Draft Annex to ISPM 27:2006: *Guignardia citricarpa* Kiely on fruit (2004-023)

Comm. no	Para. no.	Comment type	Comment	Explanation	Country
1.	*G*	Editorial	Add structure number in [47], [51],[53],[73]. E.g. add 4.1.1 to [47].	Same format as other similar standards.	China
2.	*G*	Editorial	The standard was read and there are no comments.		Lesotho*
3.	*G*	Substantive	Delete “personal communication” in text as well as related content.e.g.[52], last sentence, (Dr C. Glienke, personal communication) ; [63], last sentence,(Dr J.P. meffert, personal communication)	As international standard, it is not scientific to use the data which is never published.	China
4.	*G*	Substantive	1. To distinguish Guignardia citricarpa from other related species in terms of symptoms, culture, morphology and molecular basis, this protocol should include descriptions of other related species, such as Phyllosticta citrichinaensis which has recently been reported, and Phyllosticta citribraziliensis which is the endophyte of citrus. 2.The figure of the vertical section of pycnidium of G. citricarpa should be added. 3.The description of Morphology in section 4.1 (paragraphs 53 to 60) should be an isolated new section or moved after paragraph 38.	1.Phyllosticta citrichinaensis and Phyllosticta citribraziliensis should be reviewed by the editorial team and included in this protocol because P. citrichinaensis is a citrus pathogen which has recently been reported, and P. citribraziliensis may be an endophyte species related to Guignardia citricarpa in the process of isolation-culturing. (References) Phyllosticta citrichinaensis: Fungal Diversity (2012) 52:209-224. Phyllosticta citribraziliensis: Persoonia (2011) 26:47-56. 2.This protocol does not have any figures of pycnidia on the host even though it has figures of colonies and conidia. 3.It is not appropriate to include the description of morphology in the section of 'isolation and culturing of G. citricarpa'. Also, it is not appropriate to include the description of pseudothecia in this section as these are not formed on fruits or in culture.	Japan
5.	*G*	Technical	The name of the causal agent of citrus black spot is *Phyllosticta citricarpa* not *Guignardia citricarpa*. Note that Species Fungorum (viewed 14 August 2012) gives *P. citricarpa* as the current name and includes *G. citricarpa* in synonyms	Although ISPM 27 states that for fungi the teleomorph name should be used, the International Botanical Congress in Melbourne in July 2011 determined that after 1 January 2013, one fungus can only have one name; the system of permitting separate names to be used for anamorphs then ends. This means that all legitimate names proposed for a species, regardless of what stage they are typified by, can serve as the correct name for that species. This was clarified in Glienke et al 2011 Persoonia 26: 47-56 (page 54). http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3160796/?tool=pubmed Phyllosticta citricarpa is the priority name and as such the protocol needs to take this into account. It is suggested that the protocol is revised to take account of the name	Australia

				change. It will require subsequent rewriting and references to G. citricarpa will need to be checked and amended as necessary.	
6.	*1*	Technical	**DRAFT ANNEX to ISPM 27:2006: ~~*Guignardia citricarpa* Kiely~~ *Phyllosticta citricarpa* (McAlpine) Aa on fruit (2004-023)**	The current name should be Phyllosticta citricarpa under the amended nomenclatural code, also noted in Glienke et al 2011 Persoonia 26: 47-56 (page 54) http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3160796/?tool=pubmed Species Fungorum (viewed 14 August 2012) gives P. citricarpa as the current name and includes G. citricarpa in synonyms	Australia
7.	*8*	Editorial	*Guignardia citricarpa* Kiely, the causal agent of"citrusblack spot" disease, is a leaf-spotting and fruit-blemishing pest affecting *Citrus, Poncirus, Fortunella* and their hybrids. Except for *Citrus aurantium* L. and its hybrids and *C. latifolia* Tan., all commercially grown *Citrus* species are susceptible (Kotzé, 2000; Aguilar-Vildoso *et al.,* 2002). *Citrus limon* L. is particularly susceptible and, thus, in an ~~unaffected~~ area where the pest is absent, the disease usually appears first on *C. limon* (Kotzé, 2000).	To be consistent with ISPM 8.	Brazil
8.	*8*	Editorial	*Guignardia citricarpa* Kiely, the causal agent of"citrusblack spot" disease, is a leaf-spotting and fruit-blemishing pest affecting *Citrus, Poncirus, Fortunella* and their hybrids. Except for *Citrus aurantium* L. and its hybrids and *Citrus~~.~~ latifolia* Tan., all commercially grown *Citrus* species are susceptible (Kotzé, 2000; Aguilar-Vildoso *et al.,* 2002). *Citrus limon* L. is particularly susceptible and, thus, in an unaffected area, the disease usually appears first on *C. limon* (Kotzé, 2000).	Write out abbreviation in full for the first time where it is used e.g. "C" for "Citrus" in order to provide clarity for those who may not be familiar with the abbreviation in question.	South Africa
9.	*8*	Technical	*Guignardia citricarpa* Kiely, the causal agent of"citrusblack spot" disease, is a leaf-spotting and fruit-blemishing pest affecting *Citrus, Poncirus, Fortunella* and their hybrids. Except for *Citrus aurantium* L. and its hybrids and *C. latifolia* Tan., all commercially grown *Citrus* species are susceptible (Kotzé, 2000; Aguilar-Vildoso *et al.,* 2002). *Citrus limon* L. is particularly susceptible and, thus, in an ~~unaffected~~ area where the pest is absent, the disease usually appears first on *C. limon* (Kotzé, 2000).	To be consistent with ISPM 8.	COSAVE, Paraguay, Chile
10.	*8*	Technical	*Guignardia citricarpa* Kiely, the causal agent of"citrusblack spot" disease, is a leaf-spotting and fruit-blemishing pest affecting *Citrus, Poncirus, Fortunella* and their hybrids. Except for *Citrus aurantium* L. and its hybrids and *C. latifolia* Tan., all commercially grown *Citrus* species are susceptible (Kotzé, 2000; Aguilar-Vildoso *et al.,* 2002). *Citrus limon* L. is particularly susceptible and, thus, in an ~~unaffected~~ area where the pest is absent, the disease usually appears first on *C. limon* (Kotzé, 2000).	To be consistent with ISPM 8.	Uruguay
11.	*8*	Technical	*Guignardia citricarpa* Kiely, the causal agent of"citrusblack spot" disease, is a leaf-spotting and fruit-blemishing ~~pest~~fungus affecting *Citrus, Poncirus, Fortunella* and their hybrids. Except for *Citrus aurantium* L. and its hybrids and *C. latifolia* Tan., all commercially grown *Citrus* species are susceptible (Kotzé, 2000; Aguilar-Vildoso *et al.,* 2002). *Citrus limon* L. is particularly susceptible and, thus, in an unaffected area, the disease usually appears first on *C. limon* (Kotzé, 2000).	more specific to say "fungus"	United States of America
12.	*8*	Technical	*Guignardia citricarpa* Kiely, the causal agent of"citrusblack spot" disease, is a leaf-spotting and fruit-blemishing pest affecting *Citrus, Poncirus, Fortunella* and their hybrids. Except for *Citrus aurantium* L. and its hybrids and *C. latifolia* Tan., all commercially grown *Citrus* species are susceptible (Kotzé, 2000; Aguilar-Vildoso *et al.,* 2002). *Citrus limon* L. is particularly susceptible and, thus, in an ~~unaffected~~ area where the pest is absent, the disease usually appears first on *C. limon* (Kotzé, 2000).	To be consistent with ISPM 8.	Argentina
13.	*9*	Editorial	Citrus black spot was first recorded in Australia in 1895 on *C~~.~~itrus sinensis* (Linnaeus) Osbeck	Write out abbreviation in full for the first time where	South Africa

			(Benson, 1895). It is now present in some citrus-producing areas in Asia, Africa, Australia and South America (CABI, 2011). In March 2010, G. citricarpa was detected for the first time in a few citrus groves in Florida (USA) (NAPPO, 2010). Surveys on the distribution of the organism in the area are ongoing (USDA-APHIS, 2010).The organism has not been reported from Europe, Central America or the Caribbean region (EPPO/CABI, 1997; CABI/EPPO, 1998; CABI, 2011; NAPPO, 2010).	it is used e.g. “C” for “Citrus” in order to provide clarity for those who may not be familiar with the abbreviation in question.	
14.	*9*	Substantive	Citrus black spot was first recorded in Australia in 1895 on *C. sinensis* (Linnaeus) Osbeck (Benson, 1895). It is now present in some citrus-producing areas in Asia, Africa, Australia and South America (CABI, 2011). In March 2010, G. citricarpa was detected for the first time in a few citrus groves in Florida (USA) (NAPPO, 2010, Schubert et al, 2012). ~~Surveys on the distribution of the organism in the area are ongoing (USDA-APHIS, 2010).~~The organism has not been reported from Europe, Central America or the Caribbean region (EPPO/CABI, 1997; CABI/EPPO, 1998; CABI, 2011; NAPPO, 2010).	Insertion of new author “Schubert et al, 2012” to substantiate paragraph 9 Deletion of the sentence “Surveys on the distribution of the organism in the area are ongoing (USDA-APHIS, 2010)” since the sentence appears to be no longer relevant due to the new findings by “Schubert et al, 2012”.	South Africa
15.	*10*	Substantive	*G. citricarpa* has ~~significant~~ economic impact mainly because of the external blemishes it causes, which make citrus fruit ~~un~~less suitable for the fresh market. Severe infections may cause premature fruit drop in unmanaged groves, but little if any fruit drop occurs in commercial groves with routine control (Kotzé, 2000). Some losses due to fruit drop occur in years favourable for pest development and when fruit is held on the trees past peak maturity (CABI, 2011). In addition, latently infected (asymptomatic) fruit at harvest may still develop symptoms during transport or storage (Kotzé, 1996).	Delete "significant" as this is a subjective judgment and should be left out of a diagnostic protocol. Replace "unsuitable" with "less suitable". Black spot infected fruit is sold worldwide in local markets. It is not considered to be high enough quality for export, and thus the most significant economic impact is from this quality discrimination and from quarantines. Add "in managed groves...." because this is important information with regard to the potential impact of the fungus.	United States of America
16.	*11*	Substantive	The epidemiology of citrus black spot is influenced by the availability of inoculum, the occurrence of environmental conditions favourable for infection (warm, wet and humid conditions), the growth cycle of the citrus tree and the age of the fruit in relation to its susceptibility to infection (Kotzé, 1981, 2000). In areas where rain is confined to a single season, pseudothecia with ascospores, produced exclusively on leaf litter, are the main source of inoculum. Where rain is not confined to a single season, where out-of-season fruit with lesions remain on the trees after flowering and fruit set, or where successive and irregular flowering occurs in the cultivated citrus species and varieties, pycnidia with conidia of the anamorph *Phyllosticta citricarpa* (McAlpine) Aa are also important as inoculum sources (Kotzé, 1981; Spósito *et al*., 2008, Spósito *et al*., 2011).	Insertion of “Spósito et al., 2011” since these authors also researched on the context in paragraph 11.	South Africa
17.	*11*	Technical	The epidemiology of citrus black spot is influenced by the availability of inoculum, the occurrence of environmental conditions favourable for infection (warm, wet and humid conditions), the growth cycle of the citrus tree and the age of the fruit and leaves in relation to its susceptibility to infection (Kotzé, 1981, 2000). In areas where rain is confined to a single season, pseudothecia with ascospores, produced exclusively on leaf litter, are the main source of inoculum. Where rain is not confined to a single season, where out-of-season fruit with lesions remain on the trees after flowering and fruit set, or where successive and irregular flowering occurs in the cultivated citrus species and varieties, pycnidia with conidia of the anamorph *Phyllosticta citricarpa* (McAlpine) Aa are also important as inoculum sources (Kotzé, 1981; Spósito *et al*., 2008).	More accurate to include leaves in addition to fruit	United States of America
18.	*13*	Editorial	After infection, the fungus remains in a quiescent state until the fruit becomes fully grown or mature, with symptoms being produced many months after infection has taken place (Kotzé, 2000). Leaves remain susceptible from development up to 10 months of age (Truter *et al.,* 2007). *G. citricarpa* has two asexual states: a macroconidial state in the genus *Phyllosticta* and a microconidial in the genus *Leptodothiorella* (Kiely, 1949a). Pycnidia with conidia are produced on	A new paragraph should begin after the two first sentences which fit for both sexual reproduction (pseudothecia with ascospores) and asexual reproduction (pycnidia with conidia). The other sentences of [13] only fit for asexual reproduction.	EPPO, Georgia, Russian Federation, Netherlands, European Union

