Draft Annex to ISPM 27:2006 - *Tilletia indica* Mitra (2004-014)

Comm. no.	Para. no.	Comment type	Comment	Explanation	Language	Country
1.	G	Editorial		The symbols usage should be revised, e.g. the number and the symbols in several cases are together, while it is usual that are separated.	English	OIRSA
2.	G	Editorial		The document is well written. It will further be important to put reference on methodology.	English	South Africa
3.	G	Substantive		A global revision of the use of terms "seed" and "grain" was made, because the term "seed" should not be used to represent "grain". Both terms are defined in the glossary and both should be used when necessary to avoid confusion.	English	COSAVE, Paraguay, Chile, Brazil
4.	G	Substantive		A global revision of the use of terms "seed" and "grain" was made, because the term "seed" should not be used to represent "grain". Both terms are defined in the glossary and both should be used when necessary to avoid confusion.	English	Uruguay
5.	G	Substantive	*Tilletia indica* is considered a quality pest by many countries and grain is often traded on the basis of additional declarations for *Tilletia indica* without spore testing of consignments. In many cases, countries use bunted kernels as the basis for regulation of grain for consumption. This diagnostic protocol presents a methodology for detecting contamination of 1-5 *T. indica* teliospores at the 99%-99.99% levels of confidence. The use of spore testing at contamination and confidence levels specified in this draft diagnostic protocol to determine whether a non propagative consignment may be considered infested is precedent setting. The protocol may be interpreted to provide the basis for rejection of a 60,000 MT wheat cargo due to detection of a single spore. Substantive changes are necessary to this draft dianostic protocol to ensure that it will be useful for a full range of phytosanitary circumstances and will fully promote the goals of harmonization for the broadest range of contracting parties. In addition, the draft protocol does not conform to certain elements of the purpose and requirements of diagnostic protocols as described in ISPM 27 in that it prescribes more than the minimum requirements for reliable diagnosis of *T. indica* (ISPM 27-5); it does not present alternative diagnostic techniques applicable for different phytosanitary circumstances (ISPM 27-7); and does not consider availability of equipment and expertise and practicality when prescribing the process for detecting and identifying *Tilletia indica* (ISPM 27-8). For these reasons, we recommend that the draft protocol be reconsidered and revised to enhance i		English	United States of America

			ts usefulness for a broader range of phytosanitary circumstances.			
6.	*G*	Substantive	The molecular identification method in the standard may be attached as appendix, in which the method can be divided into three parts: conventional PCR, nested PCR and real-time fluorescence PCR, while each category can contain several parallel. The operator can choose one method under laboratory conditions, technical level and sensitivity of the method. For example, The operator can take conventional PCR or real-time PCR detection method to obtain mycelium spore germination, or take nested PCR or nested PCR + real-time fluorescence PCR to detect trace spores.	The Structure would be clearer. Referring the structure of EPPO standards of Phytophthora ramorum, C. michiganensis subsp. insidiosus, Thrips palmi, the structure is suggested as follows: Appendix XX Identification at species level by conventional PCR Conventional PCR: Method A (Frederick et al., 2000) A primer pair (Tin 3 and Tin 4) has been developed by Frederick et al. (Frederick et al., 2000) for the detection of T. indica by conventional PCR. The amplicon is 163bp. The primer sequences are: Tin 3 (5′-CAA TGT TGG CGT GGC GGC GC-3′) Tin 4 (5′-CAA CTC CAG TGA TGG CTC CG-3′). PCR for this assay uses the following mastermix (concentration per 25µl single reaction): 20.2 µl of MGW, 2.5 µl of 10X PCR buffer containing 15mM MgCl2 (Applied Biosystems)2, 0.25 µl of each dNTPs (10 mM) (final concentration of 0.1 mM each), 0.1 µl AmpliTaq (5 U/µl) (Applied Biosystems)2, 0.1 µl of each primer (25 µM), 1.0 µl neat extracted DNA obtained as described above. PCR cycling parameters: 94 °C denaturation for 1 min, 25 cycles of 94 °C for 15 s, 65 °C for 15 s, 72 °C for 15 s, and 72 °C extension step for 6 min. Conventional PCR: Method B (Wagner & Werres, 2003) For Garbelotto's primers see above. Sampling and sample preparation: if possible at least five twigs or leaves with disease symptoms are taken per plant. About five tissue pieces per twig or leaf (each 0.5 cm2, per sample about 100–200 mg total weight) are Conventional PCR: Method C (Lane et al., 2003b) The following protocol is for the	English	China

				conventional PCR identification of P. ramorum from cultures and plant material. Primers: a primer pair (Pram F1 and Pram R1) has been developed by Hughes (Lane et al., 2003b) for conventional PCR. The primer sequences are: Pram F1 : 5′-CTA TCA TGG CGA GCG CTT GA-3′ and Pram R1 : 5′-GAA GCC GCC AAC ACA AG-3′. Appendix XX Identification at species level by Real-time PCR The following two equivalent and validated methods may be used for TaqMan®-PCR identification of P. ramorum from cultures and plant material. RT-PCR: Method A (Hughes et al., 2005) Primers/TaqMan®-probe: the primer sequences are: Pram 114-FC: 5′-TCA TGG CGA GCG CTG GA-3′, Pram 1527-190-R: 5′-AGT ATA TTC AGT ATT TAG GAATGG GTT TAA AAA GT-3′, and the TaqMan®-probe is: Pram 1527-134-T: 5′-TTC GGG TCT GAG CTA GTA G-3′. RT-PCR: Method B (Hayden et al., 2004) Primers/TaqMan®-probe: the primer sequences are: Pram-5: 5′-TTA GCT TCG GCT GAA CAA TG-3′, Pram-6: 5′-CAG CTA CGG TTC ACC AGT CA-3′, and the TaqMan®-probe is: Pram-7 : 5′-ATG CTT TTT CTG CTG TGG CGG TAA-3′. Appendix XX Identification at species level by Nested PCR Nested PCR: Method A (Hughes et al., 2005) the primer sequences are: Pram 114-FC: 5′-TCA TGG CGA GCG CTG GA-3′, Pram 1527-190-R: 5′-AGT ATA TTC AGT ATT TAG GAATGG GTT TAA AAA GT-3′, and the TaqMan®-probe is: Pram 1527-134-T: 5′-TTC GGG TCT GAG CTA GTA G-3′. Nested PCR: Method B (Hayden et al., 2004)		
7.	*G*	Substantive	1. A note which indicates that 'Real-time PCR on individual teliospores has the possibility to judge	1. The wash test may detect the	English	Japan

			dead teliospores as positive' should be added. 2. The figure of *T. ehrhartae* should be added.	teliospores inactivated by agricultural chemicals and/or physical damages and it is possible to judge the dead ones as positive in Real-time PCR if it is conducted without checking the activation of the samples. Therefore, a note to alert this should be added. 2. Only T. ehrhartae does not have any figures in this protocol even though other related species (T. walkeri and T. horrida) do. (Reference) Smut Fungi of the World (APS Press): This has figures taken by optical microscope and SEM.		
8.	*G*	Substantive		A global revision of the use of terms "seed" and "grain" was made, because the term "seed" should not be used to represent "grain". Both terms are defined in the glossary and both should be used when necessary to avoid confusion.	English	Argentina
9.	*1*	Editorial	**DRAFT ANNEX to ISPM 27:2006 - ~~Tilletia indica~~*Tilletia indica* Mitra** (2004-014)	The scientific names are typed in italic	English	OIRSA
10.	*7*	Substantive	*Tilletia indica* Mitra causes the disease Karnal bunt, or partial bunt, of wheat (*Triticum* spp.). Karnal bunt was first described in Karnal, India, in 1931. *T. indica* is a serious pest for international trade. It is of economic importance because it reduces grain or seed quality; and its restricted distribution makes it a concern for those countries currently free from the pest. The pathogen is widespread in parts of South Asia and Southwest Asia (Wiese, 1987; USDA, 2007). It has also been detected in North America, including the south-western United States, and South Africa (Fuentes-Davila, 1996; Crous *et al.,* 2001).	It is not appropriate to use the term seed to refer to both seed and grain. Both are glossary terms	English	COSAVE, Paraguay, Chile, Brazil
11.	*7*	Substantive	*Tilletia indica* Mitra causes the disease Karnal bunt, or partial bunt, of wheat (*Triticum* spp.). Karnal bunt was first described in Karnal, India, in 1931. *T. indica* is a serious pest for international trade. It is of economic importance because it reduces grain or seed quality; and its restricted distribution makes it a concern for those countries currently free from the pest. The pathogen is widespread in parts of South Asia and Southwest Asia (Wiese, 1987; USDA, 2007). It has also been detected in North America, including the south-western United States, and South Africa (Fuentes-Davila, 1996; Crous *et al.,* 2001).	It is not appropriate to use the term seed to refer to both seed and grain. Both are glossary terms	English	Uruguay
12.	*7*	Substantive	*Tilletia indica* Mitra causes the disease Karnal bunt, or partial bunt, of wheat (*Triticum* spp.). Karnal bunt was first described in Karnal, India, in 1931. *T. indica* is a serious pest for international trade. It is of economic importance because it reduces grain or seed quality; and its restricted distribution makes it a concern for those countries currently free from the pest. The pathogen is widespread in parts of South Asia and Southwest Asia (Wiese, 1987; USDA, 2007). It has also been detected in North America, including the south-western United States, and South Africa (Fuentes-Davila, 1996; Crous *et al.,* 2001).	It is not appropriate to use the term seed to refer to both seed and grain. Both are glossary terms	English	Argentina
13.	*7*	Technical	*Tilletia indica* Mitra causes the disease Karnal bunt, or partial bunt, of wheat (*Triticum* spp.). Karnal bunt was first described in Karnal, India, in 1931. *T. indica* is ~~a serious pest for international trade.~~	Karnal bunt is a quality pest which causes no major impact to the grain	English	United States of America

			~~It is~~ of economic importance because it reduces grain quality; and its restricted distribution makes it a concern for those countries currently free from the pest. The pathogen is widespread in parts of South Asia and Southwest Asia (Wiese, 1987; USDA, 2007). It is under quarantine in the ~~has also been detected in North America, including the south-western~~ United States~~,~~ (restricted to the state of Arizona), it is present in some areas in Mexico and has been detected in South Africa (Fuentes-Davila, 1996; Crous *et al.,* 2001).	productivity. Yield losses from Karnal bunt are usually minor, but grain quality may be reduced because of an unpleasant fishy odor.		
14.	*7*	Technical	*Tilletia indica* Mitra causes the disease Karnal bunt, or partial bunt, of wheat (*Triticum* spp.). Karnal bunt was first described in Karnal, India, in 1931. *T. indica* is a serious pest for international trade. It is of economic importance because it reduces grain quality; and its restricted distribution makes it a concern for those countries currently free from the pest. The pathogen is widespread in parts of South Asia and Southwest Asia (Wiese, 1987; USDA, 2007). It has also been detected in North America in restricted areas of the United States and Mexico and has been detected in ~~, including the south-western United States, and~~ South Africa (Fuentes-Davila, 1996; Crous *et al.,* 2001).	More acurately depicts the distribution of Karnal bunt in North America.	English	Canada
15.	*10*	Technical	*T. indica* reduces grain quality by discolouring and imparting an objectionable odour to the grain and products made from it. It also causes a small reduction in yield. Generally *Triticum aestivum* containing more than 3% bunted kernels is considered unsatisfactory for human consumption. (Citation)	This last sentence needs reference support from a scientific source.	English	United States of America
16.	*11*	Editorial	There are other *Tilletia* species that can be confused with *T. indica* and are commonly found in harvested grain or seeds. These include *T. walkeri* (a pathogen of *Lolium perenne* and *L. multiflorum*), *T. horrida* (a pathogen of *Oryza* spp.)and *T. ehrhartae* (a pathogen of *Ehrharta calycina*). In Australia, *T. walkeri* and *T. ehrhartae* are found to contaminate harvested seed of *Triticum aestivum. T. walkeri* and *T. horrida* are present in the United States and are detected in harvested seed of *Triticum aestivum* especially where *Oryza* spp. and *Lolium* spp. are grown in rotation with *Triticum aestivum* (Castlebury, 1998; Castlebury and Carris, 1999; Pascoe *et al.,* 2005). Because of the morphological similarity of these pathogens, accurate identification ~~of the different pathogens~~ is important.	Useless repetition?	English	EPPO
17.	*11*	Editorial	There are other *Tilletia* species that can be confused with *T. indica* and are commonly found in harvested grain or seeds. These include *T. walkeri* (a pathogen of *Lolium perenne* and *L. multiflorum*), *T. horrida* (a pathogen of *Oryza* spp.)and *T. ehrhartae* (a pathogen of *Ehrharta calycina*). In Australia, *T. walkeri* and *T. ehrhartae* are found to contaminate harvested seed of *Triticum aestivum. T. walkeri* and *T. horrida* are present in the United States and are detected in harvested seed of *Triticum aestivum* especially where *Oryza* spp. and *Lolium* spp. are grown in rotation with *Triticum aestivum* (Castlebury, 1998; Castlebury and Carris, 1999; Pascoe *et al.,* 2005). Because of the morphological similarity of these pathogens, accurate identification ~~of the different pathogens~~ is important.	Useless repetition?	English	Georgia, Russian Federation, Netherlands, European Union
18.	*11*	Editorial	There are other *Tilletia* species that can be confused with *T. indica* and are commonly found in harvested grain or seeds. These include *T. walkeri* (a pathogen of *Lolium perenne* and *L. multiflorum*), *T. horrida* (a pathogen of *Oryza* spp.)and *T. ehrhartae* (a pathogen of *Ehrharta calycina*). In Australia, *T. walkeri* and *T. ehrhartae* are found to contaminate harvested seed of *Triticum aestivum. T. walkeri* and *T. horrida* are present in the United States and are detected in harvested seed of *Triticum aestivum* especially where *Oryza* spp. and *Lolium* spp. are grown in rotation with *Triticum aestivum* (Castlebury, 1998; Castlebury and Carris, 1999; Pascoe *et al.,* 2005). ~~Because of the morphological similarity of these pathogens, accurate identification of the different pathogens is important.~~The accurate identification of the different pathogens is important beacuse of the morpho logical similarity of these pathogens.		English	Saint Kitts And Nevis
19.	*11*	Editorial	There are other *Tilletia* ~~species~~ spp. that can be confused with *T. indica* and are commonly found	uniformity	English	Lesotho*

