE* **(133) Philippines (29 Sep 2016 8:06 AM)** Is this a validated protocol? if yes, the validated protocol should be part of this Annex	Considered, but not incorporated A protocol can not be added as an annex, because the protocol itself is already an annex
109	A conventional PCR test with CIRC1A/CIRC4A primers, from the ribosomal (r)DNA intergenic spacer (IGS) region, designed by Schweigkofler *et al.* (2004) can be used for direct detection of the pathogen in plant tissue or seeds as well for identification of the fungus in pure culture. In any case, verification of the nature of the PCR amplicon should be carried out by sequencing. Infection by other *Fusarium* spp. is frequent and cryptic speciation was reported in the *fujikuroi* species complex (Steenkamp *et al.*, 2002). In addition, PCR cross-reaction might occur with phylogenetically close *Fusarium* spp., especially when a large amount of *Fusarium* template DNA is used.	*Category : TECHNICAL* **(59) United States of America (21 Sep 2016 8:38 PM)** The recent work of Darryl Herron at FABI has revealed several closely species that also occur on pine. Have these species been tested against this previously designed detection method? If sequencing is going to occur anyway, suggest amplifying tef1-alpha and sequencing that.	Modified The related Fusarium species recently described by Herron et al., obetained in Colombia, have not been tested. This will be the case in the framework of a COST project. Sequencing may be a systematic option, but this means that TEF1 sequencing should be done for DNA extracted from a pure culture, which is not possible for in planta detection. In addition would amplicon sequencing (IGS) provide evidence that F. circinatum DNA was amplified and not the closely related species described by Herron et al.? (Only Tef1

Para	Text	Comment	SC's response
			and tub2 sequences are available, not IGS which is the target of conventional and real-time PCR tests described here.) The reference to Herron et al and explaining text has been provided at par 60.
114	**Table 1.** CIRC1A/CIRC4A conventional PCR master mix composition, cycling parameters and amplicons	*Category : SUBSTANTIVE* **(134) Philippines (29 Sep 2016 8:08 AM)** although it is possible to compute the volume of the PCR components reflected in this table, it is better to indicate or specify the volume to make it easier for the analyst to follow the protocol.	considered, but not incorporated The suggestion is sound, but the presentation of the tables has to stick to the guidelines of the "Draft table template format for PCR reaction conditions" of the Instructions to authors.
135	~~X~~ 94 °C for ~~X~~ 3 min	*Category : TECHNICAL* **(207) Bolivia (30 Sep 2016 3:56 AM)** To clarify	incorporated
135	~~X °C~~ 94°C for ~~X~~ 3 min	*Category : TECHNICAL* **(184) Peru (29 Sep 2016 7:23 PM)**	incorporated
135	~~X °C~~ 94°C for ~~X~~ 3 min	*Category : TECHNICAL* **(170) Brazil (29 Sep 2016 3:54 PM)**	incorporated
135	~~X~~ 94 °C for ~~X~~ 3 min	*Category : TECHNICAL* **(113) Argentina (28 Sep 2016 7:36 PM)**	incorporated
135	~~X~~ 94 °C for ~~X~~ 3 min	*Category : TECHNICAL* **(100) Chile (28 Sep 2016 5:08 PM)**	incorporated
135	~~X °C~~ 94°C for ~~X~~ 3 min	*Category : TECHNICAL* **(45) Uruguay (15 Sep 2016 8:22 PM)** To clarify	incorporated
135	~~X~~ 94 °C for ~~X~~ 3 min	*Category : TECHNICAL* **(8) COSAVE (11 Aug 2016 10:22 PM)**	incorporated
138	Denaturation	*Category : TECHNICAL* **(215) Mali (30 Sep 2016 11:43 AM)** o Les niveaux de spécificité et de sensibilité de ces techniques devraient être fournies pour permettre la comparaison avec avec d'autres techniques de diagnostic (paragraphe 232).	Considered but not incoporated. The levels of specificity and sensitivity are interesting performance value, indeed. However, indicating such values is not recommanded in the instructions to authors