			fruit, leaves, dead twigs, fruit pedicels and in abundance on leaf litter (Kotzé, 2000). They may be splash-dispersed onto the canopy or washed off from infected late-hanging fruit onto younger fruit and leaves that are still at the susceptible stage (Agostini *et al*., 2006; Spósito *et al*., 2008). The microconidial state, *Leptodothiorella* sp., also referred to as "spermogonial" state (Kiely, 1949a), usually appears on fallen leaves before pseudothecia develop. However, the role of microconidia in the biology of *G. citricarpa* is still unclear.		
19.	*13*	Technical	After infection, the fungus remains in a quiescent state until the fruit becomes fully grown or mature, with symptoms being produced many months after infection has taken place (Kotzé, 2000). Leaves remain susceptible to infection from development up to 10 months of age (Truter *et al.,* 2007). *G. citricarpa* has two asexual states: a macroconidial state in the genus *Phyllosticta* and a microconidial in the genus *Leptodothiorella* (Kiely, 1949a). Pycnidia with conidia are produced on fruit, leaves, dead twigs, fruit pedicels and in abundance on leaf litter (Kotzé, 2000). They may be splash-dispersed onto the canopy or washed off from infected late-hanging fruit onto younger fruit and leaves that are still at the susceptible stage (Agostini *et al*., 2006; Spósito *et al*., 2008). The microconidial state, *Leptodothiorella* sp., also referred to as "spermogonial" state (Kiely, 1949a), usually appears on fallen leaves before pseudothecia develop and rarely on fruit. However, the role of microconidia in the biology of *G. citricarpa* is still unclear.	Technical corrections to the text. More correct to say "leaves remain susceptibel to infection...". Also it is important to note that certain life stages rarely occur on fruit.	United States of America
20.	*15*	Editorial	It should be noted that in symptomless citrus fruit or fruit with minute spots (<2 mm in diameter) without pycnidia, the non-pathogenic endophyte *Guignardia mangiferae* A.J. Roy (anamorph *Phyllosticta capitalensis* Henn.), recorded in many plant families, may be present. The cultural, morphological and molecular characters that differentiate *G. mangiferae* from *G. citricarpa*~~, the species pathogenic to citrus,~~ have been described by Baayen *et al.* (2002). Furthermore, symptoms of *G. citricarpa* may be confused with those caused by *Phyllosticta citriasiana* Wulandari, Crous & Gruyter*,* a newly described pathogen that has so far been found only on *Citrus maxima.* The pathogenicity of *P. citriasiana* to other *Citrus* species is unknown. The cultural, morphological and molecular characters that differentiate *P. citriasiana* from *G. citricarpa,* the species pathogenic to citrus, have been described by Wulandari *et al.* (2009).	unnecessary, already stated	Australia
21.	*15*	Substantive	It should be noted that in symptomless citrus fruit or fruit with minute spots (<2 mm in diameter) without pycnidia, wide spread of the non-pathogenic endophyte ~~*Guignardia mangiferae* A.J. Roy (anamorph *Phyllosticta capitalensis* Henn.)~~, recorded in many plant families, may be present. Recently, another non-pathogenic endophyte, Phyllosticta citribraziliensis has been described from citrus in Brazil (Glienke et al., 2011).The cultural, morphological and molecular characters that differentiate *P. capitalensis* and *P. citribraziliensis* ~~*G. mangiferae*~~ from *G. citricarpa,* the species pathogenic to citrus, have been described by Baayen *et al.* (2002) and Glienke *et al*., (2011). Furthermore, symptoms of *G. citricarpa* may be confused with those caused by *Phyllosticta citriasiana* Wulandari, Crous & Gruyter*,* a newly described pathogen that has so far been found only on *Citrus maxima Merr (Wulandari et al., 2009; Wang et al., 2012).* ~~.~~The pathogenicity of *P. citriasiana* to other *Citrus* species is unknown. Recently, another new *Phyllosticta* species, *Phyllosticta citrichinaensis* X.H. Wang, K.D. Hyde & H.Y. Li causing minor leaf and fruit spots on *C. limon, C. maxima*, *Citrus reticulata* Blanco and *C, sinensis*, have been described from China (Wang *et al*., 2012).The cultural, morphological and molecular characters that differentiate *P. citriasiana and P. citrichinaensis* from *G. citricarpa,* ~~the species pathogenic to citrus,~~ have been described by Wulandari *et al.* (2009) and Wang *et al.,* (2012)..	Insertion of "wide spread of the" since the insertion makes the sentence more scientifically and grammatically correct. Deletion of "Guignardia manginiferae "l A.J Roy anamorph Phyllosticta. Capitalensis Henn" since Glienke et al. 2011 demonstrated that Guignardia mangiferae and Phyllosticta capitalensis are not the same species. G. mangiferae was only recorded from mangoes, whereas P. capitalensis is an endophyte of numerous hosts. All references to G. mangiferae as the endophyte should be replaced with P. capitalensis. Insertion of the sentence "Recently, another non-pathogenic endophyte, Phyllosticta citribraziliensis, has been described from citrus in Brazil (Glienke et al., 2011)" since this is a relevant, recent scientific finding. Insertion of the sentence "Recently, another new Phyllosticta species, Phyllosticta citrichinaensis X.H. Wang, K.D. Hyde & H.Y. Li, causing minor leaf and fruit spots on C. limon, C. maxima, Citrus reticulata Blanco and C,	South Africa

				sinensis, has been described from China (Wang et al., 2012).” Insertion of “Glienke et al. (2011) and Merr Wulandari et al., 2009; Wang et al., 2012)” as new references. Deletion of the sentence “the species pathogenic to citrus” since all three species mentioned in this sentence are pathogenic to citrus. Insertion of Wang et al. (2012) since the authors also researched on the context in question.	
22.	*15*	Technical	It should be noted that in symptomless citrus fruit or fruit with minute spots (<2 mm in diameter) without pycnidia, the non-pathogenic endophyte ~~*Guignardia mangiferae* A.J. Roy (anamorph *Phyllosticta capitalensis* Henn.)~~, recorded in many plant families, may be present. The cultural, morphological and molecular characters that differentiate *G. mangiferae* from *G. citricarpa,* the species pathogenic to citrus, have been described by Baayen *et al.* (2002). Furthermore, symptoms of *G. citricarpa* may be confused with those caused by *Phyllosticta citriasiana* Wulandari, Crous & Gruyter, a newly described pathogen that has so far been found only on *Citrus maxima.* The pathogenicity of *P. citriasiana* to other *Citrus* species is unknown. The cultural, morphological and molecular characters that differentiate *P. citriasiana* from *G. citricarpa,* the species pathogenic to citrus, have been described by Wulandari *et al.* (2009).	It is incorrectly noted that the anamorph of G. mangiferae is P. capitalensis. The non-pathogenic endophyte from symptomless citrus fruit (or fruit with minor spots) is actually P. capitalensis. This was clarified in Glienke et al 2011 Persoonia 26: 47-56 (page 54). http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3160796/?tool=pubmed Note that the text shown to amend is not the text of the draft standard - there are words missing "	Australia
23.	*17*	Editorial	Name: *Guignardia citricarpa* Kiely (teleomorph) Anamorph: *Phyllosticta citricarpa* (McAlpine) Aa (macroconidial state) Synonyms: *Phyllostictina citricarpa* (McAlpine) Petr. *Phoma citricarpa* var. *mikan* Hara *Phoma citricarpa* McAlpine Synanamorph: *Leptodothiorella* sp. (microconidial state) Taxonomic position: Eukaryota, Fungi, Ascomycota, Pezizomycotina, Dothideomycetes, Botryosphaeriales, Botryosphaeriaceae Common names: *English*: citrus black spot *French*: taches noires des agrumes; maladie des taches noires des agrumes *Spanish*: mancha negra de los ~~Citrus~~ Citricos; mancha negra de las frutas de cítricos; manchas negras de los agrios	Correct name in spanish	Mexico

			Portuguese: pinta preta dos citros Reference: MycoBank 286752		
24.	*17*	Substantive	Name: *Guignardia citricarpa* Kiely (teleomorph) Anamorph: *Phyllosticta citricarpa* (McAlpine) Aa (macroconidial state) Synonyms: *Phyllostictina citricarpa* (McAlpine) Petr. *Phoma citricarpa* var. *mikan* Hara *Phoma citricarpa* McAlpine Synanamorph: *Leptodothiorella* sp. (microconidial state) Taxonomic position: Eukaryota, Fungi, Ascomycota, Pezizomycotina, Dothideomycetes, Botryosphaeriales, Botryosphaeriaceae Common names: *English*: citrus black spot ~~*French*: taches noires des agrumes; maladie des taches noires des agrumes *Spanish*: mancha negra de los Citrus; mancha negra de las frutas de cítricos; manchas negras de los agrios *Portuguese*: pinta preta dos citros~~ Reference: MycoBank 286752 Delete the common names in French, Spanish, Portuguese. Only remain the English common name.	General format.	China
25.	*17*	Substantive	Name: ~~*Guignardia citricarpa* Kiely (teleomorph)~~Phyllosticta citricarpa (McAlpine) Aa 1973 ~~Anamorph:~~ ~~*Phyllosticta citricarpa* (McAlpine) Aa (macroconidial state)~~ Synonyms: *Phyllostictina citricarpa* (McAlpine) Petr. 1953 *Guignardia citricarpa Kiely 1948 (teleomorph)*	the current name should be Phyllosticta citricarpa, under the amended nomenclatural code (also noted in Glienke et al 2011) as given in Species Fungorum As there seems to be issues with the editorial comment for this para, note that adding the years to the author references and including G. citricarpa, as required in ISPM 27, s2.2 first dash point "name,(current scientific name, author and YEAR.	Australia