			in harvested grain or seeds. These include *T. walkeri* (a pathogen of *Lolium perenne* and *L. multiflorum*), *T. horrida* (a pathogen of *Oryza* spp.)and *T. ehrhartae* (a pathogen of *Ehrharta calycina*). In Australia, *T. walkeri* and *T. ehrhartae* are found to contaminate harvested seed of *Triticum aestivum. T. walkeri* and *T. horrida* are present in the United States and are detected in harvested seed of *Triticum aestivum* especially where *Oryza* spp. and *Lolium* spp. are grown in rotation with *Triticum aestivum* (Castlebury, 1998; Castlebury and Carris, 1999; Pascoe *et al.,* 2005). Because of the morphological similarity of these pathogens, accurate identification of the different pathogens is important.			
20.	*16*	Editorial	**Common name:** Karnal bunt or Partial bunt	Another common name for the pest	English	EPPO
21.	*16*	Editorial	**Common name:** Karnal bunt or Partial bunt	Another common name for the pest	English	Georgia, Russian Federation, Netherlands, European Union
22.	*16*	Editorial	**Common name:** English: Karnal bunt Spanish: carbón parcial	A criteria should be follow regarding the inclusion or not of common names in other languages. DP1 and DP2 (T. palmi and PPV) only include common name in English; DP3 (T. granarium) includes English, French, Spanish, Arabic; Guignardia citricarpa draft French, Spanish, Portuguese	English	COSAVE, Paraguay, Chile, Brazil
23.	*16*	Editorial	**Common name:** English: Karnal bunt Spanish: carbón parcial	A criteria should be follow regarding the inclusion or not of common names in other languages. DP1 and DP2 (T. palmi and PPV) only include common name in English; DP3 (T. granarium) includes English, French, Spanish, Arabic; Guignardia citricarpa draft French, Spanish, Portuguese	English	Uruguay
24.	*16*	Editorial	**Common name:** English: Karnal bunt Spanish: carbón parcial	A criteria should be follow regarding the inclusion or not of common names in other languages. DP1 and DP2 (T. palmi and PPV) only include common name in English; DP3 (T. granarium) includes English, French, Spanish, Arabic; Guignardia citricarpa draft French, Spanish, Portuguese	English	Argentina
25.	*20*	Editorial	If no bunted kernels are detected in the sample, the sample is then tested for the presence of teliospores by using a size-selective sieving wash test on three subsamples (section 3.2). If no teliospores are detected after the size-selective sieve wash test, the sample can be declared healthy. If teliospores are detected, the number of teliospores detected will determine what pathway can be taken for identification. If there are less than 10 teliospores detected, it is highly recommended that the size-selective sieve wash test is repeated on new subsamples. However, if	1. To be consistent with figure 3 (paragraph [129]).	English	EPPO, Georgia ,Russian Federation ,Netherlands ,European Union

			there are ~~more than~~ 10 or more teliospores present, the first step is identification of the species of the teliospores (section 4.1) by morphology; the next step is *either* isolation of the teliospores and germination (section 4.2.1) followed by the molecular protocols in sections 4.3.1–4.3.3 *or* removal of individual teliospores (section 4.2.3) followed by a direct real-time polymerase chain reaction (PCR) on the individual teliospores (section 4.3.4). (Refer A, B, C in Figure 3.)			
26.	*20*	Editorial	If no bunted kernels are detected in the sample, ~~the sample~~ it is then tested for the presence of teliospores by using a size-selective sieving wash test on three subsamples (section 3.2). If no teliospores are detected after the size-selective sieve wash test, the sample can be declared healthy. If teliospores are detected, the number of teliospores detected will determine what pathway can be taken for identification. If there are less than 10 teliospores detected, it is highly recommended that the size-selective sieve wash test is repeated on new subsamples. However, if there are more than 10 teliospores present the first step is identification of the species of the teliospores (section 4.1) by morphology; the next step is *either* isolation of the teliospores and germination (section 4.2.1) followed by the molecular protocols in sections 4.3.1–4.3.3 *or* removal of individual teliospores (section 4.2.3) followed by a direct real-time polymerase chain reaction (PCR) on the individual teliospores (section 4.3.4). (Refer A, B, C in Figure 3.)	Better wording	English	COSAVE, Paraguay, Chile, Brazil
27.	*20*	Editorial	If no bunted kernels are detected in the sample, ~~the sample~~ it is then tested for the presence of teliospores by using a size-selective sieving wash test on three subsamples (section 3.2). If no teliospores are detected after the size-selective sieve wash test, the sample can be declared healthy. If teliospores are detected, the number of teliospores detected will determine what pathway can be taken for identification. If there are less than 10 teliospores detected, it is highly recommended that the size-selective sieve wash test is repeated on new subsamples. However, if there are more than 10 teliospores present the first step is identification of the species of the teliospores (section 4.1) by morphology; the next step is *either* isolation of the teliospores and germination (section 4.2.1) followed by the molecular protocols in sections 4.3.1–4.3.3 *or* removal of individual teliospores (section 4.2.3) followed by a direct real-time polymerase chain reaction (PCR) on the individual teliospores (section 4.3.4). (Refer A, B, C in Figure 3.)	Better wording	English	Uruguay
28.	*20*	Editorial	If no bunted kernels are detected in the sample, the sample is then tested for the presence of teliospores by using a size-selective sieving wash test on three subsamples (section 3.2). If no teliospores are detected after the size-selective sieve wash test, the sample can be declared negative ~~healthy~~. If teliospores are detected, the number of teliospores detected will determine what pathway can be taken for identification. If there are less than 10 teliospores detected, it is highly recommended that the size-selective sieve wash test is repeated on new subsamples. However, if there are more than 10 teliospores present the first step is identification of the species of the teliospores (section 4.1) by morphology; the next step is *either* isolation of the teliospores and germination (section 4.2.1) followed by the molecular protocols in sections 4.3.1–4.3.3 *or* removal of individual teliospores (section 4.2.3) followed by a direct real-time polymerase chain reaction (PCR) on the individual teliospores (section 4.3.4). (Refer A, B, C in Figure 3.)	In lab test, the samples are positives or negatives	English	OIRSA
29.	*20*	Editorial	If no bunted kernels are detected in the sample, ~~the sample~~ it is then tested for the presence of teliospores by using a size-selective sieving wash test on three subsamples (section 3.2). If no teliospores are detected after the size-selective sieve wash test, the sample can be declared healthy. If teliospores are detected, the number of teliospores detected will determine what pathway can be taken for identification. If there are less than 10 teliospores detected, it is highly recommended that the size-selective sieve wash test is repeated on new subsamples. However, if there are more than 10 teliospores present the first step is identification of the species of the teliospores (section 4.1) by morphology; the next step is *either* isolation of the teliospores and germination (section 4.2.1) followed by the molecular protocols in sections 4.3.1–4.3.3 *or* removal	Better wording	English	Argentina

			of individual teliospores (section 4.2.3) followed by a direct real-time polymerase chain reaction (PCR) on the individual teliospores (section 4.3.4). (Refer A, B, C in Figure 3.)			
30.	*20*	Substantive	If no bunted kernels are detected in the sample, the sample is then tested for the presence of teliospores by using a size-selective sieving wash test on three subsamples (section 3.2). If no teliospores are detected after the size-selective sieve wash test, the diagnostic result of the tested sample is negative ~~the sample can be declared healthy~~. If teliospores are detected, the number of teliospores detected will determine what ~~pathway~~ method can be taken for identification. If there are less than 10 teliospores detected, it is highly recommended that the size-selective sieve wash test is repeated on new subsamples. However, if there are more than 10 teliospores present the first step is identification of the species of the teliospores (section 4.1) by morphology; the next step is *either* isolation of the teliospores and germination (section 4.2.1) followed by the molecular protocols in sections 4.3.1–4.3.3 *or* removal of individual teliospores (section 4.2.3) followed by a direct real-time polymerase chain reaction (PCR) on the individual teliospores (section 4.3.4). (Refer A, B, C in Figure 3.)	The sample is not declared negative, the diagnostic result is negative "Method": Suggest not to use the term pathway which under IPPC has an specific meaning.	English	COSAVE, Paraguay, Chile, Brazil
31.	*20*	Substantive	If no bunted kernels are detected in the sample, the sample is then tested for the presence of teliospores by using a size-selective sieving wash test on three subsamples (section 3.2). If no teliospores are detected after the size-selective sieve wash test, the diagnostic result of the tested sample is negative ~~the sample can be declared healthy~~. If teliospores are detected, the number of teliospores detected will determine what ~~pathway~~ method can be taken for identification. If there are less than 10 teliospores detected, it is highly recommended that the size-selective sieve wash test is repeated on new subsamples. However, if there are more than 10 teliospores present the first step is identification of the species of the teliospores (section 4.1) by morphology; the next step is *either* isolation of the teliospores and germination (section 4.2.1) followed by the molecular protocols in sections 4.3.1–4.3.3 *or* removal of individual teliospores (section 4.2.3) followed by a direct real-time polymerase chain reaction (PCR) on the individual teliospores (section 4.3.4). (Refer A, B, C in Figure 3.)	The sample is not declared negative, the diagnostic result is negative "Method": Suggest not to use the term pathway which under IPPC has an specific meaning.	English	Uruguay
32.	*20*	Substantive	If no bunted kernels are detected in the sample, the sample ~~is then~~may be tested for the presence of teliospores by using a size-selective sieving wash test on three subsamples (section 3.2). If no teliospores are detected after the size-selective sieve wash test, the sample can be declared healthy. ~~If teliospores are detected, the number of teliospores detected will determine what pathway can be taken for identification. If there~~re less than 10 teliospores detected, it is highly recommended that the size-selective sieve wash test is repeated on new subsamples. However, i~~f~~ there are more than 10 teliospores present the first step is identification of the species of the teliospores (section 4.1) by morphology; the next step is *either* isolation of the teliospores and germination (section 4.2.1) followed by the molecular protocols in sections 4.3.1–4.3.3 *or* removal of individual teliospores (section 4.2.3) followed by a direct real-time polymerase chain reaction (PCR) on the individual teliospores (section 4.3.4). (Refer A, B, C in Figure 3.)	The Annex should keep an objective view based on scientific data. It is up to the NPPO if they want to analyze subsamples any further. It would seem 10 teliospores is an arbitrary number and too prescriptive for this guideline. Where is the scientific data to support this number? Less than 10 teliospores could result from cross contamination. A bunted kernel would have far more than 10 teliospores. This is not a protocol that the U.S. would agree to.	English	United States of America
33.	*20*	Substantive	If no bunted kernels are detected in the sample, the sample is then tested for the presence of teliospores by using a size-selective sieving wash test on three subsamples (section 3.2). If no teliospores are detected after the size-selective sieve wash test, the diagnostic result of the tested sample is negative ~~the sample can be declared healthy~~. If teliospores are detected, the number of teliospores detected will determine what ~~pathway~~ method can be taken for identification. If there are less than 10 teliospores detected, it is highly recommended that the size-selective sieve wash test is repeated on new subsamples. However, if there are more than 10 teliospores present the	The sample is not declared negative, the diagnostic result is negative "Method": Suggest not to use the term pathway which under IPPC has an specific meaning.	English	Argentina

			first step is identification of the species of the teliospores (section 4.1) by morphology; the next step is *either* isolation of the teliospores and germination (section 4.2.1) followed by the molecular protocols in sections 4.3.1–4.3.3 *or* removal of individual teliospores (section 4.2.3) followed by a direct real-time polymerase chain reaction (PCR) on the individual teliospores (section 4.3.4). (Refer A, B, C in Figure 3.)			
34.	*20*	Technical	If no bunted kernels are detected in the sample, the sample is then tested for the presence of teliospores by using a size-selective sieving wash test on three subsamples (section 3.2). If no teliospores are detected after the size-selective sieve wash test, the sample can be declared healthy. If teliospores are detected, the number of teliospores detected will determine what pathway can be taken for identification. If there are less than 10 teliospores detected, it is highly recommended that the size-selective sieve wash test is repeated on new subsamples. However, if there are more than 10 teliospores present the first step is identification of the species of the teliospores (section 4.1) by morphology; the next step is *either* isolation of the teliospores and germination (section 4.2.1) followed by the molecular protocols in sections 4.3.1–4.3.3 *or* removal of individual teliospores (section 4.2.3) followed by a direct real-time polymerase chain reaction (PCR) on the individual teliospores (section 4.3.4). (Refer A, B, C in Figure 3.)	1. The detection process is complicated particularly when both morphology and molecular tests are required but the work flow does not provide guideline on when to conclude a positive or negative detection. The work flow need to address situation when there are discrepancies between results from morphological examination and molecular testing. 2. The present protocol require at least 10 teliospores to be collected if any teliospores are found. An instruction is required on what to do when less than 10 teliospores are found after repeated sampling. 3. When less than 10 teliospores are collected after repeated sampling, a positive results by PCR can be confirmed by DNA sequencing; however, negative results can be questionable and require guideline on confirming the results. 4. It is recommended to add DNA sequence analysis as a confirmation test for diagnostician to consider when required. This will be helpful if the PCR produce weak bands or the CT value of real-time PCR is close to the cut off point.	English	New Zealand
35.	*20*	Technical	If no bunted kernels are detected in the sample, the sample is then tested for the presence of teliospores by using a size-selective sieving wash test on three subsamples (section 3.2). If no teliospores are detected after the size-selective sieve wash test, the sample can be declared as non-infested with *T. indica.* ~~healthy~~. If teliospores are detected, the number of teliospores detected will determine what options ~~pathway~~ can be taken for identification. If there are less than 10 teliospores detected, it is highly recommended that the size-selective sieve wash test is repeated on new subsamples. However, if there are more than 10 teliospores present the first step is identification of the species of the teliospores (section 4.1) by morphology; the next optional step is *either* isolation of the teliospores and germination (section 4.2.1) followed by the molecular protocols in sections 4.3.1–4.3.3 *or* removal of individual teliospores (section 4.2.3) followed by a direct real-time polymerase chain reaction (PCR) on the individual teliospores	The term "healthy" is quite vague and does not adequately depict the concept that the authors are trying to express in this paragraph. It is therefore preferable to indicate that sample can be declared as non-infested with T. indica rather than healthy. The term "pathway" has a very specific meaning in the context of the IPPC and it is not used adequately in this context. If a	English	Canada

			(section 4.3.4). (Refer A, B, C in Figure 3.)	sample is contentious, molecular confirmation is required. However molecular confirmation should be optional and not mandatory. This comment is also reflected in paragraph 129 (Flow chart showing the process to be used for the detection and identification ot Tilletia indica in grain samples).		
36.	*20*	Technical	If no bunted kernels are detected in the sample, the sample is then tested for the presence of teliospores by using a size-selective sieving wash test on three subsamples (section 3.2). If no teliospores are detected after the size-selective sieve wash test, the sample can be declared ~~healthy~~ negative. If teliospores are detected, the number of teliospores detected will determine what pathway can be taken for identification. If there are less than 10 teliospores detected, it is highly recommended that the size-selective sieve wash test is repeated on new subsamples. However, if there are more than 10 teliospores present the first step is identification of the species of the teliospores (section 4.1) by morphology; the next step is *either* isolation of the teliospores and germination (section 4.2.1) followed by the molecular protocols in sections 4.3.1–4.3.3 *or* removal of individual teliospores (section 4.2.3) followed by a direct real-time polymerase chain reaction (PCR) on the individual teliospores (section 4.3.4). (Refer A, B, C in Figure 3.)	Samples are "positive or negative", and is the terminology most used in laboratory tests.	English	Mexico
37.	*22*	Editorial	**3.1 Examination of seeds**~~[1]~~	The reference to a foot note in 3.1 should be removed as there is no foot note at the bottom of that page.	English	Canada
38.	*22*	Substantive	**3.1 Examination of seeds or grains**~~[1]~~	It is not appropriate to use the term seed to refer to both seed and grain. Both are glossary terms	English	COSAVE, Paraguay, Chile, Brazil
39.	*22*	Substantive	**3.1 Examination of seeds or grains**~~[1]~~	It is not appropriate to use the term seed to refer to both seed and grain. Both are glossary terms	English	Uruguay
40.	*22*	Substantive	**3.1 Examination of seeds or grains**~~[1]~~	It is not appropriate to use the term seed to refer to both seed and grain. Both are glossary terms	English	Argentina
41.	*23*	Editorial	Direct visual examinations for bunted kernels or teliospores contaminating seed surfaces are not considered reliable methods for phytosanitary purposes. However, bunted kernels may be detected by visual examination with the naked eye and low power microscopy (10–40× magnification). The whole sample (minimum 1 kg per 30 000 tonnes of seeds) needs to be examined for bunted kernels (Figure 2) or other Poaceae seeds (for example *Lolium* spp.). ~~The sample is assessed for symptoms (Figure 2), and for presence of other Poaceae seeds.~~ The symptoms observed and the presence of the other Poaceae seeds are recorded.	To prevent a useless repetion.	English	EPPO, Georgia, Russian Federation, Netherlands
42.	*23*	Editorial	Direct visual examinations for bunted kernels or teliospores contaminating seed surfaces are not considered reliable methods for phytosanitary purposes. However, bunted kernels may be detected by visual examination with the naked eye and low power microscopy (10–40× magnification). The whole sample (minimum 1 kg per 30 000 tonnes of seeds) needs to be examined for bunted kernels or other Poaceae seeds (for example *Lolium* spp.). ~~The sample is assessed for symptoms (Figure 2), and for presence of other Poaceae seeds~~. The symptoms observed and the presence of the other Poaceae seeds are recorded.	The meaning of this sentence is duplicated in the previous one	English	COSAVE, Paraguay, Chile, Brazil