Para	Text	Comment	SC's response
144	Final elongation	*Category : TECHNICAL* **(196) Ghana (29 Sep 2016 11:46 PM)** We propose that number of cycles for denaturation, annealing and elongation steps should be placed appropriately.	Considered, but not incorporated The suggestion is sound, but the presentation of the tables has to stick to the guidelines of the "Draft table template format for PCR reaction conditions" of the Instructions to authors.
144	Final elongation	*Category : TECHNICAL* **(163) Kenya (29 Sep 2016 2:53 PM)** Paragraph 144 - Number of cycles for Denaturation, Annealing and Elongation steps should be placedappropriately	Considered, but not incorporated The suggestion is sound, but the presentation of the tables has to stick to the guidelines of the "Draft table template format for PCR reaction conditions" of the Instructions to authors.
152	The PCR products are separated by electrophoresis in a ~~1%~~ 1-2% agarose gel and visualized under UV light after staining.	*Category : TECHNICAL* **(208) Bolivia (30 Sep 2016 4:01 AM)** To clarify	incorporated
152	The PCR products are separated by electrophoresis in a ~~1%~~ 1-2 % agarose gel and visualized under UV light after staining.	*Category : TECHNICAL* **(186) Peru (29 Sep 2016 7:25 PM)**	incorporated
152	The PCR products are separated by electrophoresis in a 1% agarose gel and visualized under UV light after staining.	*Category : TECHNICAL* **(185) Peru (29 Sep 2016 7:24 PM)** To clarify	Modified. See modified text
152	The PCR products are separated by electrophoresis in a ~~1%~~ 1-2% agarose gel and visualized under UV light after staining.	*Category : TECHNICAL* **(171) Brazil (29 Sep 2016 3:55 PM)**	incorporated
152	The PCR products are separated by electrophoresis in a ~~1%~~ 1-2% agarose gel and visualized under UV light after staining.	*Category : TECHNICAL* **(114) Argentina (28 Sep 2016 7:37 PM)** To clarify	incorporated
152	The PCR products are separated by electrophoresis in a ~~1%~~ 1-2% agarose gel and visualized under UV light after staining.	*Category : TECHNICAL* **(101) Chile (28 Sep 2016 5:08 PM)** to clarify	incorporated
152	The PCR products are separated by electrophoresis in a ~~1%~~ 1 - 2% agarose gel and visualized under UV light after staining.	*Category : TECHNICAL* **(46) Uruguay (15 Sep 2016 8:23 PM)** To clarify	incorporated
152	The PCR products are separated by electrophoresis in a 1% agarose gel and visualized under UV light after staining.	*Category : TECHNICAL* **(10) COSAVE (11 Aug 2016 10:25 PM)** To clarify	see modified text
152	The PCR products are separated by electrophoresis in a ~~1%~~ 1-2% agarose gel and visualized under UV light after staining.	*Category : TECHNICAL* **(9) COSAVE (11 Aug 2016 10:24 PM)**	incorporated

Para	Text	Comment	SC's response
154	A sample will be considered positive if it produces a 360 base pair (bp) PCR product whose sequence ~~shows 100%~~ shows 100% identity with a *F. circinatum* reference sequence (section 4.2), provided that the negative amplification control and negative extraction control are negative.	*Category : SUBSTANTIVE* **(155) China (29 Sep 2016 11:22 AM)** Confirm whether 100% or 99% -100% is used for sequence homology.The 100% sequence homology in [154] is inconsistent with 99% -100% described in Line 7 of Paragraph [349] and Line 6 of Paragraph [351].	modified 99-100% should be used and will be corrected.
155	A sample will be considered negative if it does not produce a 360 bp PCR product, provided that the positive nucleic acid control and internal control are positive, or if it produces a 360 bp PCR product whose sequence does not show 100% identity with a *F. circinatum* reference sequence.	*Category : TECHNICAL* **(60) United States of America (21 Sep 2016 8:41 PM)** Is there no allelic variation in this marker in F. circinatum?	modified polymorphism occur within F. circinatum, so 99-100% should be used instead and will be corrected.
156	**3.4.4 Detection of *Fusarium circinatum* by SYBR Green® real-time PCR**	*Category : SUBSTANTIVE* **(135) Philippines (29 Sep 2016 8:10 AM)** Is this a validated Protocol? if yes, validated protocol should be included in this Annex	Considered, but not incorporated A protocol can not be added as an annex, because the protocol itself is already an annex
157	A SYBR Green® real-time PCR test with CIRC1A/CIRC4A primers designed by Schweigkofler *et al.* (2004) (see section 3.4.3 for their sequence) can be used for direct detection of the pathogen in plant tissue or seeds as well as for identification of the fungus in pure culture. In any case, verification of the nature of the PCR amplicon should be carried out by sequencing for the same reasons as those presented in section 3.4.3.	*Category : TECHNICAL* **(61) United States of America (21 Sep 2016 8:41 PM)** This method requires a targeted PCR detection method like this IGS-based method, but again, does it distinguish new species now known to occur on pine?	Modified The new species described by Herron et al. 2015 have not been tested by the authors of this protocols, and there are no published reference dealing with that. In addition, no IGS sequences are available on GenBank regarding this new species, which does not enable in silico testing. See additional text par. 3. detection
159	**Table 2.** CIRC1A/CIRC4A SYBR Green® real-time PCR master mix composition, cycling parameters and amlpicons	*Category : SUBSTANTIVE* **(136) Philippines (29 Sep 2016 8:10 AM)** although it is possible to compute the volume of the PCR components reflected in this table, it is better to indicate or specify the volume to make it easier for the analyst to follow the protocol.	considered, but not incorporated The suggestion is sound, but the presentation of the tables has to stick to the guidelines of the "Draft table template format for PCR reaction conditions" of the Instructions to authors.
182	~~X~~ 94 °C for ~~X~~ 3 min	*Category : TECHNICAL* **(210) Bolivia (30 Sep 2016 4:05 AM)** To clarify	incorporated
182	~~X °C~~ 94°C for ~~X~~ 3 min	*Category : TECHNICAL* **(187) Peru (29 Sep 2016 7:26 PM)** To clarify	incorporated