			Phoma citricarpa var. *mikan* Hara 1925 *Phoma citricarpa* McAlpine 1899 Synanamorph: *Leptodothiorella* sp. (microconidial state) Taxonomic position: Eukaryota, Fungi, Ascomycota, Pezizomycotina, Dothideomycetes, Botryosphaeriales, Botryosphaeriaceae Common names: *English*: citrus black spot *French*: taches noires des agrumes; maladie des taches noires des agrumes *Spanish*: mancha negra de los Citrus; mancha negra de las frutas de cítricos; manchas negras de los agrios *Portuguese*: pinta preta dos citros Reference: MycoBank 286752		
26.	*17*	Substantive	Name: *Guignardia citricarpa* Kiely (teleomorph) Anamorph: *Phyllosticta citricarpa* (McAlpine) Aa (macroconidial state) Synonyms: *Phyllostictina citricarpa* (McAlpine) Petr. ~~*Phoma citricarpa* var. *mikan* Hara~~ *Phoma citricarpa* McAlpine Synanamorph: *Leptodothiorella* sp. (microconidial state) Taxonomic position: Eukaryota, Fungi, Ascomycota, Pezizomycotina, Dothideomycetes, Botryosphaeriales, Botryosphaeriaceae Common names: *English*: citrus black spot *French*: taches noires des agrumes; maladie des taches noires des	Phoma citricarpa var. mikan was described in 1925, but since then its name has been changed to P. erratica var. mikan as a different species of P. citricarpa that has been distributed overseas (Hara (1960, 1961)). Therefore, it is not appropriate to describe this species as a synonym of Phoma citricarpa and Guignardia citricarpa.	Japan

			agrumes *Spanish*: mancha negra de los Citrus; mancha negra de las frutas de cítricos; manchas negras de los agrios *Portuguese*: pinta preta dos citros Reference: MycoBank 286752		
27.	*17*	Technical	Name: *Guignardia citricarpa* Kiely (teleomorph) Anamorph: *Phyllosticta citricarpa* (McAlpine) Aa (macroconidial state) Synonyms: *Phyllostictina citricarpa* (McAlpine) Petr. *Phoma citricarpa* var. *mikan* Hara *Phoma citricarpa* McAlpine Synanamorph: *Leptodothiorella* sp. (microconidial state) Taxonomic position: Eukaryota, Fungi, Ascomycota, Pezizomycotina, Dothideomycetes, Botryosphaeriales, Botryosphaeriaceae Common names: *English*: citrus black spot *French*: taches noires des agrumes; maladie des taches noires des agrumes *Spanish*: mancha negra de los Citrus; mancha negra de las frutas de cítricos; manchas negras de los agrios *Portuguese*: pinta preta dos citros Reference: MycoBank ~~286752~~MB320327	updated information	Australia
28.	*17*	Translation	Name: *Guignardia citricarpa* Kiely (teleomorph) Anamorph: *Phyllosticta citricarpa* (McAlpine) Aa (macroconidial state)	Correct name in Spanish	OIRSA

			Synonyms: *Phyllostictina citricarpa* (McAlpine) Petr. *Phoma citricarpa* var. *mikan* Hara *Phoma citricarpa* McAlpine Synanamorph: *Leptodothiorella* sp. (microconidial state) Taxonomic position: Eukaryota, Fungi, Ascomycota, Pezizomycotina, Dothideomycetes, Botryosphaeriales, Botryosphaeriaceae Common names: *English*: citrus black spot *French*: taches noires des agrumes; maladie des taches noires des agrumes *Spanish*: mancha negra de los cítricos ~~Citrus~~; mancha negra de las frutas de cítricos; manchas negras de los agrios *Portuguese*: pinta preta dos citros Reference: MycoBank 286752		
29.	*22*	Editorial	*Hard spot*: the most typical symptom of citrus black spot, consisting of shallow lesions, 3–10 mm in diameter, with a grey to tan centre and a dark brown to black margin (Figure 1A). At advanced stages of symptom development, the centre of the lesions becomes crater-like. Individual hard spot lesions may either remain small or coalesce to form larger lesions. A yellow halo, when the fruit is green, or a green halo, when the fruit is yellow or orange, may appear around these lesions. Quite often, pycnidia of the anamorph *P. citricarpa* are produced in the centre of these spots (Figure 1a) and can be detected by using a hand lens or a dissecting microscope. Hard spot usually appears when fruit starts maturing, even before colour change and on the side of the fruit most exposed to sunlight (Kotzé, 1981, 2000). ~~Hard spot symptoms with pycnidia would be the most easily identified as c~~Citrus black spot can be easily identified by hard spot symptoms with pycnidia.	clearer English	Australia
30.	*28*	Editorial	*Cracked spot*: superficial, slightly raised, variable in size, dark brown to black lesions with a cracked surface and irregular margins (Goes *et al*., 2000) (Figure 2E). The lesions are devoid of pycnidia and appear on fruit older than six months. This type of symptom has been associated with co-infestation by *Phyllocoptruta oleivora* Ashmead (FUNDECITRUS, 2005).	This sentence is not very clear. We therefore propose that the word "infestation" is replaced by the word "co-infestation" or the paragraph is clarified in some other way.	EPPO, Georgia, Russian Federation, Netherlands, European Union
31.	*30*	Substantive	In some countries in areas with high inoculum pressure, symptoms may also appear on small fruit, calyxes and peduncles. The symptoms on calyxes are red to dark brown lesions similar to freckle spots. On small fruit and peduncles, symptoms appear as small black spots (Aguilar-Vildoso *et al.,* 2002).This has only been reported in Brazil.	Insertion of “some countries in” since lesions on small fruit, calyxes and peduncles have not been observed in South Africa, even under high inoculum pressure and where no chemical control was implemented for CBS Insertion of “this has only been reported in Brazil” since the research	South Africa

				was conducted in Brazil and has not been reported anywhere in other countries.	
32.	*32*	Editorial	Citrus black spot usually occurs on leaves as quiescent infections without any visible symptoms (Sutton and Waterston, 1966). If symptoms appear, they start as pinpoint spots visible on both leaf surfaces. The spots, which may increase in size up to 3 mm in diameter, are circular with their centres becoming gray or light brown in colour surrounded by a dark brown to black ~~rim~~ margin and a yellow halo (Kotzé, 2000) (Figure 3A). Pycnidia may occasionally be present in the centre of the lesions on the adaxial leaf surface.	consistency with terminology used in para 22	Australia
33.	*35*	Substantive	Symptoms on fruit are variable in appearance and often resemble those caused by other citruspathogens (such as *P. citriasiana,* *P. citrichinaensis* *Diaporthe citri*, *Mycosphaerella citri* *F.A. Wolf*, *Alternaria alternata* pv. *citri* *Whiteside*, *Septoria* spp., *Colletotrichum* spp.), insect or mechanical damage (Snowdon, 1990; Bonants *et al*., 2003, Wulandari *et al*., 2009) or cold damage, particularly in the case of freckle spot (Dr L. Diaz, personal communication).	Insertion of ”P. citrichinaensis, F.A. Wolf as an author for Diaporthe citri, Whiteside as an author for Mycosphaerella citri and (Fr.) Keissl as an author for Alternaria alternata”. Insertion of “Wulandari et al., 2009)” as a new reference.	South Africa
34.	*38*	Editorial	This protocol describes the detection and identification of *G. citricarpa* on citrus fruit. Citrus fruit should be inspected for the detection of any symptoms typical of citrus black spot (see section 3). If suspected symptoms are present in the form of spots or lesions, they are examined with a magnifying lens or a dissecting microscope for the presence of pycnidia. However, as the pycnidia and spores of *P. citricarpa* (anamorph of *G. citricarpa*) are very similar to those of *P. citriasiana*~~, the recently described pathogen~~ on *C. maxima* (Wulandari *et al.,* 2009), the identityof *G. citricarpa* has to be confirmed by applying the diagnostic methods described below (Figure 4). The diagnostic Method A (isolation and culturing) is used for the identification of *G. citricarpa* on citrus fruit, but can also be used on leaves, twigs and pedicels, whereas Method B (molecular assays) is applied on citrus fruit only.	repetitious of para 15	Australia
35.	*38*	Substantive	[**This protocol describes the detection and identification of *G. citricarpa* on citrus fruit.**] Citrus fruit should be inspected for the detection of any symptoms typical of citrus black spot (see section 3). If suspected symptoms are present in the form of spots or lesions, they are examined with a magnifying lens or a dissecting microscope for the presence of pycnidia. However, as the pycnidia and spores of *P. citricarpa* (anamorph of *G. citricarpa*) are very similar to those of *P. citriasiana,* the recently described pathogen on *C. maxima* (Wulandari *et al.,* 2009), the identityof *G. citricarpa* has to be confirmed by applying the diagnostic methods described below (Figure 4). The diagnostic Method A (isolation and culturing) is used for the identification of *G. citricarpa* on citrus fruit, but can also be used on leaves, twigs and pedicels, whereas Method B (molecular assays) is applied on citrus fruit only.	The diagnostic protocol describes identification of the pathogen from fruit, which is fine if it is being diagnosed in a consignment or confirmed in the field. It is problematic however for detection (surveillance) purposes because the appearance of lesions on fruit occurs years after the pathogen has been introduced. By the time fruit is showing symptoms, the disease has already spread via the ascospores to nearby trees and areas where symptoms are not yet visible. In other words, the actual area of infestation is invisible. The annex does not make this critical point. Suggest that additional language be added to clarify this point better.	United States of America
36.	*39*	Editorial	If after applying Method A the cultural characteristics of the colonies grown on cherry decoction agar (CHA) and oatmeal agar (OA) media are not consistent with those of *G. citricarpa* (see section 4.2, requirements (i), (ii) and (iii)~~)~~, then the plant material is considered free of *G. citricarpa.* On *G. citricarpa*-like cultures that do not produce mature pycnidia within 14 days, application of real-time polymerase chain reaction (real-time PCR) (see section 4.3.2) or internal transcribed spacer (ITS) sequencing (see section 4.3.3) is recommended. However, isolation and culturing of the organism on appropriate media followed by a direct molecular test of the cultures is a time-consuming procedure and thus undesirable in time-critical diagnosis of consignments.		Saint Kitts And Nevis
37.	*41*	Substantive	The real-time PCR method, developed by Gent-Pelzer *et al.* (2007) (see section 4.3.2), can be used for a positive diagnosis of *G. citricarpa,* as it will give a positive signal only when *G. citricarpa* is	Insertion of ‘or P. citrichinaensis” to add another species Deletion of “two species” and replacement	South Africa