43.	*23*	Editorial	Direct visual examinations for bunted kernels or teliospores contaminating seed surfaces are not considered reliable methods for phytosanitary purposes. However, bunted kernels may be detected by visual examination with the naked eye and low power microscopy (10–40× magnification). The whole sample (minimum 1 kg per 30 000 tonnes of seeds) needs to be examined for bunted kernels or other Poaceae seeds (for example *Lolium* spp.). ~~The sample is assessed for symptoms (Figure 2), and for presence of other Poaceae seeds~~. The symptoms observed and the presence of the other Poaceae seeds are recorded.	The meaning of this sentence is duplicated in the previous one	English	Uruguay
44.	*23*	Editorial	Direct visual examinations for bunted kernels or teliospores contaminating seed surfaces are not considered reliable methods for phytosanitary purposes. However, bunted kernels may be detected by visual examination with the naked eye and low power microscopy (10–40× magnification). The whole sample (minimum 1 kg per 30 000 tonnes of seeds) needs to be examined for bunted kernels (Figure 2) or other Poaceae seeds (for example *Lolium* spp.). ~~The sample is assessed for symptoms (Figure 2), and for presence of other Poaceae seeds.~~ The symptoms observed and the presence of the other Poaceae seeds are recorded.	To prevent a useless repetition.	English	European Union
45.	*23*	Editorial	Direct visual examinations for bunted kernels or teliospores contaminating seed surfaces are not considered reliable methods for phytosanitary purposes. However, bunted kernels may be detected by visual examination with the naked eye and low power microscopy (10–40× magnification). The whole sample (minimum 1 kg per 30 000 tonnes of seeds) needs to be examined for bunted kernels or other Poaceae seeds (for example *Lolium* spp.). ~~The sample is assessed for symptoms (Figure 2), and for presence of other Poaceae seeds~~. The symptoms observed and the presence of the other Poaceae seeds are recorded.	The meaning of this sentence is duplicated in the previous one	English	Argentina
46.	*23*	Substantive	Direct visual examinations for bunted kernels or teliospores contaminating seed or grain surfaces are not considered reliable methods for phytosanitary purposes. However, bunted kernels may be detected by visual examination with the naked eye and low power microscopy (10–40× magnification). The whole sample (minimum 1 kg per 30 000 tonnes of seeds) needs to be examined for bunted kernels or other Poaceae seeds (for example *Lolium* spp.). The sample is assessed for symptoms (Figure 2), and for presence of other Poaceae seeds. The symptoms observed and the presence of the other Poaceae seeds are recorded.	See explanation on paragraph 7	English	COSAVE, Paraguay, Chile, Brazil
47.	*23*	Substantive	Direct visual examinations for bunted kernels or teliospores contaminating seed or grain surfaces are not considered reliable methods for phytosanitary purposes. However, bunted kernels may be detected by visual examination with the naked eye and low power microscopy (10–40× magnification). The whole sample (minimum 1 kg per 30 000 tonnes of seeds) needs to be examined for bunted kernels or other Poaceae seeds (for example *Lolium* spp.). The sample is assessed for symptoms (Figure 2), and for presence of other Poaceae seeds. The symptoms observed and the presence of the other Poaceae seeds are recorded.	See explanation on paragraph 7	English	Uruguay
48.	*23*	Substantive	Direct visual examinations for bunted kernels or teliospores contaminating seed or grain surfaces are not considered reliable methods for phytosanitary purposes. However, bunted kernels may be detected by visual examination with the naked eye and low power microscopy (10–40× magnification). The whole sample (minimum 1 kg per 30 000 tonnes of seeds) needs to be examined for bunted kernels or other Poaceae seeds (for example *Lolium* spp.). The sample is assessed for symptoms (Figure 2), and for presence of other Poaceae seeds. The symptoms observed and the presence of the other Poaceae seeds are recorded.	See explanation on paragraph 7	English	Argentina
49.	*23*	Technical	Direct visual examinations for bunted kernels or teliospores contaminating seed surfaces are not considered reliable methods for phytosanitary purposes. However, bunted kernels may be detected by visual examination with the naked eye and low power microscopy (10–40× magnification). The whole sample ~~(minimum 1 kg per 30 000 tonnes of seeds)~~ needs to be examined for bunted kernels (Figure 2) or other Poaceae seeds (for example *Lolium* spp.). The	Deleted text: there is not technical parameter for this recommendation "(Figure 2)": It was added because we propose to delete the next sentence (see editorial comment)	English	COSAVE, Paraguay, Chile, Brazil

			sample is assessed for symptoms (Figure 2), and for presence of other Poaceae seeds. The symptoms observed and the presence of the other Poaceae seeds are recorded.			
50.	*23*	Technical	Direct visual examinations for bunted kernels or teliospores contaminating seed surfaces are not considered reliable methods for phytosanitary purposes. However, bunted kernels may be detected by visual examination with the naked eye and low power microscopy (10–40× magnification). The whole sample ~~(minimum 1 kg per 30 000 tonnes of seeds)~~ needs to be examined for bunted kernels (Figure 2) or other Poaceae seeds (for example *Lolium* spp.). The sample is assessed for symptoms (Figure 2), and for presence of other Poaceae seeds. The symptoms observed and the presence of the other Poaceae seeds are recorded.	Deleted text: there is not technical parameter for this recommendation "(Figure 2)": It was added because we propose to delete the next sentence (see editorial comment)	English	Uruguay
51.	*23*	Technical	Direct visual examinations for bunted kernels or teliospores contaminating seed surfaces are not considered reliable methods for phytosanitary purposes. However, bunted kernels may be detected by visual examination with the naked eye and low power microscopy (10–40× magnification). The whole sample (~~minimum 1 kg per 30 000 tonnes of seeds~~) needs to be examined for bunted kernels or other Poaceae seeds (for example *Lolium* spp.). The sample is assessed for symptoms (Figure 2), and for presence of other Poaceae seeds. The symptoms observed and the presence of the other Poaceae seeds are recorded. Delete the sample size in bracket.	The sampling of “minimum 1 kg per 30 000 tonnes of seeds” is too small to make reasonable testing result, if there is huge volume seeds from different farm. Suggest to give the scientific base.	English	China
52.	*23*	Technical	Direct visual examinations for bunted kernels or teliospores contaminating seed surfaces are not considered reliable methods for phytosanitary purposes. However, bunted kernels may be detected by visual examination with the naked eye and low power microscopy (10–40× magnification). The whole sample ~~(minimum 1 kg per 30 000 tonnes of seeds)~~ needs to be examined for bunted kernels (Figure 2) or other Poaceae seeds (for example *Lolium* spp.). The sample is assessed for symptoms (Figure 2), and for presence of other Poaceae seeds. The symptoms observed and the presence of the other Poaceae seeds are recorded.	Deleted text: there is not technical parameter for this recommendation "(Figure 2)": It was added because we propose to delete the next sentence (see editorial comment)	English	Argentina
53.	*25*	Editorial	To help visualize symptoms, kernels can be soaked in 0.2% NaOH for 24 hours (h) at 20 °C. This is especially useful for chemically treated seed lots where coloured dyes may obscure symptoms (Agarwal and Mathur, 1992; Mathur and Cunfer, 1993). With severe infestation and contamination, teliospores may be seen on the surface of seeds (Mathur and Cunfer, 1993).	Write out abbreviation in full the first time where it is used to provide clarity for those who may not be familiar with the abbreviation in question.	English	South Africa
54.	*25*	Technical	To help visualize symptoms, kernels can be soaked in 0.2% NaOH for 24 h at 20 °C (it produces a mild bleaching of the endosperm that makes the blackened infection point out in stark contrast).. This is especially useful for chemically treated seed lots where coloured dyes may obscure symptoms (Agarwal and Mathur, 1992; Mathur and Cunfer, 1993). With severe infestation and contamination, teliospores may be seen on the surface of seeds (Mathur and Cunfer, 1993).	Explains how this action helps to visualize symptoms.	English	EPPO, Georgia, Russian Federation, Netherlands
55.	*25*	Technical	To help visualize symptoms, kernels can be soaked in 0.2% NaOH for 24 h at 20 °C (it produces a mild bleaching of the endosperm that makes the blackened infection point out in stark contrast). This is especially useful for chemically treated seed lots where coloured dyes may obscure symptoms (Agarwal and Mathur, 1992; Mathur and Cunfer, 1993). With severe infestation and contamination, teliospores may be seen on the surface of seeds (Mathur and Cunfer, 1993).	Explains how this action helps to visualize symptoms.	English	European Union
56.	*26*	Substantive	The absence of bunted kernels ~~does~~may not indicate that the sample is free of *T. indica* ~~is not present~~. In the absence of bunted kernel ~~It is important to proceed to~~ the size-selective sieve wash method (section 3.2) may be used for determining whether *T. indica* is present or not present in the sample. If seed of *Lolium* spp. is found contaminating the sample there is a high probability that *T. walkeri* will be detected in the sample as well.	Too prescriptive. The US regulates this pest on the basis of bunted kernels. In certain cases, after taking into consideration other risk factors, the size-selective sieve wash method is sometimes used.	English	United States of America

57.	*27*	Substantive	**3.2 Extraction of teliospores from seeds or grains**	See explanation in paragraph 7	English	COSAVE, Paraguay, Chile, Brazil
58.	*27*	Substantive	**3.2 Extraction of teliospores from seeds or grains**	See explanation in paragraph 7	English	Uruguay
59.	*27*	Substantive	**3.2 Extraction of teliospores from seeds or grains**	See explanation in paragraph 7	English	Argentina
60.	*27*	Technical	**3.2 Extraction of teliospores from seeds**	Add pictures that demonstrate the extraction methods from EPPO Diagnostic protocol PM7/29.	English	New Zealand
61.	*28*	Substantive	The size-selective sieve wash method is the most reliable method for detection of *T. indica* teliospores in a sample of *Triticum aestivum*, *Triticum durum* or *Triticum aestivum* × *Secale cereale*. Detection of teliospores suggests that diseased kernels may be present. It is important that a minimum of three replicates of 50 g are done to ensure detection of teliospores if they are present in the sample (refer to Table 1 for number of samples required to detect different numbers of teliospores). This method has, on average, an 82% efficiency of recovery, and microscopic examinations typically require only a few slides per 50 g sample. The method is described below and further details are available (Peterson *et al.,* 2000; Inman *et al.,* 2003; Wright *et al.,* 2003).	If a bunted kernel is present, a slide is made of spores removed from the bunted kernel using a dissecting needle. The size-selective sieve wash method is the most reliable method for detection of teliospores. Detection of teliospores suggests that diseased kernels may be present. The next step would be to test for the presence of bunted kernels. The bunted kernel method is the only method which clearly identifies a positive infection of the sample. Other methods may only be indicative of cross contamination.	English	United States of America
62.	*28*	Technical	The size-selective sieve wash method is the most reliable method for detection of *T. indica* teliospores in a sample of *Triticum aestivum*, *Triticum durum* or *Triticum aestivum* × *Secale cereale*. It is important that a minimum of three replicates of 50 g are done to ensure detection of teliospores if they are present in the sample (refer to Table 1 for number of samples required to detect different numbers of teliospores). When symptoms have been observed (section 3.1), as many bunted kernels as possible should be included in the three replicates. This method has, on average, an 82% efficiency of recovery, and microscopic examinations typically require only a few slides per 50 g sample. The method is described below and further details are available (Peterson *et al.,* 2000; Inman *et al.,* 2003; Wright *et al.,* 2003).	This sentence could be added in order to increase the probability of detection of T. indica teliospores.	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
63.	*29*	Editorial	Before beginning it is important that all equipment is soaked for 15 min~~utes~~ in a bleach solution (1.6% NaOCl active ingredient), to eliminate the risk of false positives by cross-contamination from previous samples. The bleach kills teliospores and makes them appear hyaline compared with the normally dark, pigmented teliospores. All equipment is rinsed with tap water after soaking.	Deletion of the word “minutes’ for the insertion of “min” since the former was already abbreviated and written in full for consistency with the rest of the text	English	South Africa
64.	*30*	Editorial	The 50 g sample is placed in an Erlenmeyer flask (250 ml) with a 100 ml of 0.01% Tween 20 aqueous solution. The samples are then placed on a shaker for ~~three~~ 3 min~~utes~~ at 200 rpm to release the teliospores from the seeds. The solution and seeds are then poured onto a 53 µm sieve, which is sitting on top of a 20 µm sieve, which is sitting inside a funnel on top of another flask (500 ml). The flasks that contained the seeds are then rinsed twice (approximately 100 ml) with sterile tap water and this is then poured over the seeds sitting in the sieve. The seeds are further washed with sterile tap water (approximately 200–300 ml) using an aspirator bottle to ensure a good removal of the teliospores from the seed. The seeds and the 53 µm sieve are	Use of the numerical 3”for consistency with other numbers.	English	South Africa