Para	Text	Comment	SC's response
182	~~X °C~~ 94°C for ~~X~~ 3 min	*Category : TECHNICAL* **(172) Brazil (29 Sep 2016 3:56 PM)** To clarify	incorporated
182	~~X °C~~ 94°C for ~~X~~ 3 min	*Category : TECHNICAL* **(115) Argentina (28 Sep 2016 7:41 PM)** To clarify	incorporated
182	~~X~~ 94 °C for ~~X~~ 3 min	*Category : TECHNICAL* **(102) Chile (28 Sep 2016 5:09 PM)** to clarify	incorporated
182	~~X °C~~ 94°C for ~~X~~ 3 min	*Category : TECHNICAL* **(47) Uruguay (15 Sep 2016 8:25 PM)** To clarify	incorporated
182	~~X~~ 94 °C for ~~X~~ 3 min	*Category : TECHNICAL* **(11) COSAVE (11 Aug 2016 10:26 PM)** To clarify	incorporated
197	$ May be directly included in a ready-to-use SYBR Green® master mix	*Category : EDITORIAL* **(211) Bolivia (30 Sep 2016 4:08 AM)** To be check	Problem with the symbol $ Editorial problem to check by IPPC editor.
197	~~$~~ * May be directly included in a ready-to-use SYBR Green® master mix	*Category : EDITORIAL* **(188) Peru (29 Sep 2016 7:28 PM)** to be check	Problem with the symbol $ Editorial problem to check by IPPC editor.
197	$ May be directly included in a ready-to-use SYBR Green® master mix	*Category : TECHNICAL* **(116) Argentina (28 Sep 2016 7:42 PM)** To be checked	Problem with the symbol $ Editorial problem to check by IPPC editor.
197	~~$~~ May be directly included in a ready-to-use SYBR Green® master mix	*Category : EDITORIAL* **(103) Chile (28 Sep 2016 5:10 PM)** to be check	Problem with the symbol $ Editorial problem to check by IPPC editor.
197	$ May be directly included in a ready-to-use SYBR Green® master mix	*Category : EDITORIAL* **(48) Uruguay (15 Sep 2016 8:27 PM)** The use of "$" sign should be checked	Problem with the symbol $ Editorial problem to check by IPPC editor.
197	$ May be directly included in a ready-to-use SYBR Green® master mix.	*Category : EDITORIAL* **(30) New Zealand (4 Sep 2016 11:01 PM)** Could not find a $ in the table! Added fullstop to sentence.	Problem with the symbol $ Editorial problem to check by IPPC editor.
197	~~$~~ $ May be directly included in a ready-to-use SYBR Green® master mix	*Category : EDITORIAL* **(12) COSAVE (11 Aug 2016 10:27 PM)** to be check	Problem with the symbol $ Editorial problem to check by IPPC editor.
203	**3.4.5 Detection and identification of *Fusarium circinatum* by real-time PCR using a hydrolysis probe**	*Category : SUBSTANTIVE* **(138) Philippines (29 Sep 2016 8:12 AM)** Is this a validated protocol? if yes, the validated protocol should be included in this Annex.	Considered, but not incorporated The IPPC protocol itselves is already an anex to ISPM 27

Para	Text	Comment	SC's response
204	Ioos *et al.* (2009) described a technique based on a real-time PCR using a hydrolysis probe designed from the rDNA IGS region to identify the anamorphic stage of *F. circinatum* in pure culture or directly in plant samples. This PCR test produces a 149 bp amplicon for *F. circinatum* (sequences of the IGS region for *F. circinatum* may be retrieved from GenBank, accession numbers AY249397 to AY249403). A *F. circinatum*-specific region of IGS is amplified using the primer pair FCIR-F/FCIR-R and is detected by a fluorescent hydrolysis probe, FCIR-P. This method has proven to be more sensitive than the conventional CIRC1A/CIRC4A PCR by detecting as little as 8 fg target DNA per reaction, and its specificity is strengthened ~~thanks to~~ by the combination of specific primers and hydrolysis probe and the high stringency conditions in the reaction (Ioos *et al.*, 2009).	*Category : EDITORIAL* **(139) Philippines (29 Sep 2016 8:13 AM)** grammar	modified see below
204	Ioos *et al.* (2009) described a technique based on a real-time PCR using a hydrolysis probe designed from the rDNA IGS region to identify the anamorphic stage of *F. circinatum* in pure culture or directly in plant samples. This PCR test produces a 149 bp amplicon for *F. circinatum* (sequences of the IGS region for *F. circinatum* may be retrieved from GenBank, accession numbers AY249397 to AY249403). A *F. circinatum*-specific region of IGS is amplified using the primer pair FCIR-F/FCIR-R and is detected by a fluorescent hydrolysis probe, FCIR-P. This method has proven to be more sensitive than the conventional CIRC1A/CIRC4A PCR by detecting as little as 8 fg target DNA per reaction, and its specificity is ~~strengthened thanks to the combination of specific primers and hydrolysis probe and the high stringency conditions in the reaction~~ higher (Ioos *et al.*, 2009).	*Category : TECHNICAL* **(31) New Zealand (4 Sep 2016 11:04 PM)** Delete unnecessary details.	incorporated
210	**Table 3.** FCIR-F/-R/-P real-time PCR using a hydrolysis probe master mix composition and cycling parameters	*Category : SUBSTANTIVE* **(137) Philippines (29 Sep 2016 8:11 AM)** although it is possible to compute the volume of the PCR components reflected in this table, it is better to indicate or specify the volume to make it easier for the analyst to follow the protocol.	Considered, not incorporated The suggestion is sound, but the presentation of the tables has to stick to the guidelines of the "Draft table template format for PCR reaction conditions" of the instruction to authors.
230	~~2 µL~~2µL (0,8-30 ng)	*Category : TECHNICAL* **(212) Bolivia (30 Sep 2016 4:10 AM)** To clarify	Considered, not incorporated The quantity (0.8-30 ng) was only measured with DNA extracted from seeds from a few different pine species by Ioos et al. 2009. These values should be adapted for other