			present, and not with any of the related species. The conventional PCR method (as described in section 4.3.1) will give amplification when either *G. citricarpa* or *P. citriasiana* *or P. citrichinaensis* is present. In this case, after a positive signal, isolation and culturing (see section 4.1) or a real-time PCR (see section 4.3.2) or ITS sequencing (see section 4.3.3) should be performed to discriminate between the ~~two~~ three species.	with "three species" as a result of addition of new species in the paragraph.	
38.	*45*	Editorial	Fruit lesions are excised with a cork borer or scalpel, dipped in 70% ethanol for 30 s, surface disinfested with 1% sodium hypochlorite (NaOCl) for 2 min, rinsed twice in sterilized distilled water, and blotted dry (Peres *et al*., 2007). For increasing the isolation frequency, lesions must be excised carefully with any asymptomatic tissue being removed prior to plating (Dr N.A. Peres, personal communication). Subsequently, the lesions are placed aseptically on Petri dishes (9 cm in diameter) with CHA or potato dextrose agar (PDA) (see below, "Culture media") or PDA amended with 50 µg ml^{-1} penicillin and 50 µg ml^{-1} streptomycin (OEPP/EPPO, 2003). If PDA is used and slow-growing, dark *G. citricarpa*-like culturesdevelop on it, they are subsequently transferred both to cherry decoction agar (CHA) dishes for testing the growth rate of the colonies and to oatmeal agar (OA) (see below, "Culture media") dishes for evaluating the yellow pigment production. At the same time, the cultures grown on PDA medium are placed under near-ultraviolet (NUV) light at 22 °C to induce pycnidium formation. Cultures that (i) grow slowly on CHA medium (see below, "Cultural characteristics") and (ii) produce the characteristic pycnidia and conidia of the anamorph *P. citricarpa* (see below, "Cultural characteristics") are identified as belonging to *G. citricarpa.*	first use of abbreviation should be spelt out	Australia
39.	*45*	Technical	Fruit lesions are excised with a cork borer or scalpel, dipped in 70% ethanol for 30 s, surface disinfested with 1% sodium hypochlorite (NaOCl) for 2 min, rinsed twice in sterilized distilled water, and blotted dry (Peres *et al*., 2007). For increasing the isolation frequency, lesions must be excised carefully with any asymptomatic tissue being removed prior to plating (Dr N.A. Peres, personal communication). Subsequently, the lesions are placed aseptically on Petri dishes (9 cm in diameter) with CHA or potato dextrose agar (PDA) (see below, "Culture media") or PDA amended with 50 µg ml^{-1} penicillin and 50 µg ml^{-1} streptomycin (OEPP/EPPO, 2003). If PDA is used and slow-growing, dark *G. citricarpa*-like culturesdevelop on it, they are subsequently transferred both to CHA dishes for testing the growth rate of the colonies and to OA (see below, "Culture media") dishes for evaluating the yellow pigment production. At the same time, the cultures grown on PDA medium ~~are~~should be placed under near-ultraviolet (NUV) light at 22 °C to ~~induce~~make easier the induction of pycnidium formation. Cultures that (i) grow slowly on CHA medium (see below, "Cultural characteristics") and (ii) produce the characteristic pycnidia and conidia of the anamorph *P. citricarpa* (see below, "Cultural characteristics") are identified as belonging to *G. citricarpa.*	"are" should be read as "should be" since based on our experience, UV light is not mandatory to induce pycnidium formation. The proposed solution is to reword this whole sentence if the new wording appears to be technically correct.	EPPO, Georgia, Russian Federation, Netherlands, European Union
40.	*48*	Editorial	*Cherry decoction agar (CHA).*Cherry juice is made by boiling 1 kg of cherries, free of stones and petioles, in 1 litre of tap water for approximately 2 h. The extract is filtered using what?, poured into bottles, sterilized for 30 min at 110 °C and stored until use. In a bottle containing 0.8 litres of distilled water, 20 g of technical agar no. 3 is this brand specific? is added and sterilized for 15 min at 121 °C. when is pH checked? Immediately after sterilization, 0.2 litres of the sterilized cherry extract (pH 4.5) is added, mixed well and sterilized for 5 min at 102 °C (Gams *et al*., 1998).	information missing and needs adding for completeness	Australia
41.	*49*	Substantive	*Oatmeal agar is commercially available. Alternatively it can be prepared by Oatmeal agar (OA).*30 g of oatmeal flakes is placed into cheesecloth and suspended in a pan containing tap water. After simmering for approximately 2 h, the flakes are squeezed, filtered through cheesecloth and the extract is sterilized for 15 min at 121 °C. In a bottle containing 1 litre of the oatmeal extract, 20 g of technical agar no. 3 is added and sterilized for 15 min at 121 °C (Gams *et al*., 1998).	Useful to point out that this media can be sourced commercially.	New Zealand
42.	*52*	Editorial	*G. citricarpa* colonies grow slowly on CHA medium with an average diameter 25–30 mm after 7 days at 22 °C in darkness (Baayen *et al*., 2002). On PDA, the colonies have irregular margins lined by a much wider translucent zone of colourless submerged mycelium (Figure 5A). The centre of the	order of sentences changed to keep all OA information together. Next sentence amended to read more clearly	Australia

			colony is dark with grey to glaucous aerial mycelium, often with numerous small tufts. The reverse of the colony is very dark in the centre surrounded by areas of grey sepia and buff (Baayen *et al*., 2002). Stromata start to develop after 7–8 days, whereas mature pycnidia with conidia are generally produced within 10–14 days (Figure 5B). On OA a distinct yellow pigment is often produced that diffuses into the medium around the colony (Figure 6D, top row) although not all isolates produce a yellow pigment (Dr C Glienke, personal communication). This yellow pigment is weakly produced ~~weakly~~ on CHA and PDA~~the other culture media mentioned above. It is also worth mentioning that not all the *G. citricarpa* isolates produce a yellow pigment on OA medium (Dr C. Glienke, personal communication).~~		
43.	*52*	Substantive	*G. citricarpa* colonies grow slowly on CHA medium with an average diameter 25–30 mm after 7 days at 22 °C in darkness (Baayen *et al*., 2002). On PDA, the colonies have irregular margins lined by a much wider translucent zone of colourless submerged mycelium (Figure 5A). The centre of the colony is dark with grey to glaucous aerial mycelium, often with numerous small tufts. The reverse of the colony is very dark in the centre surrounded by areas of grey sepia and buff (Baayen *et al*., 2002). On OA, the colonies are flat, spreading, olivaceous-grey, becoming pale olivaceous-grey towards the margin, with sparse to moderate aerial mycelium (Glienke *et al*., 2011). Stromata start to develop after 7–8 days, whereas mature pycnidia with conidia are generally produced within 10–14 days (Figure 5B). On OA a distinct yellow pigment is often produced that diffuses into the medium around the colony (Figure 6D, top row). This pigment is produced weakly on the other culture media mentioned above. It is also worth mentioning that not all the *G. citricarpa* isolates produce a yellow pigment on OA medium (Dr C. Glienke, personal communication).	Insertion of a new sentence, namely "On OA, the colonies are flat, spreading, olivaceous-grey, becoming pale olivaceous-grey towards the margin, with sparse to moderate aerial mycelium (Glienke et al., 2011)" to support the context. Suggestion that Dr Glienke should clarify if the statement provided during personal communication is still relevant after the description of the new Phyllosticta spp.	South Africa
44.	*54*	Editorial	Published data on morphology of *G. citricarpa* varies considerably, partly because of the confusion about the identity of pathogenic and "non-pathogenic" strains (Baayen *et al*., 2003 is this 2002?). The following morphological and morphometric characteristics refer to fructifications and spores of *G. citricarpa* produced mainly in cultures; they are based on Sutton and Waterston (1966) and van der Aa (1973) data, as revised and amended by Baayen *et al.* (2002).	Baayen et al 2002 reference given but not Baayen et al 2003. Is this a mistake or is the 2003 reference different, if the latter needs adding to the reference list	Australia
45.	*54*	Substantive	Published data on morphology of *G. citricarpa* varies considerably, partly because of the confusion about the identity of the different *Phyllosticta* species associated with citrus strains (Baayen et al., 2003; Wulandari *et al*., 2009; Glienke *et al*., 2011; Wang *et al*., 2012,~~pathogenic and "non-pathogenic" strains (~~Baayen *et al*., 2003). The following morphological and morphometric characteristics refer to fructifications and spores of *G. citricarpa* produced mainly in cultures; they are based on Sutton and Waterston (1966) and van der Aa (1973) data, as revised and amended by Baayen *et al.* (2002)and Glienke *et al*. (2011).	Insertion of "the different Phyllosticta species associated with citrus (Baayen et al., 2003; Wulandari et al., 2009; Glienke et al., 2011; Wang et al., 2012)" to support the scientific published data. Insertion of the Authors and "Glienke et al. (2011)" to add new references.	South Africa
46.	*58*	Substantive	*Pycnidia*: produced *in vivo* on fruit, attached leaves, dead twigs, ~~and~~ leaf litter and in culture~~r~~. They are solitary or occasionally aggregated, globose, immersed, mid-to-dark brown, 70–330 µm in diameter. The pycnidial wall is up to four cells thick, sclerotioid on the outside, pseudoparenchymatous within, ostiole darker, slightly papillate, circular and 10–15 µm in diameter.	Deletion of "and" before the word leaf litter and addition of "and in culture" to indicate other sources where Pycnidia can be produced.	South Africa
47.	*60*	Technical	*Spermatial state*: in the form-genus *Leptodothiorella*, formed both on host and in pure culture; spermatia dumbbell-shaped, rarely cylindrical, straight or slightly curved, 5–8 µm × 0.5–1 µm. Add one table to list the distinguishing characters among similar species. Move all characters in sexual stage to 1.pest information. According to the text, in this part only the	It make clear and easy to use. Make the structure more reasonable.	China

			character in asexual stage is used to make judgement.		
48.	*61*	Editorial	**4.2 Comparison of *G. citricarpa* cultural and morphological characteristics with those of similar *Guignardia* and *Phyllosticta* species**	To make the title more specific.	EPPO, Georgia, Russian Federation, Netherlands, European Union
49.	*62*	Editorial	Cultures of *G. citricarpa* are very similar to those of the endophytic, non-pathogenic to citrus *G. mangiferae* (anamorph *P. capitalensis*)and *P. citriasiana*~~, a species recently described on *C. maxima* fruit (Wulandari *et al.*,2009).~~	repetitious of para 15	Australia
50.	*62*	Substantive	Cultures of *G. citricarpa* are very similar to those of the endophytic, non-pathogenic to citrus ~~*G. mangiferae* (anamorph *P. capitalensis*)~~*P. citribraziliensis* and *P. citriasiana,* a species recently described on *C. maxima* fruit (Wulandari *et al.*,2009) and *P. citrichinaensis*, a species recently described on *C. limon, C. maxima*, *C. reticulata* and *C, sinensis* (Wang *et al*., 2012).	Deletion of "Guignardia manginiferae anamorph P. capitalensis)" since Glienke et al. 2011 demonstrated that Guignardia mangiferae (anamorph P capitalensis) and ,P. citribraziliensis, and P. citriasiana are not the same species and G. mangiferae was only recorded from mangoes. Insertion of "P. citribraziliensis" for consistency and "C. limon, C. maxima, C. reticulata and C, sinensis (Wang et al., 2012)" with Authors name to add new references.	South Africa
51.	*63*	Substantive	Identification of *G. citricarpa* colonies is possible by combining (i) the colony growth on CHA medium (the average colony diameter after 7 days at 22 °C in darkness is 25–30 mm for *G. citricarpa*, >40 mm for *G. mangiferae* and 18–20 mm for *P. citriasiana* (average of two isolates), although the ranges may overlap), (ii) the thickness of the mucoid sheath surrounding the conidia (G. citricarpa and *P. citriasiana*, >1.5 µm for *G. mangiferae*) (Figures 5C, 5D, 6A, 6B, 6C), ~~and~~ (iii) the length of the conidial appendage (4–6 µm for *G. citricarpa* and *G. mangiferae,* 7–14 µm for *P. citriasiana)* (Dr J.P. Meffert, personal communication), and iv)presence of yellow halo on OA medium.	Adding another important morphological trait	COSAVE, Paraguay, Chile, Brazil
52.	*63*	Substantive	Identification of *G. citricarpa* colonies is possible by combining (i) the colony growth on CHA medium (the average colony diameter after 7 days at 22 °C in darkness is 25–30 mm for *G. citricarpa*, >40 mm for *G. mangiferae* and 18–20 mm for *P. citriasiana* (average of two isolates), although the ranges may overlap), (ii) the thickness of the mucoid sheath surrounding the conidia (G. citricarpa and *P. citriasiana*, >1.5 µm for *G. mangiferae*) (Figures 5C, 5D, 6A, 6B, 6C), ~~and~~ (iii) the length of the conidial appendage (4–6 µm for *G. citricarpa* and *G. mangiferae,* 7–14 µm for *P. citriasiana)* (Dr J.P. Meffert, personal communication), and iv)presence of yellow halo on OA medium.	Adding another important morphological trait	Uruguay
53.	*63*	Substantive	Identification of *G. citricarpa* colonies is possible by combining (i) the colony growth on CHA medium (the average colony diameter after 7 days at 22 °C in darkness is 25–30 mm for *G. citricarpa*, >40 mm for *G. mangiferae* and 18–20 mm for *P. citriasiana* (average of two isolates), although the ranges may overlap), (ii) the thickness of the mucoid sheath surrounding the conidia (<1.5 µm for *G. citricarpa* and *P. citriasiana*, >1.5 µm for *G. mangiferae*) (Figures 5C, 5D, 6A, 6B, 6C), ~~and~~ (iii) the length of the conidial appendage (4–6 µm for *G. citricarpa* and *G. mangiferae,* 7–14 µm for *P. citriasiana)* (Dr J.P. Meffert, personal communication), and iv)presence of yellow halo on OA medium.	Adding another important morphological trait	Argentina
54.	*63*	Substantive	Identification of *G. citricarpa* colonies is possible by combining (i) the colony growth on CHA medium (the average colony diameter after 7 days at 22 °C in darkness is 25–30 mm for *G. citricarpa*, >40 mm for ~~*G. mangiferae*~~ *P. capitalensis* and 18–20 mm for *P. citriasiana* (average of two isolates), although the ranges may overlap), (ii) the thickness of the mucoid sheath surrounding the conidia (<1.5 µm for *G. citricarpa*~~and~~ *P. citriasiana*, *and P. citrichinaensis* >1.5 µm for~~ *G. man*~~*P. capitalensis and P. citribraziliensis ~~giferae~~)* (Figures 5C, 5D, 6A, 6B, 6C), and (iii) the length of the conidial	Deletion of "Guignardia manginiferae since Glienke et al. 2011 demonstrated that G mangiferae is not the same as G. citricarpa P. Citriasiana, P. citrichinaensis and P. capitalensis and G. mangiferae was only recorded from mangoes. Insertion of the new species of "P. capitalensis P.	South Africa