			removed. The 20 µm sieve is tilted to a 45° angle and, using an aspirator bottle filled with sterile tap water, the debris is washed on the sieve from the top to the bottom with a sidewards sweeping motion going backwards and forwards. This is to wash all teliospores recovered from the seeds into the lower side of the sieve. The teliospores and debris are then washed into a 15 ml conical centrifuge tube. It is important that polypropylene tubes are used as the teliospores will stick to the sides of polycarbonate tubes, giving false results. These steps are repeated until the 20 µm sieve appears clean. The final volume in the tube will be approximately 8 ml. If necessary, the 20 µm sieve can be examined under a low power microscope to check for any residual teliospores.			
65.	*30*	Substantive	The 50 g sample of untreated seed is placed in an Erlenmeyer flask (250 ml) with a 100 ml of 0.01% Tween 20 aqueous solution. The samples are then placed on a shaker for three minutes at 200 rpm to release the teliospores from the seeds. The solution and seeds are then poured onto a 53 µm sieve, which is sitting on top of a 20 µm sieve, which is sitting inside a funnel on top of another flask (500 ml). The flasks that contained the seeds are then rinsed twice (approximately 100 ml) with sterile tap water and this is then poured over the seeds sitting in the sieve. The seeds are further washed with sterile tap water (approximately 200–300 ml) using an aspirator bottle to ensure a good removal of the teliospores from the seed. The seeds and the 53 µm sieve are removed. The 20 µm sieve is tilted to a 45° angle and, using an aspirator bottle filled with sterile tap water, the debris is washed on the sieve from the top to the bottom with a sidewards sweeping motion going backwards and forwards. This is to wash all teliospores recovered from the seeds into the lower side of the sieve. The teliospores and debris are then washed into a 15 ml conical centrifuge tube. It is important that polypropylene tubes are used as the teliospores will stick to the sides of polycarbonate tubes, giving false results. These steps are repeated until the 20 µm sieve appears clean. The final volume in the tube will be approximately 8 ml. If necessary, the 20 µm sieve can be examined under a low power microscope to check for any residual teliospores.	Important to emphasize the use of untreated seeds or the diagnostic process. Although para 25 refers to procedures for examination of chemically treated seed, it is not clear whether the sieving and molecular tests can be applied to treated seed. We therefore request this is clarified	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
66.	*30*	Substantive	The 50 g sample is placed in an Erlenmeyer flask (250 ml) with a 100 ml of 0.01% Tween 20 aqueous solution. The samples are then placed on a shaker for three minutes at 200 rpm to release the teliospores from the seeds or grains. The solution and seeds or grains are then poured onto a 53 µm sieve, which is sitting on top of a 20 µm sieve, which is sitting inside a funnel on top of another flask (500 ml). The flasks that contained the seeds or grains are then rinsed twice (approximately 100 ml) with sterile tap water and this is then poured over the seeds or grains sitting in the sieve. The seeds or grains are further washed with sterile tap water (approximately 200–300 ml) using an aspirator bottle to ensure a good removal of the teliospores from the seed or grain. The seeds or grains and the 53 µm sieve are removed. The 20 µm sieve is tilted to a 45° angle and, using an aspirator bottle filled with sterile tap water, the debris is washed on the sieve from the top to the bottom with a sidewards sweeping motion going backwards and forwards. This is to wash all teliospores recovered from the seeds or grains into the lower side of the sieve. The teliospores and debris are then washed into a 15 ml conical centrifuge tube. It is important that polypropylene tubes are used as the teliospores will stick to the sides of polycarbonate tubes, giving false results. These steps are repeated until the 20 µm sieve appears clean. The final volume in the tube will be approximately 8 ml. If necessary, the 20 µm sieve can be examined under a low power microscope to check for any residual teliospores.	See explanation on paragraph 7	English	COSAVE, Paraguay, Chile, Brazil
67.	*30*	Substantive	The 50 g sample is placed in an Erlenmeyer flask (250 ml) with a 100 ml of 0.01% Tween 20 aqueous solution. The samples are then placed on a shaker for three minutes at 200 rpm to release the teliospores from the seeds or grains. The solution and seeds or grains are then poured onto a 53 µm sieve, which is sitting on top of a 20 µm sieve, which is sitting inside a funnel on top of another flask (500 ml). The flasks that contained the seeds or grains are then rinsed twice (approximately 100 ml) with sterile tap water and this is then poured over the seeds or grains sitting	See explanation on paragraph 7	English	Uruguay

			in the sieve. The seeds or grains are further washed with sterile tap water (approximately 200–300 ml) using an aspirator bottle to ensure a good removal of the teliospores from the seed or grain. The seeds or grains and the 53 µm sieve are removed. The 20 µm sieve is tilted to a 45° angle and, using an aspirator bottle filled with sterile tap water, the debris is washed on the sieve from the top to the bottom with a sidewards sweeping motion going backwards and forwards. This is to wash all teliospores recovered from the seeds or grains into the lower side of the sieve. The teliospores and debris are then washed into a 15 ml conical centrifuge tube. It is important that polypropylene tubes are used as the teliospores will stick to the sides of polycarbonate tubes, giving false results. These steps are repeated until the 20 µm sieve appears clean. The final volume in the tube will be approximately 8 ml. If necessary, the 20 µm sieve can be examined under a low power microscope to check for any residual teliospores.			
68.	*30*	Substantive	The 50 g sample of untreated seed is placed in an Erlenmeyer flask (250 ml) with a 100 ml of 0.01% Tween 20 aqueous solution. The samples are then placed on a shaker for three minutes at 200 rpm to release the teliospores from the seeds. The solution and seeds are then poured onto a 53 µm sieve, which is sitting on top of a 20 µm sieve, which is sitting inside a funnel on top of another flask (500 ml). The flasks that contained the seeds are then rinsed twice (approximately 100 ml) with sterile tap water and this is then poured over the seeds sitting in the sieve. The seeds are further washed with sterile tap water (approximately 200–300 ml) using an aspirator bottle to ensure a good removal of the teliospores from the seed. The seeds and the 53 µm sieve are removed. The 20 µm sieve is tilted to a 45° angle and, using an aspirator bottle filled with sterile tap water, the debris is washed on the sieve from the top to the bottom with a sidewards sweeping motion going backwards and forwards. This is to wash all teliospores recovered from the seeds into the lower side of the sieve. The teliospores and debris are then washed into a 15 ml conical centrifuge tube. It is important that polypropylene tubes are used as the teliospores will stick to the sides of polycarbonate tubes, giving false results. These steps are repeated until the 20 µm sieve appears clean. The final volume in the tube will be approximately 8 ml. If necessary, the 20 µm sieve can be examined under a low power microscope to check for any residual teliospores.	Important to emphasize the use of untreated seeds for the diagnostic process. Although para 25 refers to procedures for examination of chemically treated seed, it is not clear whether the sieving and molecular tests can be applied to treated seed. We therefore request this is clarified	English	European Union
69.	*30*	Substantive	The 50 g sample is placed in an Erlenmeyer flask (250 ml) with a 100 ml of 0.01% Tween 20 aqueous solution. The samples are then placed on a shaker for three minutes at 200 rpm to release the teliospores from the seeds or grains. The solution and seeds or grains are then poured onto a 53 µm sieve, which is sitting on top of a 20 µm sieve, which is sitting inside a funnel on top of another flask (500 ml). The flasks that contained the seeds or grains are then rinsed twice (approximately 100 ml) with sterile tap water and this is then poured over the seeds or grains sitting in the sieve. The seeds or grains are further washed with sterile tap water (approximately 200–300 ml) using an aspirator bottle to ensure a good removal of the teliospores from the seed or grain. The seeds or grains and the 53 µm sieve are removed. The 20 µm sieve is tilted to a 45° angle and, using an aspirator bottle filled with sterile tap water, the debris is washed on the sieve from the top to the bottom with a sidewards sweeping motion going backwards and forwards. This is to wash all teliospores recovered from the seeds or grains into the lower side of the sieve. The teliospores and debris are then washed into a 15 ml conical centrifuge tube. It is important that polypropylene tubes are used as the teliospores will stick to the sides of polycarbonate tubes, giving false results. These steps are repeated until the 20 µm sieve appears clean. The final volume in the tube will be approximately 8 ml. If necessary, the 20 µm sieve can be examined under a low power microscope to check for any residual teliospores.	See explanation on paragraph 7	English	Argentina
70.	*31*	Editorial	The collected suspension is centrifuged at 1000 *g* for 3 min (this is to collect the teliospores as they are denser than most of the debris ~~detected~~ collected during the wash test). The equation for calculating the relative centrifugal force (RCF (× *g*)) from rpm is RCF = 1.12 r_{max} $(rpm/100)^2$, where	Better word?	English	EPPO

<table>
<tr><td></td><td></td><td></td><td>r_{max} is the maximum radius (mm) from the centre of rotation to the bottom of the centrifuge tube. The supernatant is carefully removed using a new disposable Pasteur pipette, without disturbing the pellet. The pellet can now be examined under the microscope. If the pellet appears to be quite thick, extra water can be added to dilute the suspension before examination under the microscope. The pellet is stirred with a pipette tip before examining to ensure an even suspension is obtained.</td><td></td><td></td><td></td></tr>
<tr><td>71.</td><td>31</td><td>Editorial</td><td>The collected suspension is centrifuged at 1000 g for 3 min (this is to collect the teliospores as they are denser than most of the debris <s>detected</s> collected during the wash test). The equation for calculating the relative centrifugal force (RCF (× g)) from rpm is RCF = 1.12 r_{max} $(rpm/100)^2$, where r_{max} is the maximum radius (mm) from the centre of rotation to the bottom of the centrifuge tube. The supernatant is carefully removed using a new disposable Pasteur pipette, without disturbing the pellet. The pellet can now be examined under the microscope. If the pellet appears to be quite thick, extra water can be added to dilute the suspension before examination under the microscope. The pellet is stirred with a pipette tip before examining to ensure an even suspension is obtained.</td><td>Better word?</td><td>English</td><td>Georgia, Russian Federation, Netherlands, European Union</td></tr>
<tr><td>72.</td><td>33</td><td>Editorial</td><td>Table 1. Number of replicate 50 g subsamples needed to detect differing levels of contamination with specified confidences, assuming an equal distribution of teliospores (Peterson et al., 2000).</td><td>Table 1 (paragraphs [33] and [34]) should be placed immediately after paragraph [28] where it is referred to.</td><td>English</td><td>EPPO, Georgia, Russian Federation, Netherlands, European Union</td></tr>
<tr><td>73.</td><td>34</td><td>Editorial</td><td>
<table>
<tr><td></td><td colspan="3">No. of replicate samples required for detection according to level of confidence (%)</td></tr>
<tr><td>Contamination level (no. of teliospores per 50 g sample)</td><td>99%</td><td>99.9 %</td><td>99.99%</td></tr>
<tr><td>1</td><td>3</td><td>5</td><td>6</td></tr>
<tr><td>2</td><td>2</td><td>3</td><td>4</td></tr>
<tr><td>5</td><td>1</td><td>1</td><td>1</td></tr>
</table>
</td><td>Table 1 (paragraphs [33] and [34]) should be placed immediately after paragraph [28] where it is referred to.</td><td>English</td><td>EPPO</td></tr>
<tr><td>74.</td><td>34</td><td>Editorial</td><td>
<table>
<tr><td></td><td colspan="3">No. of replicate samples required for detection according to level of confidence (%)</td></tr>
<tr><td>Contamination level (no. of teliospores per 50 g</td><td>99%</td><td>99.9%</td><td>99.99%</td></tr>
</table>
</td><td>Table 1 (paragraphs [33] and [34]) should be placed immediately after paragraph [28] where it is referred to.</td><td>English</td><td>Georgia, Russian Federation, Netherlands, European Union</td></tr>
</table>

			sample)					
			1	3	5	6		
			2	2	3	4		
			5	1	1	1		
75.	36	Editorial	Identification of *Tilletia indica* is based either on symptoms on kernels and morphology of ~~the~~ teliospores, or morphology of teliospores and detection of the unique DNA sequence by PCR techniques (see Figure 3).			For consistency with the second line of this paragraph and with [129] (figure 3).	English	EPPO
76.	36	Editorial	Identification of *Tilletia indica* is based either on symptoms on kernels and morphology of ~~the~~ teliospores, or morphology of teliospores and detection of the unique DNA sequence by PCR techniques (see Figure 3).			For consistency with the second line of this paragraph and with [129] (figure 3).	English	Georgia, Russian Federation, Netherlands, European Union
77.	36	Editorial	Identification of *Tilletia indica* is based either on symptoms on kernels and morphology of the teliospores, or morphology of teliospores and detection of the unique DNA sequence by one of the PCR techniques (see Figure 3).			Please confirm if only one or all of the molecular protocols in sections 4.3.1 to 4.3.3 are required to be tested.	English	New Zealand
78.	36	Editorial	Identification of *T.~~illetia~~ indica* is based either on symptoms on kernels and morphology of the teliospores, or morphology of teliospores and detection of the unique DNA sequence by PCR techniques (see Figure 3).			Abbreviation of Tilletia as it has already been written in full in paragraph 7, for consistency.	English	South Africa
79.	37	Editorial	**4.1 Morphology of teliospores**			For consistency with [36].	English	EPPO
80.	37	Editorial	**4.1 Morphology of teliospores**			For consistency with [36].	English	Georgia, Russian Federation, Netherlands, European Union
81.	38	Editorial	When suspect teliospores are found in a wash test, the seeds in both the washed subsample(s) and the larger sample could be re-examined for symptoms. If symptoms are found, these should be confirmed by microscopic examination of the teliospores. Any grass seeds found in the sample should also be examined for signs of bunt infestation and, if found, the associated teliospores should be examined microscopically. If the teliospores found in the wash test are the same as those found on bunted kernels a diagnosis can be made. If, however, there are no bunted kernels found in the larger sample, testing with oine of the molecular tests (4.3.1-4.3.3) are recommended for identification.			Please confirm if only one or all of the molecular protocols in sections 4.3.1 to 4.3.3 are required to be tested.	English	New Zealand
82.	38	Substantive	When suspect teliospores are found in a wash test, the seeds or grains in both the washed subsample(s) and the larger sample could be re-examined for symptoms. If symptoms are found, these should be confirmed by microscopic examination of the teliospores. Any grass seeds found in the sample should also be examined for signs of bunt infestation and, if found, the associated			See explanation in paragraph 7	English	COSAVE, Paraguay, Chile, Brazil