Para	Text	Comment	SC's response
			substrates or even other pine species. Therefore, only volume should be indicated here.
230	~~2 µL~~2 µL (0,8-30 ng)	*Category : TECHNICAL* **(195) Peru (29 Sep 2016 7:46 PM)**	Considered, not incorporated The quantity (0.8-30 ng) was only measured with DNA extracted from seeds from a few different pine species by Ioos et al. 2009. These values should be adapted for other substrates or even other pine species. Therefore, only volume should be indicated here.
230	~~2 µL~~2 µL (0,8-30 ng)	*Category : TECHNICAL* **(189) Peru (29 Sep 2016 7:39 PM)**	Considered, not incorporated The quantity (0.8-30 ng) was only measured with DNA extracted from seeds from a few different pine species by Ioos et al. 2009. These values should be adapted for other substrates or even other pine species. Therefore, only volume should be indicated here.
230	2 ~~µL~~µL (0,8-30 ng)	*Category : TECHNICAL* **(173) Brazil (29 Sep 2016 3:58 PM)**	Considered, not incorporated The quantity (0.8-30 ng) was only measured with DNA extracted from seeds from a few different pine species by Ioos et al. 2009. These values should be adapted for other substrates or even other pine species. Therefore, only volume should be indicated here.
230	2 ~~µL~~µL (0,8-30 ng)	*Category : TECHNICAL* **(117) Argentina (28 Sep 2016 7:45 PM)**	Considered, not incorporated The quantity (0.8-30 ng) was only measured with DNA extracted from seeds from a few different pine species by Ioos et al. 2009. These values should be adapted for other substrates or even other pine species. Therefore, only volume should be indicated here.

Para	Text	Comment	SC's response
230	2 ~~µL~~µL (0.8-30 ng)	*Category : TECHNICAL* **(104) Chile (28 Sep 2016 5:11 PM)**	Considered, not incorporated The quantity (0.8-30 ng) was only measured with DNA extracted from seeds from a few different pine species by Ioos et al. 2009. These values should be adapted for other substrates or even other pine species. Therefore, only volume should be indicated here.
230	2 ~~µL~~µL (0,8-30 ng)	*Category : TECHNICAL* **(49) Uruguay (15 Sep 2016 8:29 PM)** This information should be added	Considered, not incorporated The quantity (0.8-30 ng) was only measured with DNA extracted from seeds from a few different pine species by Ioos et al. 2009. These values should be adapted for other substrates or even other pine species. Therefore, only volume should be indicated here.
230	2 ~~µL~~µL (0,8-30 ng)	*Category : TECHNICAL* **(13) COSAVE (11 Aug 2016 10:28 PM)**	Considered, not incorporated The quantity (0.8-30 ng) was only measured with DNA extracted from seeds from a few different pine species by Ioos et al. 2009. These values should be adapted for other substrates or even other pine species. Therefore, only volume should be indicated here.
233	~~X~~ 95 °C for ~~X~~ 10 min	*Category : TECHNICAL* **(213) Bolivia (30 Sep 2016 4:11 AM)** To clarify	incorporated
233	~~X °C~~ 95°C for ~~X~~ 10 min	*Category : TECHNICAL* **(194) Peru (29 Sep 2016 7:46 PM)** To clarify	incorporated
233	~~X °C~~ 95°C for ~~X~~ 10 min	*Category : TECHNICAL* **(190) Peru (29 Sep 2016 7:40 PM)** To clarify	incorporated
233	~~X °C~~ 95°C for ~~X~~ 10 min	*Category : TECHNICAL* **(174) Brazil (29 Sep 2016 3:58 PM)**	incorporated