			appendage (4–6 µm for *G. citricarpa* ~~and *G. mangiferae*~~,*6-8 µm for P. capitalensis,* 7–14 µm for *P. citriasiana*, *7-15 µm for P. citribraziliensis and 12-25 µm for P. citrichinaensis) (Wulandari et al., 2009; Glienke et al., 2011; Wang et al., 2012;)* ~~)~~ (Dr J.P. Meffert, personal communication).	citribraziliensis , P. citrichinaensis, and the colony size to differentiate the cultural characteristics of Guignardia citricarpa and Phyllosticta species. Figures need to be looked at taking into consideration the deletion of G. mangiferae and the addition of other species.	
55.	*65*	Editorial	Different molecular methods have been developed for the identification of *G. citricarpa* directly on pure cultures and fruit lesions (Bonants *et al*., 2003; Meyer *et al*., 2006; Gent-Pelzer *et al*., 2007; Peres *et al*., 2007; Stringari *et al.,* 2009). However, none of these methods has been validated on plant material other than fruit (e.g. leaves, twigs). Two methods, a conventional PCR assay, developed by Peres *et al*. (2007), and a real-time PCR assay, developed by Gent-Pelzer *et al*. (2007), are recommended for the identification of *G. citricarpa*. ~~It should be noted that a real-time PCR will pick up a signal from a single lesion, which is uncertain with the conventional PCR.~~ It is noted that real-time PCR will generate a positive signal from a citrus black spot lesion; whereas, in some cases conventional PCR may give inconclusive results.	This sentence needs to be reworded to clarify that real time PCR delivers better results from a single lesion than conventional PCR. They could also provide an explantion to the difference between the two methods for example, better test sensitivity.	New Zealand
56.	*65*	Editorial	Different molecular methods have been developed for the identification of *G. citricarpa* directly on pure cultures and fruit lesions (Bonants *et al*., 2003; Meyer *et al*., 2006; Gent-Pelzer *et al*., 2007; Peres *et al*., 2007; Stringari *et al.,* 2009). However, none of these methods has been validated on plant material other than fruit (e.g. leaves, twigs). Two methods, a conventional PCR assay, developed by Peres *et al*. (2007), and a real-time PCR assay, developed by Gent-Pelzer *et al*. (2007), are recommended for the identification of *G. citricarpa*. It should be noted that a real-time PCR will pick up a signal from a single lesion on fruit, which is uncertain with the conventional PCR.	presumably the lesion is on fruit	Australia
57.	*65*	Substantive	Different molecular methods have been developed for the identification of *G. citricarpa* directly on pure cultures and fruit lesions (Bonants *et al*., 2003; Meyer *et al*., 2006; Gent-Pelzer *et al*., 2007; Peres *et al*., 2007; Stringari *et al.,* 2009). However, none of these methods has been validated on plant material other than fruit (e.g. leaves, twigs). Two methods, a conventional PCR assay, developed by Peres *et al*. (2007), and a real-time PCR assay, developed by Gent-Pelzer *et al*. (2007), are ~~recommended~~ described for the identification of *G. citricarpa*. It should be noted that a real-time PCR will pick up a signal from a single lesion, which is uncertain with the conventional PCR.	By stating that Peres et al is recommended, it may be interpreted as excluding the other tests, in particular Bonants et al 2003. Proposal to change "recommended" to "described". However we understand there may be reasons for the recommendation of one method and, if so, these should be explained.	EPPO, Georgia, Russian Federation, Netherlands, European Union
58.	*65*	Substantive	Different molecular methods have been developed for the identification of *G. citricarpa* directly on pure cultures and fruit lesions (Bonants *et al*., 2003; Meyer *et al*., 2006; Meyer *et al*., 2012 ; Gent-Pelzer *et al*., 2007; Peres *et al*., 2007; Stringari *et al.,* 2009). However, none of these methods has been validated on plant material other than fruit (e.g. leaves, twigs). Two methods, a conventional PCR assay, developed by Peres *et al*. (2007), and a real-time PCR assay, developed by Gent-Pelzer *et al*. (2007), are recommended for the identification of *G. citricarpa*. It should be noted that a real-time PCR will pick up a signal from a single lesion., ~~which is uncertain with the conventional PCR.~~	Insertion of "Meyer et al., 2012" to add new reference in support of methods for molecular assays. Sentence 3 needs to be clarified since Meyer et al. 2012 have extensively tested the methods on leaves and twigs. Deletion of "which is uncertain with the conversional PCR" since Meyer et al., 2012 had excellent results from single lesions with conventional PCR. It should be noted that real-time PCR should be preferred due to the 5 Phyllosticta species associated with citrus, which a single primer pair will not distinguish (e.g. those of Peres et al., 2007).	South Africa
59.	*67*	Editorial	Specificity (analytical specificity) was assessed in a study with 36 isolates of *G. citricarpa*, 13 isolates of *G. mangiferae* and isolates of common citrus pests, including *A. alternata*, *C. acutatum*, *C. gloeosporioides*, *D. citri*, *M. citri* and *Penicillium digitatum*. Only *G. citricarpa* gave a positive reaction. Sensitivity (analytical sensitivity; detection limit) is 1 pg DNA/µl (Peres *et al.*, 2007). The method will give amplification in the presence of either *G. citricarpa* or *P. citriasiana.* There are three	For consistency with [100].	EPPO

			methods available to discriminate between the two species: isolation and culturing (see section 4.1), real-time PCR assay (see section 4.3.2), or ITS sequencing (see section 4.3.3).		
60.	*67*	Editorial	Specificity (analytical specificity) was assessed in a study with 36 isolates of *G. citricarpa*, 13 isolates of *G. mangiferae* and isolates of common citrus pests, including *A. alternata*, *C. acutatum*, *C. gloeosporioides*, *D. citri*, *M. citri* and *Penicillium digitatum*. Only *G. citricarpa* gave a positive reaction. Sensitivity (analytical sensitivity; detection limit) is 1 pg DNA/µl (Peres *et al.*, 2007). The method will give amplification in the presence of either *G. citricarpa* or *P. citriasiana.* There are three methods available to discriminate between the two species: isolation and culturing (see section 4.1), real-time PCR assay (see section 4.3.2), or ITS sequencing (see section 4.3.3).	For consistency with [100].	Georgia, Russian Federation, Netherlands, European Union
61.	*67*	Editorial	Specificity (analytical specificity) was assessed in a study with 36 isolates of *G. citricarpa*, 13 isolates of *G. mangiferae* and isolates of common citrus pests, including *A. alternata*, *C. acutatum*, *C. gloeosporioides*, *D. citri*, *M. citri* and *Penicillium digitatum*. Only *G. citricarpa* gave a positive reaction. Sensitivity (analytical sensitivity; detection limit) is 1 pg DNA/µl (Peres *et al.*, 2007). The method will amplify either G. citricarpa or P. citriasiana DNA~~The method will give amplification in the presence of either *G. citricarpa* or *P. citriasiana.*~~ There are three methods available to discriminate between the two species: isolation and culturing (see section 4.1), real-time PCR assay (see section 4.3.2), or sequencing (see section 4.3.3).	For conciseness.	New Zealand
62.	*67*	Editorial	Specificity (analytical specificity) was assessed in a study with 36 isolates of *G. citricarpa*, 13 isolates of *G. mangiferae* and isolates of common citrus pests, including *A. alternata* *?pv. citri*, *C. acutatum*, *C. gloeosporioides*, *D. citri*, *M. citri* and *Penicillium digitatum*. Only *G. citricarpa* gave a positive reaction. Sensitivity (analytical sensitivity; detection limit) is 1 pg DNA/µl (Peres *et al.*, 2007). The method will give amplification in the presence of either *G. citricarpa* or *P. citriasiana.* There are three methods available to discriminate between the two species: isolation and culturing (see section 4.1), real-time PCR assay (see section 4.3.2), or sequencing (see section 4.3.3).	cosnistency with para 35 clarity - what is the in C. gloesporioides Citrus or Collectotrichum as both genera have previously been mentioned and C used for Citrus?	Australia
63.	*67*	Substantive	Specificity (analytical specificity) was assessed in a study with 36 isolates of *G. citricarpa*, 13 isolates of *G. mangiferae* and isolates of common citrus pests, including *A. alternata*, *C. acutatum*, *C. gloeosporioides*, *D. citri*, *M. citri* and *Penicillium digitatum*. Only *G. citricarpa* gave a positive reaction. Sensitivity (analytical sensitivity; detection limit) is 1 pg DNA/µl (Peres *et al.*, 2007). The method will give amplification in the presence of either *G. citricarpa* or *P. citriasiana.* There are three methods available to discriminate between the two species: isolation and culturing (see section 4.1), real-time PCR assay (see section 4.3.2), or sequencing (see section 4.3.3). Suggest to use the PCR method reported by Bonants et al. 2003, among which more information are included in ITS sequence.	This PCR method can multiply longer sequence, among which more information are included in ITS sequence. The method has been used in country of China, et al.	China
64.	*74*	Editorial	DNA is extracted either from fungal cultures grown for 7 days in potato-dextrose broth or from single fruit lesions. In the second case, the symptomatic tissue is dissected out, leaving behind as much mesocarp (albedo)~~pith~~ and outer rind as possible.	consistency with para 25	Australia
65.	*75*	Substantive	DNA extraction from mycelium is done using commercially available DNA extraction kits following the manufacturer's instructions. For the extraction of DNA from single fruit lesions, the following alkaline lysis DNA extraction protocol (Klimyuk *et al*., 1993) followed by purification using a dipstick method can be used as it proved to be the most effective (Peres *et al*., 2007). Need to specify some examples of commercially available DNA extraction kits that do work for G. citricarpa mycelium.	Not all kits will extract G. citricarpa mycelium in suitable quality for PCR amplification.	New Zealand
66.	*76*	Editorial	*Alkaline lysis protocol*. Symptomatic fruit tissue is placed into sterile 2-ml ~~eppendorf~~ microtubes containing 40 µl of 0.25 M NaOH and incubated in a boiling (100 °C) water bath for 30 s (critical period). The content of the tubes is neutralized by addition of 40 µl 0.25 M HCl, 20 µl 0.5 M Tris-HCl,	"eppendorf" is a brand, so it should be replaced with "microtubes" here and throughout the standard.	EPPO, Georgia, Russian Federation,