			teliospores should be examined microscopically. If the teliospores found in the wash test are the same as those found on bunted kernels a diagnosis can be made. If, however, there are no bunted kernels found in the larger sample, molecular tests are recommended for identification.			
83.	*38*	Substantive	When suspect teliospores are found in a wash test, the seeds or grains in both the washed subsample(s) and the larger sample could be re-examined for symptoms. If symptoms are found, these should be confirmed by microscopic examination of the teliospores. Any grass seeds found in the sample should also be examined for signs of bunt infestation and, if found, the associated teliospores should be examined microscopically. If the teliospores found in the wash test are the same as those found on bunted kernels a diagnosis can be made. If, however, there are no bunted kernels found in the larger sample, molecular tests are recommended for identification.	See explanation in paragraph 7	English	Uruguay
84.	*38*	Substantive	When suspect teliospores are found in a wash test, the seeds or grains in both the washed subsample(s) and the larger sample could be re-examined for symptoms. If symptoms are found, these should be confirmed by microscopic examination of the teliospores. Any grass seeds found in the sample should also be examined for signs of bunt infestation and, if found, the associated teliospores should be examined microscopically. If the teliospores found in the wash test are the same as those found on bunted kernels a diagnosis can be made. If, however, there are no bunted kernels found in the larger sample, molecular tests are recommended for identification.	See explanation in paragraph 7	English	Argentina
85.	*39*	Editorial	Table 2 lists morphological characteristics of *T. ~~illetia~~ indica* teliospores and includes the common *Tilletia* species that can be found in seeds or grain shipments and confused with *T. indica.*	Abbreviation of Tilletia as it has already been written in full in paragraph 7, for consistency.	English	South Africa
86.	*41*	Technical	*Tilletia indica* teliospores are globose to subglobose, sometimes with a small hyphal fragment (more common on immature teliospores, but occasionally on mature teliospores), mostly 22–61~~47~~ µm in diameter, occasionally larger (mean 35–41 µm); pale orange/brown to dark, reddish brown; mature teliospores are black and opaque (Figures 4 and 5); densely ornamented with sharply pointed to truncate spines, occasionally with curved tips, 1.4~~5~~–7~~5~~.0 µm high, which in surface view appear as either individual spines (densely echinulate) or as closely spaced, narrow ridges (finely cerebriform) (Figures 4 and 5); the spines are covered by a thin hyaline membrane (CMI, 1983; Carris *et al.,* 2006).	Range in the diameter size of teliosposres and projections is slightly larger than what is referrred to in the current text - reference: NAPPO RSPM 21- Harmonized Procedure for Morphologically Distinguishing Teliospores of Karnal Bunt from Ryegrass Bunt, Rice Smut and Similar Smuts available at http://www.nappo.org/en/data/files/download/PDF/RSPM21-Rev10-08-09-e.pdf	English	Canada
87.	*42*	Editorial	Sterile cells of *T. indica* are globose, subglobose to lachrymiform (tear-shaped), yellowish brown, 10–28 µm × 48 µm, with or without an apiculus (short stalk), with smooth walls up to 7 µm thick and laminated. Sterile cells are likely to be uncommon in sieved washings (CMI, 1983; Carris *et al.,* 2006).	Recommend to add a picture of sterile cells for morphological identification.	English	New Zealand
88.	*45*	Editorial	Other tuberculate-spored *Tilletia* species may be confused with *T. indica* (Durán and Fischer, 1961; Durán, 1987; Pimentel *et al.,* 1998). These species are less likely to be found as contaminants of *Triticum aestivum*, but they include *Tilletia barclayana sensu lato* (smut of various Poaceae, e.g. *Panicum* and *Paspalum*), *Tilletia eragrostidis* (on *Eragrostis*), *Tilletia ehrhartae* (on *Ehrharta calycina*), *Tilletia inolens* (on *Lachnagrostis filiformis*), *Tilletia rugispora* (on *Paspalum*), *Tilletia bouteloua*e (on *Bouteloua gracilis*). However, Pascoe *et al.* (2005) showed that in Australia, *T. walkeri* and *T. ehrhartae* are common contaminants of harvested *Triticum aestivum*. None of these morphologically similar species have been found to naturally ~~infect~~ infest *Triticum aestivum*. In the United States, the morphologically and genetically similar fungus *Tilletia walkeri* and also *Tilletia horrida* are known contaminants of harvested *Triticum aestivum* (Smith *et al.,* 1996; Castlebury and Carris, 1999; Cunfer and Castlebury, 1999). The most important morphological characters that	1. in ISPM 5: "infest"/infestation" is used instead of "infect"/"infection". 2. Delete "exospore" to be consistent with the word used in table 2, [56].	English	EPPO, Georgia, Russian Federation, Netherlands, European Union

			discriminate *T. indica*, *T. walkeri, T. horrida* and *T. ehrhartae* are teliospore size (range and mean), ~~exospore~~ surface ornamentation and colour (Table 2; Figures 4–8). The literature on spore sizes is often variable. Spore size is affected by the mounting medium and by heating treatments.			
89.	*45*	Editorial	Other tuberculate-spored *Tilletia* species may be confused with *T. indica* (Durán and Fischer, 1961; Durán, 1987; Pimentel *et al.,* 1998). These species are less likely to be found as contaminants of *Triticum aestivum*, but they include *Tilletia barclayana sensu lato* (smut of various Poaceae, e.g. *Panicum* and *Paspalum*), *Tilletia eragrostidis* (on *Eragrostis*), *Tilletia ehrhartae* (on *Ehrharta calycina*), *Tilletia inolens* (on *Lachnagrostis filiformis*), *Tilletia rugispora* (on *Paspalum*), *Tilletia bouteloua*e (on *Bouteloua gracilis*). However, Pascoe *et al.* (2005) showed that in Australia, *T. walkeri* and *T. ehrhartae* are common contaminants of harvested *Triticum aestivum*. None of these morphologically similar species have been found to naturally infect *Triticum aestivum*. In the United States, the morphologically and genetically similar fungus *Tilletia walkeri* and also *Tilletia horrida* are known contaminants of harvested *Triticum aestivum* (Smith *et al.,* 1996; Castlebury and Carris, 1999; Cunfer and Castlebury, 1999). The most important morphological characters that discriminate *T. indica*, *T. walkeri, T. horrida* and *T. ehrhartae* are teliospore size (range and mean), exospore ornamentation and colour (Table 2; Figures 4–8). The literature on spore sizes is often variable. Spore size is affected by the mounting medium and by heating treatments.	'Exospore' is correct but the simpler term 'spore' is more appropriate here.	English	New Zealand
90.	*48*	Editorial	There are now two methods available to confirm the identification of spores detected in the sieve wash test (section 3.2). There is the standard procedure of recovering the teliospores from the slide and inducing their germination (section 4.2.1), and a new procedure developed by Tan *et al.* (2009) which enables PCR to be done directly on ~~a~~ single teliospores recovered from the slide (section 4.2.3).	1. Consistency 2. Consistency with [49] (section 4.2.1). 3.Consistency with [60].	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
91.	*48*	Editorial	There are now two methods available to confirm the identification of spores detected in the sieve wash test. There is the standard procedure of recovering the spores from the slide (section 4.2.1) and a new procedure developed by Tan *et al.* (2009) which enables PCR to be done directly on a single spore (section 4.2.3).	Suggest -There are now two methods available to confirm the identification of recover spores from detected in the sieve wash test. There is the standard procedure of recovering the spores from the slide (section 4.2.1) and a new procedure developed by Tan et al. (2009) which enables PCR to be done directly on a single spore (section 4.2.3). The methods are for the recovery of spores rather than for the confirmation of identification.	English	New Zealand
92.	*50*	Editorial	*T. indica* is a facultative biotroph. To produce cultures, teliospores are soaked in water, quickly surface-sterilized and then germinated on water-agar plates. The teliospores can be recovered from the slides and cover slips by washing them with distilled water over the 20 µm sieve and then into a clean sterile conical centrifuge tube (as in section 3.2). The volume should be approximately 3–5 ml. The tubes are incubated overnight at 21 °C to hydrate the teliospores and make fungal and bacterial contaminants more susceptible to subsequent surface sterilization. After incubating overnight, pellet the teliospores by centrifuging at 1200 *g* for 3 min.	The two first sentences of [50] should be a distinct paragraph, because they introduce the paragraphs that follow (including the other sentences of [50]).	English	EPPO, Georgia, Russian Federation, Netherlands, European Union

<table>
<tr><td>93.</td><td>52</td><td>Technical</td><td>The pellet is then plated onto 2% water agar with antibiotics (WA+A) by resuspending in 1 ml of SDW and placing 200 µl of the teliospore suspension aseptically onto the plates and spreading with a sterile spreader. Incubate the WA+A plates at 21 °C with a 12 h light cycle. Leave for about 5 days before sealing plates or placing the plates inside clear polyethylene bags.</td><td>It would be useful to get examples of antibiotics and concentrations. We note that 60 mg penicillin G (Na salt) and 200 mg streptomycin sulphate per litre of agar has been used (EPPO protocol).</td><td>English</td><td>EPPO, Georgia, Russian Federation, Netherlands, European Union</td></tr>
<tr><td>94.</td><td>55</td><td>Editorial</td><td>Table 2. Morphological characteristics of teliospores of Tilletia indica, Tilletia walkeri, Tilletia horrida and Tilletia ehrhartae, and hosts associated with these four species.</td><td>1. Table 2 (paragraphs [55] and [56]) should be located immediately after paragraph [39] where it is referred to. – 2. Introduction of "teliospores of" for consistency with [39]. 3. The presence of the column "host" in the table should be taken into account in the title.</td><td>English</td><td>EPPO, Georgia, Russian Federation, Netherlands, European Union</td></tr>
<tr><td>95.</td><td>56</td><td>Editorial</td><td>
<table>
<tr><th>Species</th><th>~~Spore~~Teliospore size range (µm)</th><th>~~Spore~~Teliospore size mean (µm)</th><th>~~Spore~~Teliospore colour</th><th>~~Spore~~Teliospore shape</th><th>~~Spore~~Teliospore sheath</th><th>~~Spore~~Teliospore spines</th><th>Hosts</th></tr>
<tr><td>T. indica[a]</td><td>28–54</td><td>35–41</td><td>Brown to dark reddish brown, opaque</td><td>Globose to subglobose</td><td>Present</td><td>1.4–5 µm

In surface view, densely echinulate or as closely spaced, narrow ridges (finely cerebriform).

In median view, smoother more complete outline due to spines being densely arranged</td><td>Triticum spp.</td></tr>
</table>
</td><td>1 Table 2 (paragraphs [55] and [56]) should be located immediately after paragraph [39] where it is referred to. 2 "Teliospore" is the word used in [39]. 3.They are often several hosts for one Tilletia species. 4.Two full stops are missing for T. ehrhartae in the column "Spore spines" (after "spines" and after "scales").</td><td>English</td><td>EPPO, Georgia, Russian Federation, Netherlands, European Union</td></tr>
</table>

								occasionally with curved tips				
			T. walkeri[b]	28–35	30–31	Pale yellow to dark reddish brown (never black/opaque)	Globose	Present. Extending to tips of projections, hyaline to yellowish brown	3–6 µm Coarse +/- cerebriform. Wide incompletely cerebriform ridges in surface view. In median view, profile is irregular with gaps between spines	*Lolium perenne* and *L. multiflorum*		
			T. horrida[c]	14–36 (mature		Light to dark chestnut brown, can be semi-opaque	Globose to subglobose	Present. Extending to the ends of the spines, hyaline to tinted	1.5–4 µm Frequently curved, and appear as polygonal scales in surface view	*Oryza* spp.		
			T. ehrhartae[d]	17–25		Very dark olivaceous brown when mature. Can be opaque because of melanization of the scales.	Globose to subglobose	Present. Extending to the apex of the spines or slightly beyond	1–2.5 µm Cylindrical or slightly tapered spines. In surface view, rarely cerebriform. Larger, acute polygonal	*Ehrharta calycina*		

			scales. In median view, broadly truncated to slightly rounded at apex			

96.	*56*	Editorial	**Species**	**Spore size range (µm)**	**Spore size mean (µm)**	**Spore colour**	**Spore shape**	**Spore sheath**	**Spore spines**	**Host**	Suggest to present Table 2 in landscape page setup.	English	New Zealand
			T. indica[a]	28–54	35–41	Brown to dark reddish brown, opaque	Globose to subglobose	Present	1.4–5 µm In surface view, densely echinulate or as closely spaced, narrow ridges (finely cerebriform). In median view, smoother more complete outline due to spines being densely arranged occasionally with curved tips	*Triticum* spp.			

				T. walkeri[b]	28–35	30–31	Pale yellow to dark reddish brown (never black/ opaque)	Globose	Present. Extending to tips of projections, hyaline to yellowish brown	3–6 µm Coarse +/- cerebriform. Wide incompletely cerebriform ridges in surface view. In median view, profile is irregular with gaps between spines	*Lolium perenne* and *L. multiflorum*			
				T. horrida[c]	14–36 (mature <25)		Light to dark chestnut brown, can be semi-opaque	Globose to subglobose	Present. Extending to the ends of the spines, hyaline to tinted	1.5–4 µm Frequently curved, and appear as polygonal scales in surface view	*Oryza* spp.			
				T. ehrhartae[d]	17–25		Very dark olivaceous brown when mature. Can be opaque because of melan	Globose to subglobose	Present. Extending to the apex of the spines or slightly beyond	1–2.5 µm Cylindrical or slightly tapered spines In surface view, rarely cerebriform. Larger, acute polygonal scales In median view,	*Ehrharta calycina*			

							ization of the scales .			broadly truncated to slightly rounded at apex			
97.	*56*	Substantive									The mean spore size of T. horrida and T. ehrhartae should be added. The cell of mean spore size of T. horrida and T. ehrhartae is blank in Table 2.	English	Japan

Species	**Spore size range (µm)**	**Spore size mean (µm)**	**Spore colour**	**Spore shape**	**Spore sheath**	**Spore spines**	**Host**
T. indica[a]	28–54	35–41	Brown to dark reddish brown, opaque	Globose to subglobose	Present	1.4–5 µm In surface view, densely echinulate or as closely spaced, narrow ridges (finely cerebriform). In median view, smoother more complete outline due to spines being densely arranged occasionally with curved tips	*Triticum* spp.
T. walkeri[b]	28–35	30–31	Pale yellow to dark reddish brown (never black/	Globose	Present. Extending to tips of projections, hyaline to yellowish brown	3–6 µm Coarse +/- cerebriform. Wide incompletely cerebriform	*Lolium perenne* and *L. multiflorum*

					opaque)			ridges in surface view. In median view, profile is irregular with gaps between spines			
			T. horrida[c]	14–36 (mature <25)		Light to dark chestnut brown, can be semi-opaque	Globose to subglobose	Present. Extending to the ends of the spines, hyaline to tinted	1.5–4 µm Frequently curved, and appear as polygonal scales in surface view	*Oryza* spp.	
			T. ehrharta e[d]	17–25		Very dark olivaceous brown when mature. Can be opaque because of melanization of the scales.	Globose to subglobose	Present. Extending to the apex of the spines or slightly beyond	1–2.5 µm Cylindrical or slightly tapered spines In surface view, rarely cerebriform. Larger, acute polygonal scales In median view, broadly truncated to slightly rounded at apex	*Ehrharta calycina*	
98.	*62*	Editorial	On another slide place a single piece of a cover slip (1 × 1 mm^2) that has been sterilized (autoclave at 121 °C for 15 min). Place a 1 µl drop of Tris-EDTA (TE) buffer onto this piece of cover slip.								The text is confusing by referring to teliospores (plural). As the real time	English	EPPO, Georgia, Russian