Para	Text	Comment	SC's response
233	~~X~~ 95 °C for ~~X~~ 10 min	*Category : TECHNICAL* **(118) Argentina (28 Sep 2016 7:46 PM)** To clarify	incorporated
233	~~X~~ 95 °C for X 10 min	*Category : TECHNICAL* **(105) Chile (28 Sep 2016 5:11 PM)** to clarify	incorporated
233	~~X °C~~ 95°C for ~~X~~ 10 min	*Category : TECHNICAL* **(50) Uruguay (15 Sep 2016 8:30 PM)** To clarify	incorporated
233	~~X~~ 95 °C for ~~X~~ 10 min	*Category : TECHNICAL* **(14) COSAVE (11 Aug 2016 10:29 PM)** To clarify	incorporated
253	**Positive nucleic acid control.** This control is used to monitor the efficiency of the test method (apart from the extraction). Pre-prepared (stored) genomic DNA from a reference strain of *F. circinatum* or subcloned *F. circinatum* PCR product (CIRC1A/CIRC4A for conventional PCR and SYBR Green® real-time PCR; FCIR-F/FCIR-R for real-time PCR with a hydrolysis probe) may be used.	*Category : SUBSTANTIVE* **(140) Philippines (29 Sep 2016 8:15 AM)** where to obtain this? is there a commercially available reference material?	Considered, but not incorporated Reference strains can be purchased to international collection. Plasmidic controls can be home-made, using reference strains.
256	For conventional PCR and SYBR Green® real-time PCR, ITS1 ~~andITS4~~ and ITS4 primers targeting the internal transcribed spacers located in the fungal ribosomal DNA (White et al., 1990) may be used in place of the CIRC1A/CIRC4A primers, under the same PCR conditions except for an annealing temperature of 50 °C. The primers are:	*Category : EDITORIAL* **(141) Philippines (29 Sep 2016 8:15 AM)**	incorporated
256	For conventional PCR and SYBR Green® real-time PCR, ITS1 andITS4 primers targeting the internal transcribed spacers located in the fungal and plant ribosomal DNA (White et al., 1990) may be used in place of the CIRC1A/CIRC4A primers, under the same PCR conditions except for an annealing temperature of 50 °C. The primers are:	*Category : TECHNICAL* **(32) New Zealand (4 Sep 2016 11:09 PM)** The primer set can amplify both fungi and plant DNA.	incorporated
266	**4. Identification** The requirement for identification of *Fusarium circinatum* is outlined in the flow chart in Figure 1. However, if the outcome is critical (e.g. post-entry quarantine samples, new host record, new country record), the fungus should be isolated and confirmed by sequence analysis.	*Category : SUBSTANTIVE* **(33) New Zealand (4 Sep 2016 11:13 PM)** It is recommended to add the following sentence after section 266.	modified: additional text has been added at par. 60 text par. 266 revised: The requirement for identification of Fusarium circinatum is outlined in the flow chart in Figure 1. However, morphological characters of species in the Fusarium fujikuroi Species Complex (FFSC) might be very similar and PCR cross-reaction might occur with phylogenetically close Fusarium spp. Therefore, it is recommended

Para	Text	Comment	SC's response
			that the fungus is isolated and confirmed by sequence analysis.
268	**4.1.1 Cultural and morphological characteristics**	*Category : EDITORIAL* **(142) Philippines (29 Sep 2016 8:30 AM)** This is a procedural manual/protocol, tenses of verbs should not be in past tense.	considered, but not incorporated Method descriptions are not written as standard operation procedures (instruction to authors)
270	To study the formation and type of microconidia, macroconidia and conidiogenous cells the isolates are grown on SNA; plates are incubated at 22 °C ± 6 °C under near UV light or daylight. All isolates are examined after ten days and confirmed as *F. circinatum* based on the morphological features described by Nirenberg and O'Donnell (1998) and Britz *et al.* (2002). On SNA, microconidia are aggregated in false heads, with branched conidiophores, monophialidic and polyphialidic conidiophores, and obovoid microconidia in aerial mycelium, mostly non-septate or occasionally one-septate (Figure 6A). Macroconidia are typically three-septate, with walls that are slightly curved, an apical cell that narrows to an inwardly (i.e. toward the ventral side) curved tip, and a foot-shaped basal cell (Figure 6B). Chlamydospores are absent. The sterile hyphae (coiled or not distinctively coiled) are characteristic of *F. circinatum* and are observed clearly on this medium (Figure 7). The epithet "circinatum" refers to these typical coiled hyphae, also called "circinate" hyphae (Figure 7A). These circinate hyphae should not be confused with the commonly observed "spiral-wrapped" hyphae at the surface of the agar, which may be produced by several species of *Fusarium*, especially *F. pseudocircinatum* (Figure 8).	*Category : SUBSTANTIVE* **(156) China (29 Sep 2016 11:25 AM)** Add the description of the characteristics of the pest in the sexual stage.Sexual stage is also the important identification reference of the pest.	Considered , but not incorporated. The sexual state is not common in culture. Also changed in par. 44
270	To study the formation and type of microconidia, macroconidia and conidiogenous cells the isolates are grown on SNA; plates are incubated at 22 °C ± 6 °C under near UV light or daylight. On the other hand, some strains form sterile hyphae only under dark condition (Aoki et al.,2001). Therefore, incubation under dark condition may be needed for some strains to form sterile hyphae. All isolates are examined after ten days and confirmed as *F. circinatum* based on the morphological features described by Nirenberg and O'Donnell (1998) and Britz *et al.* (2002). On SNA, microconidia are aggregated in false heads, with branched conidiophores, monophialidic and polyphialidic conidiophores, and obovoid microconidia in aerial mycelium, mostly non-septate or occasionally one-septate (Figure 6A). Macroconidia are typically three-septate, with walls that are slightly curved, an apical cell that narrows to an inwardly (i.e. toward the ventral side) curved tip, and a foot-shaped basal cell (Figure 6B). Chlamydospores are absent. The sterile	*Category : TECHNICAL* **(83) Japan (27 Sep 2016 11:00 AM)** Aoki. et al. (2001) reported that there are some strains which sterile hyphae formed in the dark, not observed under continuous black light. To confirm formation of sterile hyphae is very important for the identification of Fusarium circinatum. Aoki. T. et al. (2001) Fusarium fractiflexum sp. nov. and two other species within the Gibberella fujikuroi species complex recently discovered in Japan that from aerial conidia in false heads. Mycoscience 42: 461-478.	Incorporated In the text, steril hyphe are given as characteristic.