			pH 8.0 and 0.25% (v/v) Nonidet P-40 and the tubes are placed again in the boiling water bath for 2 min. This material can be either used directly for purification by applying the dipstick method (see below) or stored at 4 °C for several weeks. Prior to purification after storage, the samples are incubated in a boiling water bath for 2 min.		Netherlands, European Union
67.	*78*	Substantive	Alternatively, DNA can be extracted from fruit lesions using commercially available DNA extraction kits, according to the manufacturer's instructions. Need to specify some examples of commercially available DNA extraction kits that do work for citrus fruit infected with G. citricarpa.	Not all kits will extract G. citricarpa infected citrus fruit in suitable quality for PCR amplification.	New Zealand
68.	*79*	Editorial	**Polymerase ~~c~~Chain ~~r~~Reaction**		Saint Kitts And Nevis
69.	*80*	Editorial	The master mix (concentration per 20 µl single reaction) is composed as follows: *x* µl of MGW, 8 µl of 2.5× Eppendorf® MasterMix (Taq DNA polymerase at 0.06 U μl^{-1}), 2.5× Taq reaction buffer (4 mM Mg^{2+}, 500 µM of each dNTP), 0.8 µl of each primer at 10 µM (final concentration 0.4 µM) and 2 µl of template DNA. The PCR cycling parameters are 94 °C denaturation for 2 min, 39 cycles of 94 °C for 30 s, 64 °C for 30 s and 72 °C for 1 min and 72 °C extension step for 10 min. A PCR product of 300 bp indicates the presence of *G. citricarpa* DNA.	First sentence: "x" should be replaced by the appropriate figure in "x µl of MGW".	EPPO, Georgia, Russian Federation, Netherlands, European Union
70.	*80*	Editorial	The master mix (concentration per 20 µl single reaction) is composed as follows: *x* µl of MGW, 8 µl of 2.5× Eppendorf® MasterMix (Taq DNA polymerase at 0.06 U μl^{-1}), 2.5× Taq reaction buffer (4 mM Mg^{2+}, 500 µM of each dNTP), 0.8 µl of each primer at 10 µM (final concentration 0.4 µM) and 2 µl of template DNA. The PCR cycling parameters are 94 °C denaturation for 2 min, 39 cycles of 94 °C for 30 s, 64 °C for 30 s and 72 °C for 1 min and 72 °C extension step for 10 min. A PCR product of 300 bp indicates the presence of *G. citricarpa* DNA. First sentance needs to specify the volume pf MGW instead of "x ul".		New Zealand
71.	*80*	Technical	The master mix (concentration per 20 µl single reaction) is composed as follows: *x* µl of MGW, 8 µl of 2.5× Eppendorf® MasterMix (Taq DNA polymerase at 0.06 U μl^{-1}), 2.5× Taq reaction buffer (4 mM Mg^{2+}, 500 µM of each dNTP), 0.8 µl of each primer at 10 µM (final concentration 0.4 µM) and 2 µl of template DNA. The PCR cycling parameters are 94 °C denaturation for 2 min, 39 cycles of 94 °C for 30 s, 64 °C for 30 s and 72 °C for 1 min and 72 °C extension step for 10 min. A PCR product of 300 bp indicates the presence of *G. citricarpa* DNA.	First sentence: the mastermix compositions, here and elsewhere in the draft, should be rewritten in such a way that only the final concentration of each compound is indicated. This would prevent any mistake when trying to prepare the mastermix using chemicals with initial contentrations different from those indicated. The table has been prepared by J. Meffert and is presented separately to the IPPC as unable to insert in OCS.	EPPO, Georgia, Russian Federation, Netherlands, European Union
72.	*82*	Substantive	After amplification, 10 µl of the reaction mixture is loaded along with a molecular weight marker (100 bp DNA Ladder) onto a 1% agarose gel, separated by electrophoresis, stained with ethidium bromide or alternative reagents, viewed and photographed under UV light (Sambrook *et al*., 1989). Normally a 300bp DNA fragment would be run out on an agarose gel > 1.5% to obtain better resolution.		New Zealand
73.	*82*	Technical	After amplification, 10 µl of the reaction mixture is mixed with 2 µl of 6x DNA loading buffer (Promega) and loaded along with a molecular weight marker (100 bp DNA Ladder) onto a 1% agarose gel, separated by electrophoresis, stained with ethidium bromide or alternative reagents, viewed and photographed under UV light (Sambrook *et al*., 1989).	Beginning of the first sentence: "After amplification, 10 µl of the reaction mixture": A loading buffer should preferably be added to the PCR product to secure its location in the bottom of the well.	EPPO, Georgia, Russian Federation, Netherlands, European Union

74.	*82*	Technical	After amplification, 10 µl of the reaction mixture is loaded along with a molecular weight marker (100 bp DNA Ladder) onto a 1% agarose gel, separated by electrophoresis, stained with ethidium bromide or alternative reagents, viewed and photographed under UV light (Sambrook *et al*., 1989). Suggest to increase the concentration of 1 % agarose gel .	As for 100bp, 1 % agarose gel is too low.	China
75.	*83*	Substantive	DNA from a reference strain of *G. citricarpa* (positive control) must also be included as an additional sample to ensure that amplification has been successful. PCR amplification must also be performed on a sample, where the *G. citricarpa* DNA extract has been replaced with the DNA extract of other related species or with a sample run using healthy exocarp (negative control). To monitor possible reagent contamination and false positives, a sample must be substituted by water (reaction control). An internal amplification control (IAC) to check for false negative reactions caused by inhibition of the amplification reaction is not described by Peres *et al.* (2007), and must therefore be developed and tested. A reference strain of G.citricarpa should be specified and where it can be sourced from for example, CBS, ICMP culture collections.		New Zealand
76.	*83*	Substantive	DNA from a reference strain of *G. citricarpa* (positive control) must also be included as an additional sample to ensure that amplification has been successful. PCR amplification must also be performed on a sample, where the *G. citricarpa* DNA extract has been replaced with the DNA extract of other related species or with a sample run using healthy exocarp (negative control). To monitor possible reagent contamination and false positives, a sample must be substituted by water (reaction control). ~~An internal amplification control (IAC) to check for false negative reactions caused by inhibition of the amplification reaction is not described by Peres *et al.* (2007), and must therefore be developed and tested.~~	It is doubtful whether IAC is essential in this protocol. Furthermore, it is inappropriate to add IAC in the section of 'Essential procedural information' because it has not been developed.	Japan
77.	*85*	Substantive	Specificity (analytical specificity) was assessed with the *G. citricarpa* reference strain CBS 111.20 (representative for 10 *G. citricarpa* isolates ITS sequence group I; Baayen *et al.*, 2002), the ~~*G. mangiferae*~~ P. capitalensis reference strain GC14 (representative for 22 ~~*G. mangiferae*~~ P. capitaliensis isolates ITS sequence group II; Baayen *et al.*, 2002), 12 other citrus pests (*Alternaria* spp., *Penicillium* spp., *Colletotrichum* spp.), *Phyllosticta artocarpina* and *Guignardia bidwellii*. Only *G. citricarpa* gave a positive reaction. The sensitivity (analytical sensitivity; detection limit) is 10 fg DNA per reaction and the diagnostic sensitivity is 100% (Gent-Pelzer *et al*., 2007).	the "G. mangiferae" should be referred to as P. capitalensis	Australia
78.	*93*	Editorial	DNA is extracted either from plugs of mycelium (0.5 cm in diameter) taken from the edges of a colony grown on CHA (see section 4.1, “Culture media”) at 22 °C in darkness or from fruit lesions. Lesions are dissected from the peel, removing as much as possible of the surrounding albedo and peel tissue. Mycelium plugs or lesions are cut into small pieces and placed in a 1.5-ml microcentrifuge tube with a secure-fitting flat-top cap containing a stainless steel bead (3.2 mm in diameter) and 125 µl of extraction buffer (0.02M phosphate-buffered-saline (PBS), 0.5% Tween 20, 2% polyvinylpyrrolidone (PVP), 0.2% bovine serum albumin). The tube is shaken in a bead beater for 80 s at 5 000 ~~bpm.~~ rpm The mixture is centrifuged for 5 s at maximum speed (16 100 × *g*) in a microcentrifuge and 75 µl of the resulting supernatant is used for DNA extraction. DNA can be extracted using commercially available DNA extraction kits, according to the manufacturer’s instructions. The final volume of the DNA solution is 50 µl. The DNA is further purified over spin columns filled with PVP. The columns are prepared by filling Axygen Multi-Spin columns with 0.5 cm PVP, placing it on an empty reaction tube and washing twice with 250 µl MGW by centrifuging the column for 5 min at 4 000 × *g*. The DNA suspension is applied to a PVP column and centrifuged for 5 min at 4 000 × *g*. The flow-through fraction is used as input for the PCR assay. Purified DNA can	Typo?	New Zealand

			be used immediately; store overnight at 4 °C or at −20 °C for longer periods.		
79.	*93*	Technical	DNA is extracted either from plugs of mycelium (0.5 cm in diameter) taken from the edges of a colony grown on CHA (see section 4.1, "Culture media") at 22 °C in darkness or from fruit lesions. Lesions are dissected from the peel, removing as much as possible of the surrounding albedo and peel tissue. Mycelium plugs or lesions are cut into small pieces and placed in a 1.5-ml microcentrifuge tube with a secure-fitting flat-top cap containing a stainless steel bead (3.2 mm in diameter) and 125 µl of extraction buffer (0.02M phosphate-buffered-saline (PBS), 0.5% Tween 20, 2% polyvinylpyrrolidone (PVP), 0.2% bovine serum albumin). The tube is shaken in a bead beater for 80 s at 5 000 bpm. The mixture is centrifuged for 5 s at maximum speed (16 100 × *g*) in a microcentrifuge and 75 µl of the resulting supernatant is used for DNA extraction. DNA can be extracted using commercially available DNA extraction kits, according to the manufacturer's instructions. The final volume of the DNA solution is 50 µl. The DNA is further purified over spin columns filled with polyvinylpolypyrrolidon (PVP). The columns are prepared by filling Axygen Multi-Spin columns with 0.5 cm PVP, placing it on an empty reaction tube and washing twice with 250 µl MGW by centrifuging the column for 5 min at 4 000 × *g*. The DNA suspension is applied to a PVP column and centrifuged for 5 min at 4 000 × *g*. The flow-through fraction is used as input for the PCR assay. Purified DNA can be used immediately; store overnight at 4 °C or at −20 °C for longer periods.	PVP is used as soluble compound in the extraction buffer. PVPP is cross-linked PVP and is used as insoluble filtration material.	EPPO, Georgia, Russian Federation, Netherlands
80.	*93*	Technical	DNA is extracted either from plugs of mycelium (0.5 cm in diameter) taken from the edges of a colony grown on CHA (see section 4.1, "Culture media") at 22 °C in darkness or from fruit lesions. Lesions are dissected from the peel, removing as much as possible of the surrounding albedo and peel tissue. Mycelium plugs or lesions are cut into small pieces and placed in a 1.5-ml microcentrifuge tube with a secure-fitting flat-top cap containing a stainless steel bead (3.2 mm in diameter) and 125 µl of extraction buffer (0.02M phosphate-buffered-saline (PBS), 0.5% Tween 20, 2% polyvinylpyrrolidone (PVP), 0.2% bovine serum albumin). The tube is shaken in a bead beater for 80 s at 5 000 bpm. The mixture is centrifuged for 5 s at maximum speed (16 100 × *g*) in a microcentrifuge and 75 µl of the resulting supernatant is used for DNA extraction. DNA can be extracted using commercially available DNA extraction kits, according to the manufacturer's instructions. The final volume of the DNA solution is 50 µl. The DNA is further purified over spin columns filled with polyvinylpolypyrrolidone (PVPP). The columns are prepared by filling Axygen Multi-Spin columns with 0.5 cm PVP, placing it on an empty reaction tube and washing twice with 250 µl MGW by centrifuging the column for 5 min at 4 000 × *g*. The DNA suspension is applied to a PVP column and centrifuged for 5 min at 4 000 × *g*. The flow-through fraction is used as input for the PCR assay. Purified DNA can be used immediately; store overnight at 4 °C or at −20 °C for longer periods.	PVP is used as soluble compound in the extraction buffer. PVPP is cross-linked PVP and is used as insoluble filtration material. Check for consistent use of PVP/PVPP in this paragraph.	European Union
81.	*94*	Editorial	**Polymerase ~~c~~Chain ~~r~~Reaction**		Saint Kitts And Nevis
82.	*95*	Editorial	The master mix (concentration per 30µl single reaction) is composed as follows: 12.5 µl of MGW, 15.0 µl of 2× Premix Ex Taq (Takara)[2], 0.15 µl of each primer (50 mM), final concentration 250 nM, 0.60 µl of ~~TaqMan~~hydrolysis probe (5 mM), final concentration 100 nM, 0.60 µl of 50× ROX Reference Dye if applicable, 1.0 µl extracted DNA, obtained as described above.	Taqman is one of the commercial names of a type of probe that is called "hydrolysis probe", so "hydrolysis probe" should be used here or a footnote for Taqman added.	EPPO, Georgia, Russian Federation, Netherlands, European Union
83.	*96*	Technical	The PCR cycling parameters are 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min. ~~A cycle threshold (Ct) valueG. citrica~~rpaDNA.The cycle cut off value was obtained using the ABI PRISM 7700 or 7900 Sequence Detector (Applied Biosystems) and materials and chemistry used as	Second sentence: From a technical and scientific stand point, it is not sound to state that a "Ct value < 40 indicates the presence of G. citricarpa DNA", given that only 40 cycles of reaction are programmed. More reaction cycles should be	EPPO, Georgia, Russian Federation, Netherlands,