			Under either a compound or dissecting microscope, a single teliospore~~s are~~ is picked off with a very fine needle and placed into the TE buffer droplet. The teliospore~~s~~ will transfer to the droplet. Using forceps place another small piece of a cover slip on top to make a sandwich. The teliospore~~s are~~ is crushed by using the forceps to press down on the cover slip, and then the glass sandwich is transferred into a 0.2 ml PCR tube. The procedure then followed is as described in section 4.3.4.1.	PCR method applies to amplification of an individual teliospore, we suggest the text refers to single spores.		Federation, Netherlands, European Union
99.	*62*	Editorial	On another slide place a single piece of a cover slip (1 × 1 mm^2) that has been sterilized (autoclave at 121 °C for 15 min). Place a 1 µl drop of Tris-EDTA (TE) buffer onto this piece of cover slip. Under either a compound or dissecting microscope, single teliospores are picked off with a very fine needle and placed into the TE buffer droplet. The teliospores will transfer to the droplet. Using forceps place another small piece of a cover slip on top to make a sandwich. The teliospores are crushed by using the forceps to press down on the cover slip, and then the glass sandwich is transferred into a 0.2 ml PCR tube. The procedure then followed is as described in section 4.3.4.1.	On another slide place a single piece of a cover slip (1 × 1 mm2) that has been sterilized (autoclave at 121 °C for 15 min or heat at 170°C for 2 h). Place a 1 µl drop of Tris-EDTA (TE) buffer onto this piece of cover slip. Under either a compound or dissecting microscope, single teliospores are picked off with a very fine needle and placed into the TE buffer droplet. The teliospores will transfer to the droplet. Using forceps place another small piece of a cover slip on top to make a sandwich. The teliospores are crushed by using the forceps to press down on the cover slip, and then the glass sandwich is transferred into a 0.2 ml PCR tube. Crush the cover slip further wit h a pipette tip. The procedure then followed is as described in section 4.3.4.1. 1. The method for sterilizing cover slip in the original paper is to heat at 170°C for 2 h. Is this method inappropriate? 2. The original paper described an additional step to crush the cover slip in PCR tube with a pipette tip.	English	New Zealand
100.	*62*	Substantive	On another slide place a single piece of a cover slip (1 × 1 mm^2) that has been sterilized (autoclave at 121 °C for 15 min). Place a 1 µl drop of Tris-EDTA (TE) buffer onto this piece of cover slip. Under either a compound or dissecting microscope, single teliospores are picked off with a very fine needle and placed into the TE buffer droplet. The teliospores will transfer to the droplet. Using forceps place another small piece of a cover slip on top to make a sandwich. The teliospores are crushed by using the forceps to press down on the cover slip, and then the glass sandwich is transferred into a 0.2 ml PCR tube. The procedure then followed is as described in section 4.3.4.1.	Clarity is required pertaining to the referenced cover slip of 1 x1 mm2. The size of the cover slip is too small and it could be very difficult to work with it.	English	South Africa
101.	*64*	Editorial	There are a number of molecular methods available for *T. indica*. ~~The three main protocols described rely upon the germination of the teliospores, so that DNA can be extracted from the mycelial mat produced. The germination of the teliospores can take up to three weeks.~~ Any ~~one of the~~ method~~s~~ described below may be used~~. I~~, however, it is essential that reference material (positive controls) have been obtained from experts in this area (refer to section 6).	Proposed rearrangement of the paragraph for clarity and to ensure that concepts applying to all four methods are clear.	English	EPPO, Georgia, Russian Federation, Netherlands, European Union

			It should be noted that the first three protocols described rely ~~These methods work well, but are dependent~~ upon the germination of the teliospores so that enough DNA can be extracted from the mycelial mat produced ~~for the protocols to work~~. Peterson *et al.* (2000) found that the average teliospore germination rate was 55%, which severely reduces the chances of identifying the teliospores by these three molecular methods.			
102.	*64*	Editorial	There are a number of molecular methods available for *T. indica*. The three main protocols described rely upon the germination of the teliospores, so that DNA can be extracted from the mycelial mat produced. The germination of the teliospores can take up to three weeks. Any one of the methods described below may be used. It is essential that reference material (positive controls) have been obtained from experts in this area (refer to section 6). These methods work well, but are dependent upon the germination of the teliospores so that enough DNA can be extracted for the protocols to work. Peterson *et al.* (2000) found that the average teliospore germination rate was 55%, which severely reduces the chances of identifying the teliospores by molecular methods.	There are a number of molecular methods available for T. indica. The first three main protocols described below rely upon the germination of the teliospores, so that DNA can be extracted from the mycelial mat produced. The germination of the teliospores can take up to three weeks. Any one of the methods described below may be used. It is essential that reference material (positive controls) have been obtained from experts in this area (refer to section 6). These molecular methods work well, but are dependent upon the germination of the teliospores so that enough DNA can be extracted for the protocols to work. Peterson et al. (2000) found that the average teliospore germination rate was 55%, which severely reduces the chances of identifying the teliospores by molecular methods. There are a total of four molecular protocols described in this section.	English	New Zealand
103.	*65*	Editorial	Diagnostically significant differences exist between *T. indica*, *T. walkeri* and *T. horrida* in their nuclear and mitochondrial DNA (mtDNA). Interspecific polymorphisms have been identified using various PCR methods, including random amplification of polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) methods (Laroche *et al.,* 1998; Pimentel *et al.,* 1998). In the nuclear ribosomal (rDNA) internal transcribed spacer (ITS) ITS1 and ITS2 regions~~,~~ there is a > 98% similarity between *T. walkeri* and *T. indica* sequences (Levy *et al.,* 1998). However, within the ITS1 region, *T. walkeri* has a diagnostically important restriction enzyme site (Sca 1) that is not present with *T. indica*, *T. horrida* or other closely related species (Levy *et al.,* 1998; Pimentel *et al.,* 1998). With mtDNA, sequence differences have enabled species-specific primers to be designed for *T. indica* and *T. walkeri* (Frederick *et al.,* 2000). These primers can be used in conventional PCR assays, ~~or~~ in a TaqMan system in conjunction with a probe (Frederick *et al*., 2000) or real-time assay with five probes (Tan et al., 2009).	1. "MtDNA" is used on the second last sentence of this paragraph. 2. Need to refer to the direct real time PCR on teliospores as this is an introductory section	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
104.	*66*	Substantive	**4.3.1 Restriction enzyme analysis of the ITS1 region**	Include Figures 16 and 17 (PCR-RFLP patterns) from EPPO protocol PM7/29.	English	New Zealand

105.	*67*	Editorial	The target gene region is the ITS region of the nuclear ribosomal RNA gene (Pimentea~~l~~ *et al*., 1998). The PCR amplicon produced includes both ITS1 and ITS2 and the conserved fragment 5.8 S. This amplicon is approximately 670 base pairs (bp) including primer sequences. Oligonucleotides used:	Typo	English	OIRSA
106.	*67*	Editorial	The target gene region is the ITS region of the nuclear ribosomal RNA gene (Pimentea~~l~~ *et al*., 1998). The PCR amplicon produced includes both ITS1 and ITS2 and the conserved fragment 5.8 S. This amplicon is approximately 670 base pairs (bp) including primer sequences. Oligonucleotides used:	The correct name in spanish is Pimentel	English	Mexico
107.	*71*	Editorial	PCR to produce restriction amplicon uses the following master mix (concentration per 50 µl single reaction): 28.75 µl of MGW, 5.0 µl of 10X PCR buffer containing 15 mM $MgCl_2$ (Applied Biosystems)[2], 1.0 µl each of dNTPs (10 mM) (final concentration of each of these is 0.2 mM), 1.25 µl AmpliTaq (5U/µl) (Applied Biosystems)[2], 5.0 µl of each primer (5 µM), and 1 µl neat extracted DNA. PCR cycling parameters: 94 °C denaturation for 2 min, 30 cycles of 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min, and then a 72 °C extension step for 10 min.	PCR for this assay uses the following mastermix (concentration per 25µl single reaction): 20.2 µl of MGW, 2.5 µl of 10X PCR buffer containing 15mM MgCl2 (Applied Biosystems)2, 0.25 µl of each dNTPs (10 mM) (final concentration of 0.1 mM each), 0.1 µl AmpliTaq (5 U/µl) (Applied Biosystems)2, 0.1 µl of each primer (25 µM), 1.0 µl neat extracted DNA obtained as described above. PCR cycling parameters: 94 °C denaturation for 1 min, 25 cycles of 94 °C for 15 s, 65 °C for 15 s, 72 °C for 15 s, and followed by a final extension step of 72 °C extension step for 6 min. Clarity	English	New Zealand
108.	*71*	Substantive	PCR to produce restriction amplicon uses the following master mix (concentration per 50 µl single reaction): 28.75 µl of MGW, 5.0 µl of 10X PCR buffer containing 15 mM $MgCl_2$ (Applied Biosystems)[2], 1.0 µl each of dNTPs (10 mM) (final concentration of each of these is 0.2 mM), 1.25 µl AmpliTaq (5U/µl) (Applied Biosystems)[2], 5.0 µl of each primer (5 µM), and 1 µl ~~neat~~ extracted DNA. PCR cycling parameters: 94 °C denaturation for 2 min, 30 cycles of 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min, and then a 72 °C extension step for 10 min.	Deletion of the word “neat” since there is only purified or extracted DNA; The National Plant Protection Organization of South Africa seeks clarity on why the word “neat” has been used.	English	South Africa
109.	*71*	Technical	PCR to produce restriction amplicon uses the following master mix (concentration per 50 µl single reaction): 28.75 µl of MGW, 5.0 µl of 10X PCR buffer containing 15 mM $MgCl_2$ (Applied Biosystems)[2], 1.0 µl each of dNTPs (10 mM) (final concentration of each of these is 0.2 mM), 1.25 µl AmpliTaq (5U/µl) (Applied Biosystems)[2], 5.0 µl of each primer (5 µM), and 1 µl neat extracted DNA. PCR cycling parameters: 94 °C denaturation for 2 min, 30 cycles of 94 °C for 1 min, 54 °C for 1 min, 72 °C for 1 min, and then a 72 °C extension step for 10 min.	First sentence: For consistency with the other mix compositions, the final concentration and volume per reaction of each compound should be given, instead of volumes and initial concentrations. Such information is important for the lab to reproduce the test and to avoid any errors.	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
110.	*75*	Editorial	The assay was designed by Frederick*et al*. (2000) using the mitochondrial DNA producing an amplicon of ~~163~~ 414 bp. Oligonucleotides used for *T. indica* are:	According to the original publication of Frederick et al, the primers used (Tin3 and Tin4) produce an amplicon of 414 bp	English	EPPO, Georgia, Russian Federation, Netherlands,
111.	*75*	Substantive	The assay was designed by Frederick*et al*. (2000) using the mitochondrial DNA producing an amplicon of ~~163~~414 bp. Oligonucleotides used for *T. indica* are:	According to the original publication of Frederick et al, the primers used	English	European Union

				(Tin3 and Tin4) produce an amplicon of 414 bp		
112.	*75*	Technical	The assay was designed by Frederick*et al*. (2000) using the ~~mitochondrial~~ genomic DNA producing an amplicon of 163 bp. Oligonucleotides used for *T. indica* are: Change ”mitochondrial DNA” (Frederick et al. 2000) to ”genomic DNA”	The proposal may be wrong. The reason:(a) in GenBank, three of T. indica mitochondrial DNA sequence (Ferreira MA et al. Submitted,the accession number AF218058, AF218059 and AF218060), this mitochondrial sequence share low homology with the T. indica mitochondrial DNA sequence (accession number DQ993184). BLAST found they were only about 30% homology. (b)The base composition of the AT content in mitochondrial DNA is higher than the GC content which is generally 30 40% (Kurtzman, 1985), but AT content of three sequence in the GenBank submitted in Ferreirais 43.5%, which were lower than that of GC(56.55%). (C) The primer Tin3/Tin4 can not amplification of mitochondrial DNA to give the desired strip, in which the primer is derived from the extracted and purified of indica mitochondrial DNA Therefore, this three logged mitochondrial sequence is not T. indica mitochondrial sequence, but should be genomic sequence. Reference: Kurtzman C P. Molecular taxonomy of the fungi. In: Gene manipulations in fungi. Academic Press, Inc., Orlando, Fla, 1985.	English	China
113.	*75*	Technical	The assay was designed by Frederick*et al*. (2000) using the mitochondrial DNA producing an amplicon of ~~163~~ 414 bp. Oligonucleotides used for *T. indica* are:	According to Frederick et al. (2000), it is 414bp.	English	Japan
114.	*78*	Editorial	DNA is extracted from mycelium. This is done by grinding 0.5–1.0 g of mycelium in a 1.5 ml microcentrifuge tube with 75 µl~~L~~ lysis buffer and grinding with a sterile pestle attached to a power drill. An additional 75 µ~~L~~l lysis buffer is added before extracting DNA using a proprietary DNA extraction kit for fungi. No DNA cleanup is required. Either use extracted DNA immediately or store overnight at 4 °C or at −20 °C for longer periods.	Removal of caps “L” where it appears and replace with “l” for consistency	English	South Africa
115.	*79*	Substantive	PCR for this assay uses the following mastermix (concentration per 25µl single reaction): 20.2 µl of MGW, 2.5 µl of 10X PCR buffer containing 15mM $MgCl_2$ (Applied Biosystems)[2], 0.25 µl of each dNTPs (10 mM) (final concentration of 0.1 mM each), 0.1 µl AmpliTaq (5 U/µl) (Applied Biosystems)[2], 0.1 µl of each primer (25 µM), 1.0 µl ~~neat~~ extracted DNA obtained as described	Deletion of the word “neat” since there is only purified or extracted DNA; and further clarity is requested on why the word “neat”	English	South Africa

			above. PCR cycling parameters: 94 °C denaturation for 1 min, 25 cycles of 94 °C for 15 s, 65 °C for 15 s, 72 °C for 15 s, and 72 °C extension step for 6 min.	has been used.		
116.	*79*	Technical	PCR for this assay uses the following mastermix (concentration per 25µl single reaction): 20.2 µl of MGW, 2.5 µl of 10X PCR buffer containing 15mM $MgCl_2$ (Applied Biosystems)[2], 0.25 µl of each dNTPs (10 mM) (final concentration of 0.1 mM each), 0.1 µl AmpliTaq (5 U/µl) (Applied Biosystems)[2], 0.1 µl of each primer (25 µM), 1.0 µl neat extracted DNA obtained as described above. PCR cycling parameters: 94 °C denaturation for 1 min, 25 cycles of 94 °C for 15 s, 65 °C for 15 s, 72 °Cfor 15 s, and 72 °C extension step for 6 min.	First sentence: For consistency with the other mix compositions, the final concentration and volume per reaction of each compound should be given, instead of volumes and initial concentrations. Such information is important for the lab to reproduce the test and to avoid any errors.	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
117.	*80*	Substantive	As required 10 µl of reaction products ~~is mixed with a running~~ can be loaded with a suitable marker and run on a 2% agarose gel.	Deletion of “is mixed with a running” and insertion of “can be loaded and a suitable” due to fact that the PCR product and marker is loaded into one well.	English	South Africa
118.	*81*	Editorial	When testing for *T. walkeri* the following primer is used. Replace Tin 3 with 0.1 µl of forward primer Tin 11 (5′-TAA TGT TGG CGT GGC GGC AT-3′) (25 µM). This produces an amplicon of ~~163~~ 414bp.	The product size mentioned in Frederick et al. (2000) is 414 bp; however, the EPPO protocol PM7/29 stated the size is 163 bp.	English	New Zealand
119.	*81*	Substantive	When testing for *T. walkeri* the following primer is used. Replace Tin 3 with 0.1 µl of forward primer Tin 11 (5′-TAA TGT TGG CGT GGC GGC AT-3′) (25 µM). This produces an amplicon of ~~163~~ 414 bp.	According to the original publication of Frederick et al, the primers used (Tin11 and Tin4) produce an amplicon of 414 bp. The primers used in annex are Tin3 and Tin4, so the size of the amplicon is 414 bp and not 163, but in Fredericks et al, the primers for TaqMan assay are Tin3/Tin10 that produce an 212 amplicon. Tin3/Tin4 may work but this is not validated in the paper. The primers and size of amplicons should be carefully checked against the original publication.	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
120.	*81*	Technical	When testing for *T. walkeri* the following primer is used. Replace Tin 3 with 0.1 µl of forward primer Tin 11 (5′-TAA TGT TGG CGT GGC GGC AT-3′) (25 µM). This produces an amplicon of ~~163~~ 414 bp.	According to Frederick et al. (2000), it is 414bp.	English	Japan
121.	*82*	Substantive	Positive reactions produce a single amplicon of 414 bp for both for *T. indica* (primers Tin 3/Tin 4) and *T. walkeri* (primers Tin 11/Tin 4). If the *T. walkeri* and *T. indica* specific primers do not produce positive results for the test samples (but positive control DNA samples are positive), then the sample extractions belong to another *Tilletia* species, such as *T. horrida*. Restriction enzyme analysis may enable further species identification of these samples if required (section 4.3.1). Alternatively no amplification can result from poor-quality DNA. This can be checked by testing extracts with the universal primers (ITS1 and ITS4) described in section 4.3.1. If the samples contain good-quality DNA, and hence test samples are not *T. indica* or *T. walkeri* but another *Tilletia* species, then a single band (c. 670 bp) will be produced when PCR amplicons are ~~run~~ out on an agarose gel. However, if amplification is still not produced, fresh DNA should then be	Deletion of “out” due to fact that agarose does not run out but can only run. If agarose runs out, results cannot be obtained.	English	South Africa