Para	Text	Comment	SC's response
	hyphae (coiled or not distinctively coiled) are characteristic of *F. circinatum* and are observed clearly on this medium (Figure 7). The epithet "circinatum" refers to these typical coiled hyphae, also called "circinate" hyphae (Figure 7A). These circinate hyphae should not be confused with the commonly observed "spiral-wrapped" hyphae at the surface of the agar, which may be produced by several species of *Fusarium*, especially *F. pseudocircinatum* (Figure 8).		
270	To study the formation and type of microconidia, macroconidia and conidiogenous cells the isolates are grown on SNA; plates are incubated at 22 °C ± 6 °C under near UV light or daylight. All isolates are examined after ten days and confirmed as *F. circinatum* based on the morphological features described by Nirenberg and O'Donnell (1998) and Britz *et al.* (2002). On SNA, microconidia are aggregated in false heads, with branched conidiophores, monophialidic and polyphialidic conidiophores, and obovoid microconidia in aerial mycelium, mostly non-septate or occasionally one-septate (Figure 6A). Macroconidia are typically three-septate, with walls that are slightly curved, an apical cell that narrows to an inwardly (i.e. toward the ventral side) curved tip, and a foot-shaped basal cell (Figure 6B). Chlamydospores are absent. The ~~sterile hyphae (coiled or not distinctively coiled)~~ aforementioned characters are ~~characteristic~~ typical of several species within the FFSC, particularly *F. subglutinans*. The production of distinctive flexuous/sinuous sterile hyphae, referred to as "coiled" or "circinate" hypae, distinguishes F. circinatum and some other species in the FFSC, including some recently described species from pine, from F. subglutinans. ~~*F. circinatum* and are observed clearly on this medium (Figure 7). The epithet "circinatum" refers to these typical coiled hyphae, also called "circinate" hyphae (Figure 7A).~~ These ~~circinate~~ sinuous hyphae should not be confused with the commonly observed ~~"spiral-wrapped"~~ truly coiled hyphae (likely perithecial initials) at the surface of the agar, which may be produced by several species of *Fusarium*, ~~especially~~including *F. pseudocircinatum* (Figure 8).	*Category : TECHNICAL* **(62) United States of America (21 Sep 2016 8:46 PM)** Overall: This character and the way it's described is a seriously confusing issue. These hyphae should never have been referred to as "coiled" or "circinate"– they are not coiled at all . Look at Figure 7, which accurately documents them – those are not coils. They are flexuous or sinuous – repeatedly curved does not mean coiled. F. pseudocircinatum DOES produce flexuous hyphae that are very similar and probably developmentally related to those produced by F. circinatum, and indeed they are distinct from the true coils (likely perithecial initials, referred to as spiral wrapped hyphae)) depicted in Figure 8, that you might see in any number of Fusarium species. The true flexuous hyphae are right there in the original description of F. pseudocircinatum, and I myself have seen them. Specific to "(coiled or not distinctively coiled)": this term is a misnomer. Better descriptors would be flexuous or sinuous hyphae.	incorporated
271	The isolate observed in pure culture can reliably and confidently be assigned to the species *F. circinatum* if all the morphological features described above are observed. Table 4 presents a comparison of *F. circinatum* with other *Fusarium* species that have similar characteristics and that *F. circinatum* may therefore be confused with. In case of doubt, or if at least one characteristic cannot be clearly observed, then a DNA sequence analysis should be conducted (section 4.2).	*Category : TECHNICAL* **(63) United States of America (21 Sep 2016 8:47 PM)** This may not be true - see Herron et al. 2015	Modified, see additional text 3. Detection
276	**Presence of sterile ~~coiled~~ sinuous hyphae**	*Category : TECHNICAL* **(64) United States of America (21 Sep**	incorporated