			described above • The amplification curve should be exponential. • A sample will be considered positive if it produces a Ct value of <40 and provided that the contamination controls are negative. • A sample will be considered negative, if it produces a Ct of 40 or more and provided that the assay and extraction inhibition controls are positive. The cycle cut off value needs to be verified in each laboratory when implementing the test for the first time	performed to ascertain the quality of Ct values close to 40. Moreover, cut off values may be indicated in that section, but the type of equipment used to determine these cut off values should be added. Different qPCR machines with different softwares will generate distinct Ct values, even with identical chemical and DNA templates. Additional text is proposed to address this.	
84.	*96*	Technical	Suggest to clarify the number scope of Ct.	The Criteria: Ct < 40 is too broad in practice. Eg. Ct 38-39	China
85.	*96*	Technical	The PCR cycling parameters are 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min. ~~A cycle threshold (Ct) valueG. citrica~~The cycle cut off value was obtained using the ABI PRISM 7700 or 7900 Sequence Detector (Applied Biosystems) and materials and chemistry used as described above • The amplification curve should be exponential. • A sample will be considered positive if it produces a Ct value of <40 and provided that the contamination controls are negative. • A sample will be considered negative, if it produces a Ct of 40 or more and provided that the assay and extraction inhibition controls are positive. The cycle cut off value needs to be verified in each laboratory when implementing the test for the first time	Second sentence: From a technical and scientific stand point, it is not sound to state that a "Ct value < 40 indicates the presence of G. citricarpa DNA", given that only 40 cycles of reaction are programmed. More reaction cycles should be performed to ascertain the quality of Ct values close to 40. Moreover, cut off values may be indicated in that section, but the type of equipment used to determine these cut off values should be added. Different qPCR machines with different softwares will generate distinct Ct values, even with identical chemical and DNA templates. Additional text is proposed to address this.	European Union
86.	*98*	Technical	DNA from a reference strain of *G. citricarpa* (positive control) must also be included as an additional sample to ensure that amplification has been successful. PCR amplification must also be performed on a sample, where the *G. citricarpa* DNA extract has been replaced with the DNA extract of other related species (e.g. P. citriasiana) or with a sample run using healthy exocarp (negative control). To monitor possible reagent contamination and false positives, a sample must be substituted by water (reaction control).	"e.g. P. citriasiana" should be added here.	EPPO
87.	*98*	Technical	DNA from a reference strain of *G. citricarpa* (positive control) must also be included as an additional sample to ensure that amplification has been successful. PCR amplification must also be performed on a sample, where the *G. citricarpa* DNA extract has been replaced with the DNA extract of other related species (e.g. P. citriasiana) or with a sample run using healthy exocarp (negative control). To monitor possible reagent contamination and false positives, a sample must be substituted by water (reaction control).	"e.g. P. citriasiana" should be added here.	EPPO, Georgia, Russian Federation, Netherlands, European Union
88.	*98*	Technical	DNA from a reference strain of *G. citricarpa* (positive control) must also be included as an additional sample to ensure that amplification has been successful. PCR amplification must also be performed		New Zealand

			on a sample, where the *G. citricarpa* DNA extract has been replaced with the DNA extract of other related species or with a sample run using healthy exocarp (negative control). To monitor possible reagent contamination and false positives, an additional sample must be substituted by water (reaction control).		
89.	*99*	Substantive	To check for false negative reactions caused by inhibition of the amplification reaction, 12.5 fg of an internal amplification control, 75 nM of IAC forward primer FIAC (5'-TGG CCC TGT CCT TTT ACC AG-3'), 75 nM of IAC reverse primer RIAC (5'-TTT TCG TTG GGA TCT TTC GAA-3'), 50 nM of IAC MGB Taqman probe (5'-ACA CAA TCT GCC-3'), VIC™ label and quencher dye Eclipse Dark Quencher can be added to the reaction mixes. The IAC is a plasmid, containing a green fluorescent protein gene (GFP) that can be obtained from Dr P.J.M. Bonants (Plant Research International, Wageningen, the Netherlands). Suggest considering the inclusion of the COX internal control assay developed by Weller et al. (2000). This protocol is also widely used to determine if extract plant DNA is suitable for PCR amplification.	Weller, S. A., Elphinstone, J. G., Smith, N. C., Boonham, N., and Stead, D. E. 2000. Detection of Ralstonia solanacearum strains with a quantitative, multiplex, real-time, fluorogenic PCR (TaqMan) assay. Appl. Environ. Microbiol. 66:2853-2858.	New Zealand
90.	*99*	Substantive	To check for false negative reactions caused by inhibition of the amplification reaction, 12.5 fg of an internal amplification control, 75 nM of IAC forward primer FIAC (5'-TGG CCC TGT CCT TTT ACC AG-3'), 75 nM of IAC reverse primer RIAC (5'-TTT TCG TTG GGA TCT TTC GAA-3'), 50 nM of IAC MGB Taqman probe (5'-ACA CAA TCT GCC-3'), VIC™ label and quencher dye Eclipse Dark Quencher can be added to the reaction mixes. ~~The IAC is a plasmid, containing a green fluorescent protein gene (GFP) that can be obtained from Dr P.J.M. Bonants (Plant Research International, Wageningen, the Netherlands).~~ Delete the 2ed sentence.	IAC is a plasmid. If it has to be obtained from one person in Netherland, it will be hard for member country to get.	China
91.	*108*	Editorial	**Polymerase ~~c~~Chain ~~r~~Reaction**		Saint Kitts And Nevis
92.	*108*	Substantive	**Polymerase chain reaction** It would be useful to include the PCR product size expected to be amplified from G. citricarpa DNA using the ITS primers.		New Zealand
93.	*109*	Editorial	Total reaction volume of a single PCR reaction is 50 µL, and is composed as follows: 37.5 µl of molecular grade water, 5.0 µl 10× PCR reaction buffer (+15 mM $MgCl_2$) (Roche)[3], 4.0 µl dNTPs (10 mM each), 0.6 µl primer ITS1 (10.0 µM), 0.6 µl primer ITS4 (10.0 µM), 0.3 µl DNA Taq-polymerase (5 U/µl) (Roche)[3] and 1.0 µl of DNA extract.	Please see comment to paragraph [80]. We propose that the text is replaced by a table. Tabulated format submitted to IPPC separately as unable to insert in OCS.	EPPO, Georgia, Russian Federation, Netherlands, European Union
94.	*109*	Editorial	Total reaction volume of a single PCR reaction is 50 µL, and is composed as follows: 37.5 µl of ~~molecular grade water~~ MGW, 5.0 µl 10× PCR reaction buffer (+15 mM $MgCl_2$) (Roche)[3], 4.0 µl dNTPs (10 mM each), 0.6 µl primer ITS1 (10.0 µM), 0.6 µl primer ITS4 (10.0 µM), 0.3 µl DNA Taq-polymerase (5 U/µl) (Roche)[3] and 1.0 µl of DNA extract.	Deletion of the words Molecular grade water and writing it as “MGW” since the wording was previously written in full and initiallised in this document.	South Africa
95.	*110*	Substantive	The PCR cycle parameters are: 94 °C for 30 s, 40 cycles (94 °C for 15 s, 55 °C for 60 s, 72 °C for 30 s), 72 °C for 5 min, ~~20 °C for 1 min.~~	Suggest that the step “20 °C for 1 min” be deleted, the reason being that the procedure ends at 72°C for 5 minutes (Dr. Trutter, personal communication).	South Africa

96.	*115*	Technical	Defrost extracted DNA, if necessary; prepare enough reaction mix for testing at least one sample of the unknown isolate, a positive control containing amplifiable DNA and negative control reactions of reaction mix loaded with water rather than DNA. Resolve samples on a 1.5% agarose gel. Compare consensus sequences for test samples (excluding primer sequences) with a confirmed strain for ~~*G. citricarpa*, such as CBS 111.20 (GenBank ref FJ538314)~~the ex-epitype of P. citricarpa CBS 127454 (GenBank ref JF343583) on the NCBI database GenBank (http://www.ncbi.nlm.nih.gov). The level of identity should be between 99% and 100%.	the sample ITS sequence should be compared to the ex-epitype of P. citricarpa CBS 127454 (GenBank ref JF343583)	Australia
97.	*126*	Substantive	**Aa, H.A. van der.** 1973. Studies in *Phyllosticta* I. *Studies in Mycology*, 5: 1–110. **Agostini, J.P., Peres, N.A., Mackenzie, S.J., Adaskaveg, J.E. & Timmer, L.W.** 2006. Effect of fungicides and storage conditions on postharvest development of citrus black spot and survival of *Guignardia citricarpa* in fruit tissues. *Plant Disease,* 90: 1419–1424. **Aguilar-Vildoso, C., Baldini, J., Feichtenberger, E., de Goes, A. & Spósito, M.** 2002. *Manual técnico de procedimentos da mancha preta dos Citros*. Brasilia, Brazil, Ministério da Agricultura, Pecuária e Abastecimiento, Departamento de Defesa e Inspeção Vegetal. Projeto CE-MERCOSUL ALA 93/143. 59 pp. **Baayen, R.P., Bonants, P.J.M., Verkley, G., Carroll, G.C., van der Aa, H.A., de Weerdt, M., van Brouwershaven, I.R., Schutte, G.C., Maccheroni Jr., W., Glienke de Blanco, C. & Azevedo, J.L.** 2002. Nonpathogenic isolates of the citrus black spot fungus, *Guignardia citricarpa*, identified as a cosmopolitan endophyte of woody plants, *G. mangiferae* (*Phyllosticta capitalensis*). *Phytopathology,* 92: 464–477. **Baldassari, R.B., Reis, R.F. & de Goes, A.** 2006. Susceptibility of fruits of the 'Valência' and 'Natal' sweet orange varieties to *Guignardia citricarpa* and the influence of the coexistence of healthy and symptomatic fruits. *Fitopatologia Brasiliera*, 31: 337–341. **Benson, A.H.** 1895. Some fruit pests: Black spot of the orange. *Agricultural Gazette of New South Wales*, 6: 249–251. **Bonants, P.J.M., Carroll, G.C., de Weerdt, M., van Brouwershaven, I.R. & Baayen, R.P.** 2003. Development and validation of a fast PCR-based detection method for pathogenic isolates of the Citrus Black Spot fungus, *Guignardia citricarpa*. *European Journal of Plant Pathology*,109: 503–513. **CABI.** 2011. *Guignardia citricarpa. Crop Protection Compendium,* 2011 Edition, Wallingford, UK,CAB International. www.cabi.org/isc/?compid=5&dsid=26154&loadmodule=datasheet&page=481&site=144 **CABI/EPPO.** 1998. *Guignardia citricarpa*. *Distribution maps of quarantine pests for Europe,* no. 204. Wallingford, UK, CAB International. **EPPO/CABI.** 1997. *Guignardia citricarpa. In* I.M. Smith, D.G. McNamara, P.R. Scott & M.		South Africa