			extracted and retested.			
122.	*84*	Editorial	The assay was designed by Fredericket *al.* (2000)using the mitochondrial DNA producing an amplicon of ~~163~~ 212bp. Oligonucleotides used:	From Frederick et al. (2000), the size of the amplicon is 212 bp for primers Tin 3 and Tin 4.	English	New Zealand
123.	*84*	Substantive	The assay was designed by Fredericket *al.* (2000)using the mitochondrial DNA producing an amplicon of ~~163~~ 414 bp. Oligonucleotides used:	The primers used in annex are Tin3 and Tin4, so the size of the amplicon is 414 bp and not 163, but in Fredericks et al, the primers for TaqMan assay are Tin3/Tin10 that produce an 212 amplicon. Tin3/Tin4 may work but this is not validated in the paper. The primers and size of amplicons should be carefully checked against the original publication.	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
124.	*84*	Technical	The assay was designed by Fredericket *al.* (2000)using the ~~mitochondrial~~ genomic DNA producing an amplicon of 163 bp. Oligonucleotides used: Change "mitochondrial DNA" (Frederick et al. 2000) to "genomic DNA"	Same as the above 75.	English	China
125.	*84*	Technical	The assay was designed by Fredericket *al.* (2000)using the mitochondrial DNA producing an amplicon of ~~163~~ 414 bp. Oligonucleotides used:	According to Frederick et al. (2000), it is 414bp.	English	Japan
126.	*85*	Editorial	The forward and reverse primers are the same as for conventional PCR (Tin 3 and Tin 410).	From Frederick et al. (2000), the primer set specific to Tilletia controversa is Tin 3 and Tin 10.	English	New Zealand
127.	*88*	Substantive	PCR for this assay uses the following mastermix (concentration per 25µl single reaction): 8.5 µl of MGW, 12.5 µl of 2X universal TaqMan master mix, 1.0 µl of each primer (10 µM), 1.0 µl of TaqMan probe (10 µM), and 1.0 µl ~~neat~~ extracted DNA (obtained as in section 4.3.2). PCR cycling parameters: 50 °C for 2 min, 95 °C for 10 min, 34 cycles of 95 °C for 15 s and 60 °C for 1 min.	Deletion of the word "neat" since there is only purified or extracted DNA; and further clarity is requested on why the word "neat" has been used.	English	South Africa
128.	*88*	Technical	PCR for this assay uses the following mastermix (concentration per 25µl single reaction): 8.5 µl of MGW, 12.5 µl of 2X universal TaqMan master mix, 1.0 µl of each primer (10 µM), 1.0 µl of TaqMan probe (10 µM), and 1.0 µl neat extracted DNA (obtained as in section 4.3.2). PCR cycling parameters: 50 °C for 2 min, 95 °Cfor 10 min, 34 cycles of 95 °Cfor 15 s and 60 °Cfor 1 min.	1. First sentence: For consistency with the other mix compositions, the final concentration and volume per reaction of each compound should be given, instead of volumes and initial concentrations. Such information is important for the lab to reproduce the test and to avoid any errors. 2. Last sentence: "34 cycles": see comment in [91].	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
129.	*90*	Editorial	When testing for *T. walkeri*, replace Tin 3 with 1.0 µl of forward primer Tin 11 (5′-TAA TGT TGG CGT GGC GGC AT-3′) (25 µM) to test for *T. walkeri*, which produces an amplicon of 212~~163~~ bp.	From Federick et al. (2000), the size of the amplicon is 212 bp.	English	New Zealand
130.	*90*	Technical	When testing for *T. walkeri*, replace Tin 3 with 1.0 µl of forward primer Tin 11 (5′-TAA TGT TGG CGT GGC GGC AT-3′) (25 µM) to test for *T. walkeri*, which produces an amplicon of 163 bp.	The primers used and size of amplicons should be carefully checked against the original publication.	English	EPPO, Georgia, Russian Federation, Netherlands, European Union

131.	*90*	Technical	When testing for *T. walkeri*, replace Tin 3 with 1.0 µl of forward primer Tin 11 (5′-TAA TGT TGG CGT GGC GGC AT-3′) (25 µM) to test for *T. walkeri*, which produces an amplicon of ~~163~~ 414bp.	According to Frederick et al. (2000), it is 414bp.	English	Japan
132.	*91*	Editorial	*T. indica* produces amplification with primers Tin 3/Tin 4 while *T. walkeri* needs primers Tin 11/Tin 4. If neither primer set produces amplification but control samples react as expected, then the sample extractions belong to another *Tilletia* species, such as *T. horrida*. For example, when testing for *T. indica* and the threshold cycle (Ct) of the sample is > 40, the result indicates that it is negative for *T. indica* and is highly likely to be another species of *Tilletia*. Likewise, when testing for *T. walkeri* and the Ct > 40, the result indicates that it is negative for *T. walkeri* and is highly likely to be another species of *Tilletia*. Restriction enzyme analysis may enable further species identification of these samples if required (section 4.3.1).	T. indica produces amplification with primers Tin 3/Tin 410 while T. walkeri needs primers Tin 11/Tin 410. If neither primer set produces amplification but control samples react as expected, then the sample extractions belong to another Tilletia species, such as T. horrida. For example, when testing for T. indica and the threshold cycle (Ct) of the sample is > 4033, the result indicates that it is negative for T. indica and is highly likely to be another species of Tilletia. Likewise, when testing for T. walkeri and the Ct > 4033, the result indicates that it is negative for T. walkeri and is highly likely to be another species of Tilletia. Restriction enzyme analysis may enable further species identification of these samples if required (section 4.3.1) From Frederick et al. (2000), the primer set specific to T. indica is Tin 3/Tin 10 and the set specific to T. walkeri is Tin 11/Tin10. The Ct value for both real-time PCR tests were 33. If the Ct value is set higher at 40, this can cause false positive results.	English	New Zealand
133.	*91*	Technical	*T. indica* produces amplification with primers Tin 3/Tin 4 while *T. walkeri* needs primers Tin 11/Tin 4. If neither primer set produces amplification but control samples react as expected, then the sample extractions belong to another *Tilletia* species, such as *T. horrida*. For example, when testing for *T. indica* and the threshold cycle (Ct) of the sample is > 40, the result indicates that it is negative for *T. indica* and is highly likely to be another species of *Tilletia*. Likewise, when testing for *T. walkeri* and the Ct > 40, the result indicates that it is negative for *T. walkeri* and is highly likely to be another species of *Tilletia*. Restriction enzyme analysis may enable further species identification of these samples if required (section 4.3.1).	"the threshold cycle (Ct) of the sample is > 40", however this is impossible if the number of cycles is 34 (see [88]). If the negative is greater than 40 cycles, the number of cycles should be 40. It might be better to indicate what a positive should be.	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
134.	*92*	Substantive	Alternatively no amplification can result from poor-quality DNA. This can be checked by testing extracts with the universal primers (ITS1 and ITS4) described in section 4.3.1. If the samples contain good-quality DNA and hence test samples are not *T. indica* or *T. walkeri* but another *Tilletia* species, then a single band (c. 670 bp) will be produced when PCR amplicons are run ~~out~~ on an agarose gel. However, if amplification is still not produced, fresh DNA should then be extracted and retested.	Deletion of “out” due to fact that agarose does not run out but can only run. If agarose runs out, results cannot be obtained.	English	South Africa
135.	*92*	Technical	Alternatively no amplification can result from poor-quality DNA. This can be checked by testing extracts with the universal primers (ITS1 and ITS4) described in section 4.3.1. If the samples	The internal control using ITS1 and ITS4 primers should be included as	English	New Zealand

			contain good-quality DNA and hence test samples are not *T. indica* or *T. walkeri* but another *Tilletia* species, then a single band (c. 670 bp) will be produced when PCR amplicons are run out on an agarose gel. However, if amplification is still not produced, fresh DNA should then be extracted and retested.	part of the test rather than as an optional test. Inclusion of internal control can avoid false negative results.		
136.	*93*	Editorial	The sensitivity limits of both the *T. indica* and *T. walkeri* assays were found to be 5 pg of total DNA. This concentration produced detectable levels of fluorescence (Frederick *et al*.,2000). The species specificity of the assays was tested against DNA extracted from *T. barclayana*, *T. tritici*, *T. laevis*, *T. controversa* or *T. fusca.* None of these isolates amplified in either the *T. indica* or *T. walkeri* specific assays (Frederick *et al*.,2000).	Typo	English	OIRSA
137.	*93*	Editorial	The sensitivity limits of both the *T. indica* and *T. walkeri* assays were found to be 5 pg of total DNA. This concentration produced detectable levels of fluorescence (Fredrick *et al*.,2000). The species specificity of the assays was tested against DNA extracted from *T. barclayana*, *T. tritici*, *T. laevis*, *T. controversa* or *T. fusca.* None of these isolates amplified in either the *T. indica* or *T. walkeri* specific assays (Frederick *et al*.,2000).	The correct name is Frederick	English	Mexico
138.	*95*	Substantive	This assay was designed by Tan *et al.* (2009) to use the ITS region that occurs between the nuclear small- and large-subunit ribosomal DNA (rDNA). ~~It was found that the *Tilletia* species have two variable regions (ITS1 and ITS2) separated by the conserved 5.8S rRNA gene (Levy *et al.*, 2001; Tan and Murray, 2006).~~ The protocol is designed to initially amplify *Tilletia*-specific DNA and then using real-time PCR and fluorescent probes identify the species of *Tilletia*. The ITS1 region in rDNA was targeted in this study for the design of the multiplex assay, which enables a five-plex fluorescent PCR assay to identify closely related *Tilletia* species detected in grain.	Deletion of sentence " It was found that the Tilletia species have two variable regions (ITS1 and ITS2) separated by the conserved 5.8S rRNA gene (Levy et al., 2001; Tan and Murray, 2006)." The reason being that all fungi have 2 variable regions and it's not necessary to provide this background information.	English	South Africa
139.	*98*	Editorial	Amplification of *Tilletia*-specific DNA of various *Tilletia* species is performed with primers MK56 and Tilletia-R (Tan and Murray, 2006). Each PCR reaction is performed in 20 µ~~L~~l (single reaction). The mixture consists of 20 µ~~L~~l of 1.5 mM $MgCl_2$, 200 µM of each of the four deoxynucleotides dATP, dTTP, dCTP and dTTP, 0.5 µM of each of the primer pair and 0.5 U Taq DNA Polymerase (Invitrogen[4],USA) in 1X buffer L (50 mM Tris, pH 9.0, 20 mM NaCl, 1% Triton X-100, 0.1% gelatin).	Removal of caps "L" where it appears and replace with "l" for consistency Deletion of "T" on "dTTP" and replace it with "G" in order to read as "dTTP", because it was incorrectly written.	English	South Africa
140.	*98*	Substantive	Amplification of *Tilletia*-specific DNA of various *Tilletia* species is performed with primers MK56 and Tilletia-R (Tan and Murray, 2006). Each PCR reaction is performed in 20 µL (single reaction). The mixture consists of 20 µL of 1.5 mM $MgCl_2$, 200 µM of each of the four deoxynucleotides dATP, dTTP, dCTP and d~~T~~GTP, 0.5 µM of each of the primer pair and 0.5 U Taq DNA Polymerase (Invitrogen[4],USA) in 1X buffer L (50 mM Tris, pH 9.0, 20 mM NaCl, 1% Triton X-100, 0.1% gelatin).	Deletion of "T" on "dTTP" and replace it with "G" in order to read as "dTTP", because it was incorrectly written.	English	South Africa
141.	*98*	Technical	Amplification of *Tilletia*-specific DNA of various *Tilletia* species is performed with primers MK56 and Tilletia-R (Tan and Murray, 2006). Each PCR reaction is performed in 20 µL (single reaction). The mixture consists of 20 µL of 1.5 mM $MgCl_2$, 200 µM of each of the four deoxynucleotides dATP, dTTP, dCTP and dTTP, 0.5 µM of each of the primer pair and 0.5 U Taq DNA Polymerase (Invitrogen[4],USA) in 1X buffer L (50 mM Tris, pH 9.0, 20 mM NaCl, 1% Triton X-100, 0.1% gelatin).	Third sentence: For consistency with the other mix compositions, the final concentration and volume per reaction of each compound should be given, instead of volumes and initial concentrations. Such information is important for the lab to reproduce the test and to avoid any errors.	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
142.	*98*	Technical	Amplification of *Tilletia*-specific DNA of various *Tilletia* species is performed with primers MK56 and Tilletia-R (Tan and Murray, 2006). Each PCR reaction is performed in 20 µL (single reaction). The mixture consists of 20 µL of 1.5 mM $MgCl_2$, 200 µM of each of the four deoxynucleotides dATP,	Please provide sequence details of primers MK56 and Tilletia-R.	English	New Zealand