Para	Text	Comment	SC's response
		2016 8:47 PM)	
280	Yes, more or less clearly ~~circinate~~sinuous, depending on the isolate	*Category : TECHNICAL* **(65) United States of America (21 Sep 2016 8:47 PM)**	incorporated
305	Identification of doubtful isolates in pure culture may be ascertained by analysis of the sequence of a barcode or of another relevant phylogenetic marker. In the case of *Fusarium*, several genes may be used for identification with a high level of certainty. The *EF-1alpha* sequence is sufficient to assign the identity of a *Fusarium* strain to *F. circinatum* (O'Donnell *et al.*, 1998; Geiser, 2004) but other markers may be useful (e.g. largest RNA polymerase II B-subunit (RPB1), second largest RNA polymerase II B-subunit (RPB2), beta-tubulin, IGS) (Steenkamp *et al.*, 2002; ~~EPPO, 2005;~~ O'Donnell *et al.*, 2010). The universal barcode ITS, while very useful for fungi in general, should not be used for the *Fusarium* genus as it is not sufficiently polymorphic for several closely related species, including *F. circinatum*. Moreover, species within the *fujikuroi* species complex possess non-orthologous copies of the ITS2 region, which can lead to incorrect phylogenetic inferences (O'Donnell and Cigelnik, 1997).	*Category : TECHNICAL* **(79) Japan (26 Sep 2016 5:39 PM)** Sequence analysis is not described in EPPO(2005). Gibberella circinata. EPPO Bulletin, 35: 383–386.	incorporated
305	Identification of doubtful isolates in pure culture may be ascertained by analysis of the sequence of a barcode or of another relevant phylogenetic marker. In the case of *Fusarium*, several genes may be used for identification with a high level of certainty. The *EF-1alpha* sequence is sufficient to assign the identity of a *Fusarium* strain to *F. circinatum* (O'Donnell *et al.*, 1998; Geiser, 2004) but other markers may be useful (e.g. largest RNA polymerase II B-subunit (RPB1), second largest RNA polymerase II B-subunit (RPB2), beta-tubulin, IGS) (Steenkamp *et al.*, 2002; EPPO, 2005; O'Donnell *et al.*, 2010). The universal barcode ITS, while very useful for fungi in general, should not be used for the *Fusarium* genus as it is not sufficiently polymorphic for several closely related species, including *F. circinatum*. Moreover, species within the *fujikuroi* species complex possess non-orthologous copies of the ITS2 region, which can lead to incorrect phylogenetic inferences (O'Donnell and Cigelnik, 1997). Sequence analysis should be used to verify positive results from all PCR tests.	*Category : SUBSTANTIVE* **(34) New Zealand (4 Sep 2016 11:17 PM)** Positive results from all 3 PCR tests in this protocol should be verified by sequence analysis due to cross reactions.	Modified Added: It recommended to verify positive results from all PCR tests by sequence analysis. see also par. 60
311	**Table 5.** EF1/EF2 conventional PCR master mix composition, cycling parameters and amplicons	*Category : SUBSTANTIVE* **(143) Philippines (29 Sep 2016 8:31 AM)** although it is possible to compute the volume of the PCR components reflected in this table, it is better to indicate or specify	considered, but not incorporated The suggestion is sound, but the presentation of the tables has to stick

Para	Text	Comment	SC's response
		the volume to make it easier for the analyst to follow the protocol.	to the guidelines of the "Draft table template format for PCR reaction conditions" of the Instructions to authors.
332	~~X~~ ? °C for ~~X~~ ? min	*Category : EDITORIAL* **(193) Peru (29 Sep 2016 7:46 PM)** To be check	Modified
332	~~X~~ ? °C for ~~X~~ ? min	*Category : EDITORIAL* **(192) Peru (29 Sep 2016 7:46 PM)** To be check	Modified
332	~~X~~ ? °C for ~~X~~ ? min	*Category : EDITORIAL* **(191) Peru (29 Sep 2016 7:44 PM)** To be check	Modified
332	~~X~~ ?°C for ~~X~~ ?min	*Category : EDITORIAL* **(175) Brazil (29 Sep 2016 4:01 PM)** To be check. It's necessary to define the parameters.	Modified
332	~~X~~ ? °C for ~~X~~ ? min	*Category : TECHNICAL* **(119) Argentina (28 Sep 2016 7:47 PM)** To be checked	Modified
332	~~X~~ ? °C for ~~X~~ ? min	*Category : EDITORIAL* **(106) Chile (28 Sep 2016 5:12 PM)** to be check	Modified
332	~~X~~ ?°C for ~~X~~ ? min	*Category : TECHNICAL* **(51) Uruguay (15 Sep 2016 8:32 PM)** This should be checked	Modified
332	~~X~~ ? °C for ~~X~~ ? min	*Category : EDITORIAL* **(15) COSAVE (11 Aug 2016 10:30 PM)** To be check	Modified
354	~~In cases where other contracting parties may be affected by the results of the diagnosis, records and evidence should be kept for at least one year in a manner that ensures traceability.~~ Records and evidence should be retained as described in section 2.5 of ISPM 27 (Diagnostic protocols for regulated pests).	*Category : SUBSTANTIVE* **(35) New Zealand (4 Sep 2016 11:26 PM)** Understand this is a quote from sect 2.5 of ISPM 27 but would prefer use of same sentence as in para 461 of Phytoph draft protocol.	incorporated
372	**Gerlach, W. & Nirenberg, H.I.** 1982. *The genus* Fusarium: *A pictorial atlas.* Mitteilungen aus der Biologischen Bundesanstalt fur Land- und Forstwirtschaft 209. Berlin-Dahlem, Germany, Kommissionsverlag P. Parey. 406 pp.	*Category : EDITORIAL* **(197) Ghana (29 Sep 2016 11:50 PM)** The font size is 10, it should be in conformity with the other font sizes which is 11	incorporated
379	**ISTA** (International Seed Testing Association). ~~2002~~2016. *Detection of* Fusarium moniliforme *var.* subglutinans *Wollenw. & Reinke on* Pinus taeda *and* P. elliotii *(Pine).* International rules for testing 7-009. Bassersdorf, Switzerland, ISTA.	*Category : TECHNICAL* **(162) EPPO (29 Sep 2016 11:47 AM)** Reference should be updated to latest version of the ISTA rules for testing.	incorporated