			Holderness, eds. *Quarantine Pests for Europe*, 2ndedition, pp. 773–781. Wallingford, UK, CAB International. 1440 pp. **FUNDECITRUS.** 2005. Manual de Pinta Preta. Brazil, Fundo de Defesa da Citricultura. http://www.fundecitrus.com.br/manuais/fundec_manual_ppreta_0806.pdf **Gams, W., Hoekstra, E.S. & Aptroot, A.** 1998. *CBS Course of Mycology*, 4th edition. Baarn/Delft, The Netherlands, Centraal Bureau voor Schimmelcultures. 165 pp. **Gent-Pelzer, M.P.E. van, van Brouwershaven, I.R., Kox, L.F.F. & Bonants, P.J.M.** 2007. A TaqMan PCR method for routine diagnosis of the quarantine fungus *Guignardia citricarpa* on citrus fruit. *Journal of Phytopathology,* 155: 357–363. **Goes, A. de, Baldassari, R.B., Feichtenberger, E., Aguilar-Vildoso, C.I. & Spósito, M.B.** 2000. Cracked spot, a new symptom of citrus black spot in Brazil. *In Abstracts of the 9th Congress of the International Society of Citriculture*. Orlando, USA, University of Florida. p. 145. **Goes, A. de.** 2001. Mancha preta dos Citros: Situação atual e perspectivas futuras. *Ciência e Prática, Bebedouro, 20 December 2001*. pp. 5–7. **Hawksworth, D.L., Kirk, P.M., Sutton, B.C. & Pegler, D.N.** 1995. *Ainsworth & Bisby's Dictionary of the Fungi.* 8th edition. Oxon, UK, CAB International. 650 pp. **Hughes, K.J.D., Inman, A.J. & Cooke, D.E.L.** 2000. Comparative testing of nested PCR-based methods with bait-plant tests for detecting *Phytophthora fragariae* var. *fragariae* in infected strawberry roots from fruit crops in the UK. *Bulletin OEPP/EPPO Bulletin*, 30: 533–538. **ISPM 27**. 2006. *Diagnostic protocols for regulated pests*. Rome, IPPC, FAO. **Kiely, T.B.** 1949a. Preliminary studies on *Guignardia citricarpa* n. sp., the ascigerous stage of *Phoma citricarpa* McAlp., and its relation to black spot of citrus. *Proceedings of the Linnean Society of New South Wales,* 73: 249–292. **Kiely, T.B.** 1949b. Black spot of citrus. *The Agricultural Gazette of New South Wales*, 60: 17–20. **Kiely, T.B.** 1960. Speckled blotch of citrus. *The Agricultural Gazette of New South Wales*, 71: 474–476. **Klimyuk, V.I., Carroll, B.J., Thomas, C.M. & Jones, J.D.** 1993. Alkali treatment for rapid		

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98.	*126*	Technical	**Aa, H.A. van der.** 1973. Studies in *Phyllosticta* I. *Studies in Mycology*, 5: 1–110. **Agostini, J.P., Peres, N.A., Mackenzie, S.J., Adaskaveg, J.E. & Timmer, L.W.** 2006. Effect of fungicides and storage conditions on postharvest development of citrus black spot and survival of *Guignardia citricarpa* in fruit tissues. *Plant Disease,* 90: 1419–1424. **Aguilar-Vildoso, C., Baldini, J., Feichtenberger, E., de Goes, A. & Spósito, M.** 2002. *Manual técnico de procedimentos da mancha preta dos Citros*. Brasilia, Brazil, Ministério da Agricultura, Pecuária e Abastecimiento, Departamento de Defesa e Inspeção Vegetal. Projeto CE-MERCOSUL ALA 93/143. 59 pp. **Baayen, R.P., Bonants, P.J.M., Verkley, G., Carroll, G.C., van der Aa, H.A., de Weerdt, M., van Brouwershaven, I.R., Schutte, G.C., Maccheroni Jr., W., Glienke de Blanco, C. & Azevedo, J.L.** 2002. Nonpathogenic isolates of the citrus black spot fungus, *Guignardia citricarpa*, identified as a cosmopolitan endophyte of woody plants, *G. mangiferae* (*Phyllosticta capitalensis*). *Phytopathology,*		South Africa

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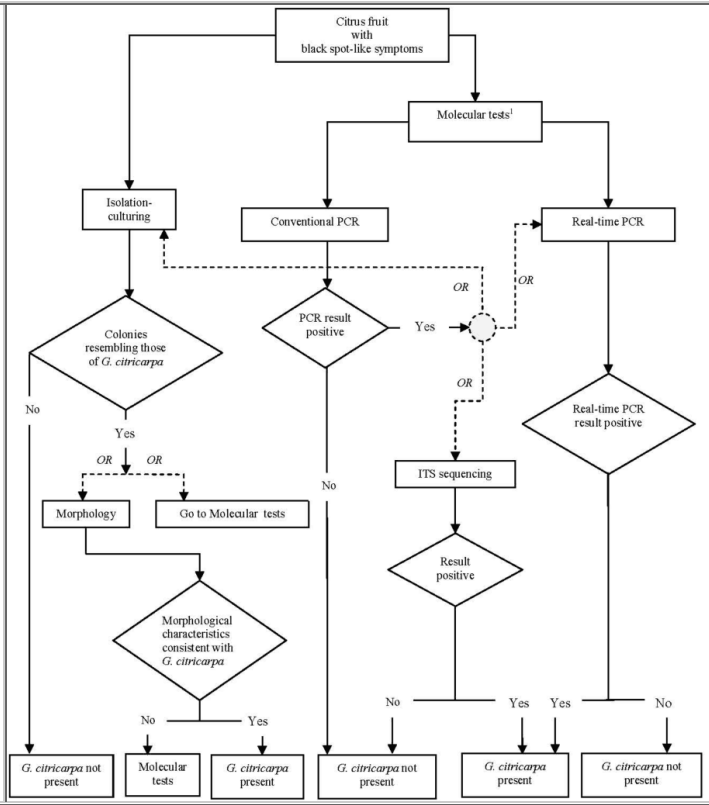
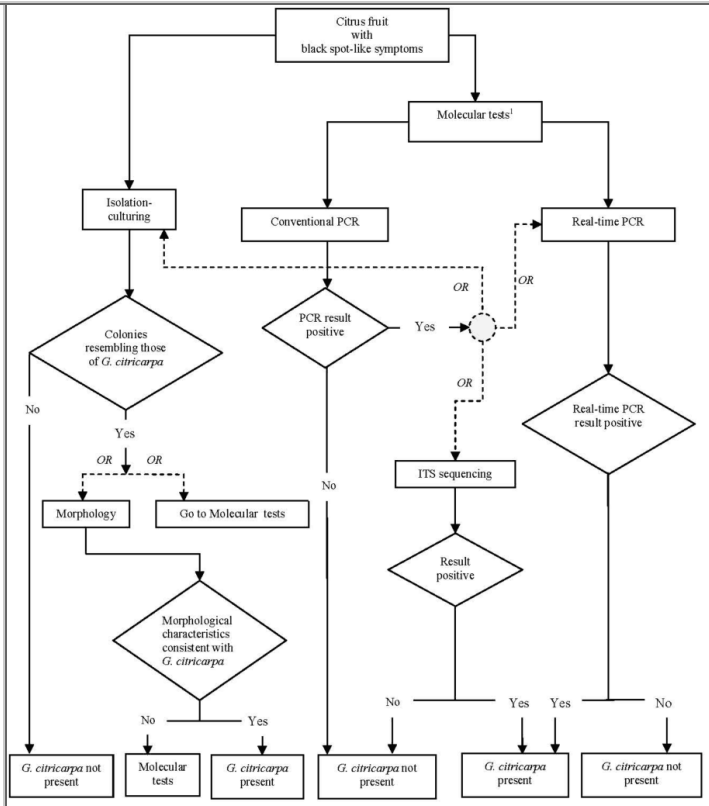
99.	*134*	Substantive		From the flow diagram for the diagnosis of G. citricarpa on citrus fruit it would appear that a diagnosis could be accepted based on morphological characteristics alone. Given that the morphological characteristics can be confused with other similar species or even Colletotricum species (as documented in paras 46, 61 and 62) it is recommended that the diagnosis is confirmed by molecular tests. The detection of G. citricarpa on citrus fruit consignments can have significant quarantine and trade consequences. Identifications of such importance should not be based on morphological characteristics alone.	New Zealand

100.	*135*	Substantive		1. A new diagnostic method should be added as the first step of the flow of Fig. 4 on the basis of the typical symptom (hard spot) on fruit and the presence of pycnidia. (We will send you proposed amendment of fig.4(1) in the PDF format by email from our contact point.) In paragraph 15, it is stated that 'It should be noted that in symptomless citrus fruit or fruit with minute spots (<2 mm in diameter) without pycnidia, the non-pathogenic endophyte Guignardia mangiferae A.J. Roy (anamorph Phyllosticta capitalensis Henn.), recorded in many plant families, may be present. '. And in paragraph 22, it is stated that 'Hard spot symptoms with pycnidia would be the most easily identified as citrus black spot.'. So, if there is a symtom which has typical hard spots ≥2mm in diameter and pycnidia, it is possible that the fruit is infested by Guignardia citricarpa. Therefore, we propose to add a new diagnostic method as the first step of the flow of Fig. 4. It is a quick diagnostic method and is practical and beneficial for countries which do not have any special measures for fresh fruits other than visual inspection. 2. In the diagnostic method using molecular tests, Conventional PCR for distinguishing Guignardia citricarpa from Phyllosticta citriasiana should be added after the first Conventional PCR. (We will send you proposed amendment of fig.4(2) in the PDF format by email from our contact point.) In paragraph 67, it is stated that 'The method will give amplification in the presence of either G. citricarpa or P. citriasiana.', but it was reported that the two species can be distinguished by Conventional PCR (Xinghong W. et al. (2012)). According to this paper, a PCR procedure to detect P. citriasiana has been developed. 3. The method which uses colonies resembling those of Guignardia citricarpa or Phyllosticta sp. (≥1.0 cm diameter) for ITS sequencing should be added before the step confirming color of colony in the process of isolation-culturing. (We will send you proposed amendment of fig.4(3) in the PDF format by email from our contact point.) In the molecular tests done directly from fruit symptom, it is described that ITS sequencing is one of the options to define Guignardia citricarpa after	Japan

				conventional PCR "positive". But, it is inappropriate to perform ITS sequencing directly from fruit symptom because of the possibility of superinfection in the symptom. So, it is necessary to do isolation-culturing which is time consuming before ITS sequencing. We think it is practical and quicker to add the new flow which makes it possible to use [before the step of confirming its color] colonies produced in the isolation-culturing process for ITS sequencing.	