Para	Text	Comment	SC's response
379	**ISTA** (International Seed Testing Association). ~~2002~~2016. *Detection of* Fusarium moniliforme *var.* subglutinans *Wollenw. & Reinke on* Pinus taeda *and* P. elliotii *(Pine).* International rules for testing 7-009. Bassersdorf, Switzerland, ISTA.	*Category : TECHNICAL* **(90) European Union (27 Sep 2016 1:24 PM)** Reference should be updated to latest version of the ISTA rules for testing.	incorporated
379	**ISTA** (International Seed Testing Association). ~~2002~~2016. *Detection of* Fusarium moniliforme *var.* subglutinans *Wollenw. & Reinke on* Pinus taeda *and* P. elliotii *(Pine).* International rules for testing 7-009. Bassersdorf, Switzerland, ISTA.	*Category : TECHNICAL* **(75) EPPO (25 Sep 2016 1:57 PM)** Reference should be updated to latest version of the ISTA rules for testing.	incorporated
390	**Sutton, B.C., 1980.**	*Category : EDITORIAL* **(198) Ghana (29 Sep 2016 11:52 PM)** We think this is an incomplete referencing, it should be corrected with the title etc..	incorporated
390	**Sutton, B.C., 1980.**	*Category : EDITORIAL* **(164) Kenya (29 Sep 2016 2:53 PM)** Reference is incomplete	incorporated
390	**Sutton, B.C., 1980.**	*Category : EDITORIAL* **(157) China (29 Sep 2016 11:26 AM)** Complete the document information.	incorporated
390	**Sutton, B.C., 1980~~.~~.** The Coelomycetes. Fungi Imperfecti with pycnidia, acervuli and stromata. CMI, Kew. 696 pp. **Swett C.L. & Gordon T.R.** 2012. First Report of Grass Species (Poaceae) as Naturally Occurring Hosts of the Pine Pathogen *Gibberella circinata. Plant Disease* 96: 908. **Swett C.L., Porter B., Fourie G., Steenkamp E.T., Gordon T.R., & Wingfield M.J**. (2014) Association of the pitch canker pathogen *Fusarium circinatum* with grass hosts in commercial pine production areas of South Africa. *Southern Forests: a Journal of Forest Science* 76:161-166.	*Category : SUBSTANTIVE* **(36) New Zealand (4 Sep 2016 11:31 PM)** Missing details on the reference Sutton (1980) and addition of two references.	incorporated
394		*Category : SUBSTANTIVE* **(37) New Zealand (4 Sep 2016 11:40 PM)** Below diamond with "Real-time PCR positive" add box with "Confirm with one of other methods" MPI PHEL has found that the real-time PCR using the hydrolysis probe developed by Ioos et al. (2009) cross reacted with a few isolates that belongs to different species of Fusarium in a project that compared these PCR tests. Therefore, Figure 1 should be revised to include a confirmatory step when there are positive results from the real-time PCR using	Modified Note added Note 1 at 'Plant tissue or seeds': it is recommended that the fungus is isolated and confirmed by sequence analysis.

Para	Text	Comment	SC's response
		hydrolysis probe developed by Ioos et al. (2009).	
403		*Category : SUBSTANTIVE* **(144) Philippines (29 Sep 2016 8:34 AM)** please provide a clearer picture and another picture showing salmon-colored tinge in the middle and/or purple to dark violet or yellow pigment in the agar as discussed in 4.1.1	considered but not incorporated There are no other pictures available by the authors, however, is there one to include?
406	There is no scale in the figure, and the related characteristics in the figure is not clear enough.	*Category : EDITORIAL* **(158) China (29 Sep 2016 11:27 AM)** Add the scale in the figure and it is suggested to replace them by clear pictures （referrence：Viljoen A, Wingfield M J, Marasas W H O. Characterization of Fusarium subglutinans f. sp. pini on causing root disease of Pinus patula seedling in South Africa[J]. Mycol.Res, 1997, 101:437-445）.	considered but not incorporated The pictures can be changed if there are pictures available from the publication mentioned
Ange the pictu res if there are pictu res avail able for mthe publi catio n ment ioine d	**There is no scale in the figure, and the related characteristics in the figure is not clear enough.**	*Category : EDITORIAL* **(159) China (29 Sep 2016 11:28 AM)** Add the scale in the figure and it is suggested to replace them by clear pictures （referrence：Viljoen A, Wingfield M J, Marasas W H O. Characterization of Fusarium subglutinans f. sp. pini on causing root disease of Pinus patula seedling in South Africa[J]. Mycol.Res, 1997, 101:437-445）.	considered but not incorporated The pictures can be changed if there are pictures available from the publication mentioned
412	**There is no scale in the figure, and the related characteristics in the figure is not clear enough.**	*Category : EDITORIAL* **(160) China (29 Sep 2016 11:28 AM)** Add the scale in the figure and it is suggested to replace them by clear pictures （Viljoen A, Wingfield M J, Marasas W H O. Characterization of Fusarium subglutinans f. sp. pini on causing root disease of Pinus patula seedling in South Africa[J]. Mycol.Res, 1997, 101:437-445）.	considered but not incorporated The pictures can be changed if there are pictures available from the publication mentioned

Para	Text	Comment	SC's response
412		*Category : SUBSTANTIVE* **(147) Philippines (29 Sep 2016 8:38 AM)** provide figures for molecular assay and sequence analysis expected results	considered but not incorporated these figures are not requiered in the IPPC protocols