			dTTP, dCTP and dTTP, 0.5 µM of each of the primer pair and 0.5 U Taq DNA Polymerase (Invitrogen[4], USA) in 1X buffer L (50 mM Tris, pH 9.0, 20 mM NaCl, 1% Triton X-100, 0.1% gelatin).			
143.	*100*	Editorial	If required, the restricted products are stored at 4 °C before visualizing on a gel. When required, load ~~mix~~ 10 µl of reaction products with a running marker and run on a 2% gel. The expected fragment size is 260 bp. This fragment will not be visible if the PCR is done on a single teliospore, as there will not be enough DNA present.	Delete the word “mix” and replace it with “load” since it is scientifically incorrect to use mix; load is scientifically used in terms of loading DNA.	English	South Africa
144.	*100*	Technical	~~If required, t~~The restricted products may be ~~are~~ stored at 4 °C. ~~before~~If visualizing on a gel. ~~When~~is required, mix 10 µl of reaction products with a running marker and run on a 2% gel. The expected fragment size is 260 bp. However, ~~T~~this fragment will not be visible if the PCR is done on a single teliospore, as there will not be enough DNA present.	We propose a modification to the paragraph to prevent confusion regarding options presented in paragraph 20.	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
145.	*102*	Editorial	Real-time PCR assays with the dual-labelled probes and oligonucleotide primers (Table 3) in 20 µ~~L~~l reactions in 0.1 ml microfuge tubes are performed in the Rotor-Gene 6000 instrument (QIAGEN[5], Australia). The five-plex reaction mixture consists of 1X ImmoBuffer (Bioline[6], UK, 5 mM $MgCl_2$, 200 µM of each of the four deoxynucleotides dATP, dTTP, dCTP and dTTP, 1 U Immolase DNA Polymerase (Bioline[5,] UK)and 0.2 µM, 0.4 µM and 0.9 µM of each of the dual-labelled probes, the four forward primers and the four reverse primers respectively (Table 3). The template DNA is 1 µ~~L~~l of PCR product from the PCR amplification of *Tilletia*-specific DNA or a known DNA concentration of a *Tilletia* spp.	Removal of caps “L” where it appears and replace with “l” for consistency	English	South Africa
146.	*102*	Substantive	Real-time PCR assays with the dual-labelled probes and oligonucleotide primers (Table 3) in 20 µL reactions in 0.1 ml microfuge tubes are performed in the Rotor-Gene 6000 instrument (QIAGEN[5], Australia). The five-plex reaction mixture consists of 1X ImmoBuffer (Bioline[6], UK, 5 mM $MgCl_2$, 200 µM of each of the four deoxynucleotides dATP, dTTP, dCTP and dG~~T~~TP, 1 U Immolase DNA Polymerase (Bioline[5,] UK)and 0.2 µM, 0.4 µM and 0.9 µM of each of the dual-labelled probes, the four forward primers and the four reverse primers respectively (Table 3). The template DNA is 1 µL of PCR product from the PCR amplification of *Tilletia*-specific DNA or a known DNA concentration of a *Tilletia* spp.	Deletion of “T” on “dTTP” and replace it with “G” in order to read as “dTTP”, because it was incorrectly written.	English	South Africa
147.	*102*	Technical	Real-time PCR assays with the dual-labelled probes and oligonucleotide primers (Table 3) in 20 µL reactions in 0.1 ml microfuge tubes are performed in the Rotor-Gene 6000 instrument (QIAGEN[5], Australia). The five-plex reaction mixture consists of 1X ImmoBuffer (Bioline[6], UK, 5 mM $MgCl_2$, 200 µM of each of the four deoxynucleotides dATP, dTTP, dCTP and dTTP, 1 U Immolase DNA Polymerase (Bioline[5,] UK)and 0.2 µM, 0.4 µM and 0.9 µM of each of the dual-labelled probes, the four forward primers and the four reverse primers respectively (Table 3). The template DNA is 1 µL of PCR product from the PCR amplification of *Tilletia*-specific DNA or a known DNA concentration of a *Tilletia* spp. as a positive control.	End of the last sentence: "or a known DNA concentration of a Tilletia spp.": It is not clear whether this relates to positive controls or specifies that this real-time-PCR test can be used directly on Tilletia DNA if the DNA concentration is sufficient. Proposal suggested for clarification.	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
148.	*104*	Technical	The sensitivity of the test for single spores was 10–40% (i.e. out of known positive *T. indica* spores only 10–40% gave positive PCR results) (Tan and Wright, 2009). This is because of a number of reasons, including all *T. indica* spores and bunted grain had to be autoclaved twice for the work to be done, so there may have been a loss in genetic material. The specificity of the probe for *T. indica* was investigated in a DNA mixture of *T. indica*:*T. walkeri* or *T. ehrhartae* or *T. caries,* in ratios of 1:0.1 pg and 0.1:1 pg (appropriate concentration range indicated from single-spore analysis). The specificity of the primers was tested and found not to react with other *Tilletia* species.	For consistency with the other PCR methods a new paragraph on the requirements for a positive or a negative result is required after [104].	English	EPPO, Georgia, Russian Federation, Netherlands, European Union
149.	*105*	Editorial	**Table 3.** Sequences and modifications of the primers and probes used in the multiplex diagnostic assay for *T. indica* and other related *Tilletia* spp.	Table 3 (paragraphs [105] to [108]) should be located immediately after	English	EPPO

<table>
<tr><td></td><td></td><td></td><td></td><td>paragraph [102] where it is referred to.</td><td></td><td></td></tr>
<tr><td>150.</td><td>105</td><td>Editorial</td><td>Table 3. Sequences and modifications of the primers and probes used in the multiplex diagnostic assay for T. indica and other related Tilletia spp.</td><td>Table 3 (paragraphs [105] to [108]) should be located immediately after paragraph [102] where it is referred to.</td><td>English</td><td>Georgia, Russian Federation, Netherlands, European Union</td></tr>
<tr><td>151.</td><td>106</td><td>Editorial</td><td>
<table>
<tr><th>Primer pairs (sequence 5′-3′)</th><th>Probes (modifications 5′, 3′)</th><th>Channel</th><th>Target</th></tr>
<tr><td rowspan="2">KB-DL-For: CTTCGGAAGAGTCTCCTT (nt. 64–81[a])
KB-DL- Rev: CCGGACAGGTACTCAG (nt. 127–142)</td><td>ACGGAAGGAACGAGGC (nt. 105–120)
(6-FAM, BHQ1)</td><td>Green</td><td>T. indica</td></tr>
<tr><td>ACGGAAGGAACAAGGC (nt. 67–82[b])
(JOE, BHQ1)</td><td>Yellow</td><td>T. walkeri</td></tr>
<tr><td>Hor-DL-For: GGCCAATCTTCTCTACTATC (nt. 40–59[c])
Hor-DL-Rev: CCGGACAGGATCACTA (nt. 87–102)</td><td>CAACCCAGACTACGGAGGGTGA (nt. 60–81)
(CAL Fluor Red 610, BHQ2)</td><td>Orange</td><td>T. horrida</td></tr>
<tr><td>Tri-DL-For: ATTGCCGTACTTCTCTTC (nt. 56–73[d])
Tri-DL-Rev: GTAGTCTTGTGTTTGGATAATAG (nt. 99–112)</td><td>AGAGGTCGGCTCTAATCCCATCA (nt. 75–97)
(Quasar 670, BHQ2)</td><td>Red</td><td>Broad range[1]</td></tr>
<tr><td>Ehr-DL-For: CGCATTCTTATGCTTCTTG (nt. 72–90[e])
Ehr-DL-Rev: GTTAGGAACCAAAGCCATC (nt. 128–146)</td><td>CAGAGTCATTGGTTCTTCGGAGC (nt. 104–126)
(Quasar 705, BHQ2)</td><td>Crimson</td><td>T. ehrhartae</td></tr>
</table>
</td><td>Table 3 (paragraphs [105] to [108]) should be located immediately after paragraph [102] where it is referred to.</td><td>English</td><td>EPPO</td></tr>
<tr><td>152.</td><td>106</td><td>Editorial</td><td>
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</table>

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<tr><td rowspan="2">KB-DL-For: CTTCGGAAGAGTCTCCTT (nt. 64–81[a])
KB-DL- Rev: CCGGACAGGTACTCAG (nt. 127–142)</td><td>ACGGAAGGAACGAGGC (nt. 105–120)
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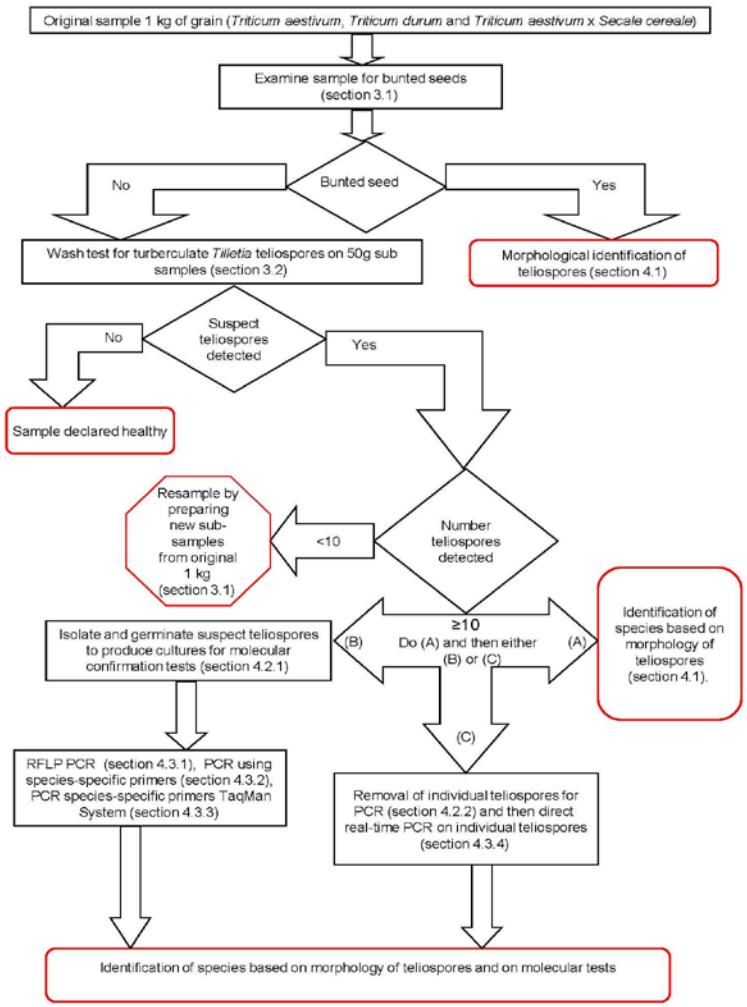
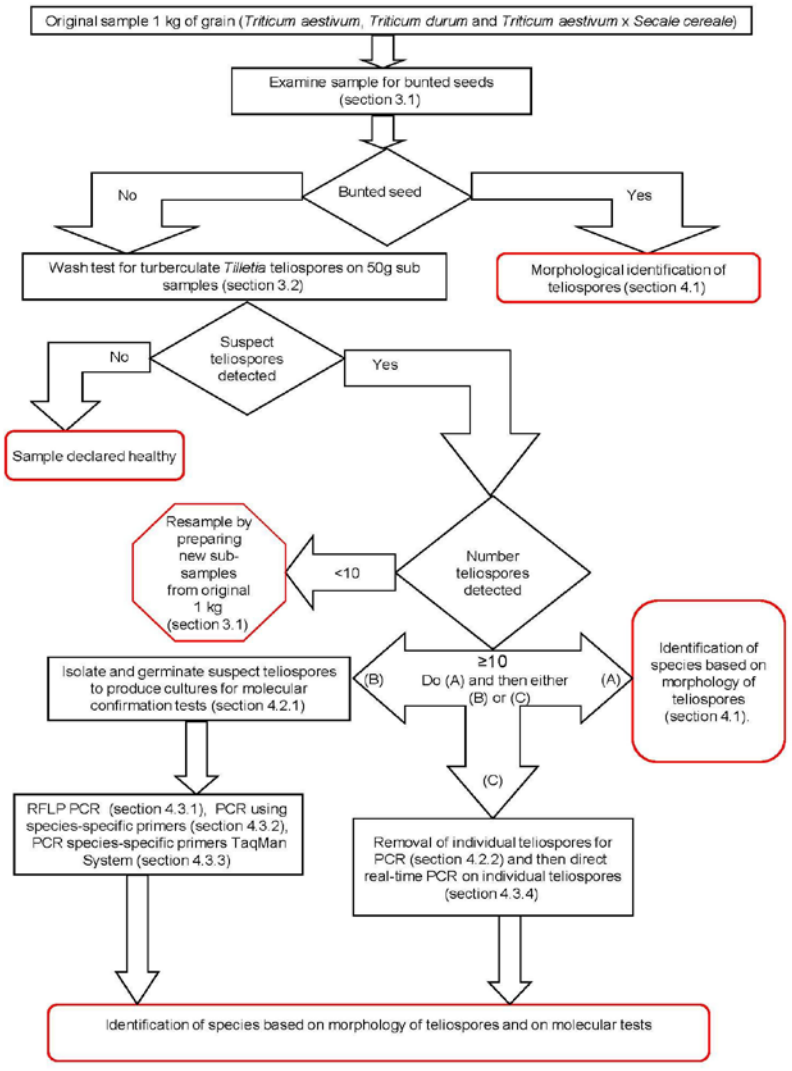
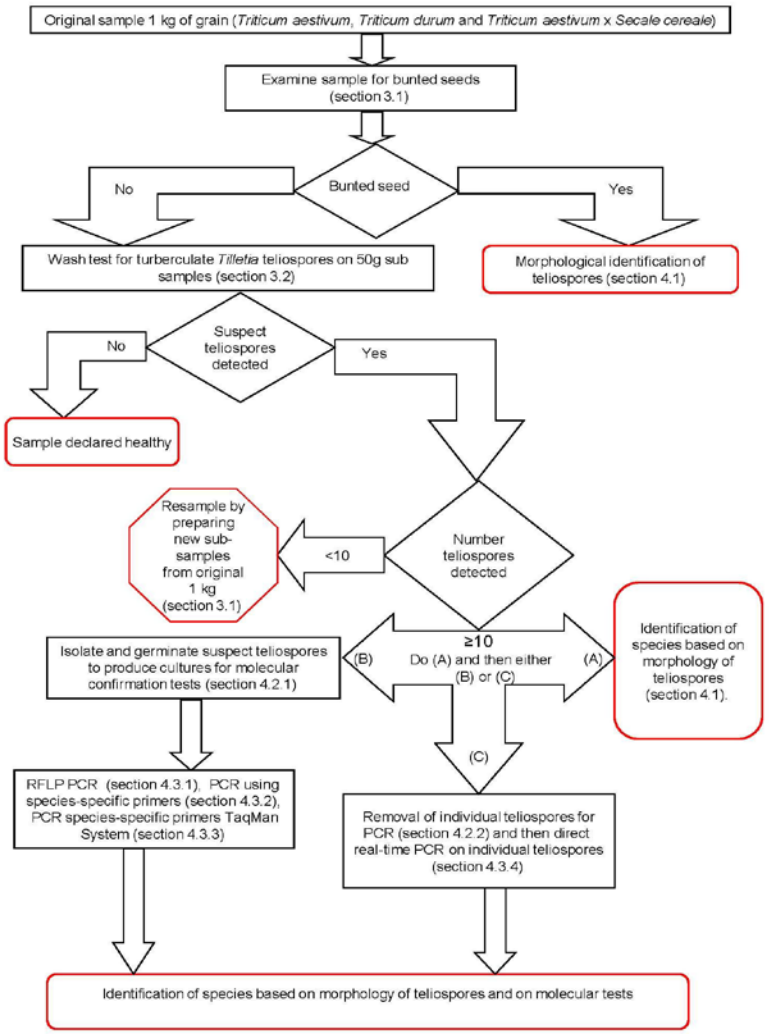
(Quasar 705, BHQ2)</td><td>Crimson</td><td>T. ehrhartae</td></tr>
</table>
</td><td>to.</td><td></td><td>Netherlands, European Union</td></tr>
<tr><td>153.</td><td>106</td><td>Editorial</td><td>It is suggested that there should retain only one probe, that is T. indica, and get rid of the other four probe.</td><td>1) the standard is only for T. indica; 2) other organizations or international standards mostly use one specie as a formality. 3) and the primers and probes of T. horrida, are not for all strains of the T. horrida, but only a part of the strains of T. horrida can be detected as positive.</td><td>English</td><td>China</td></tr>
</table>

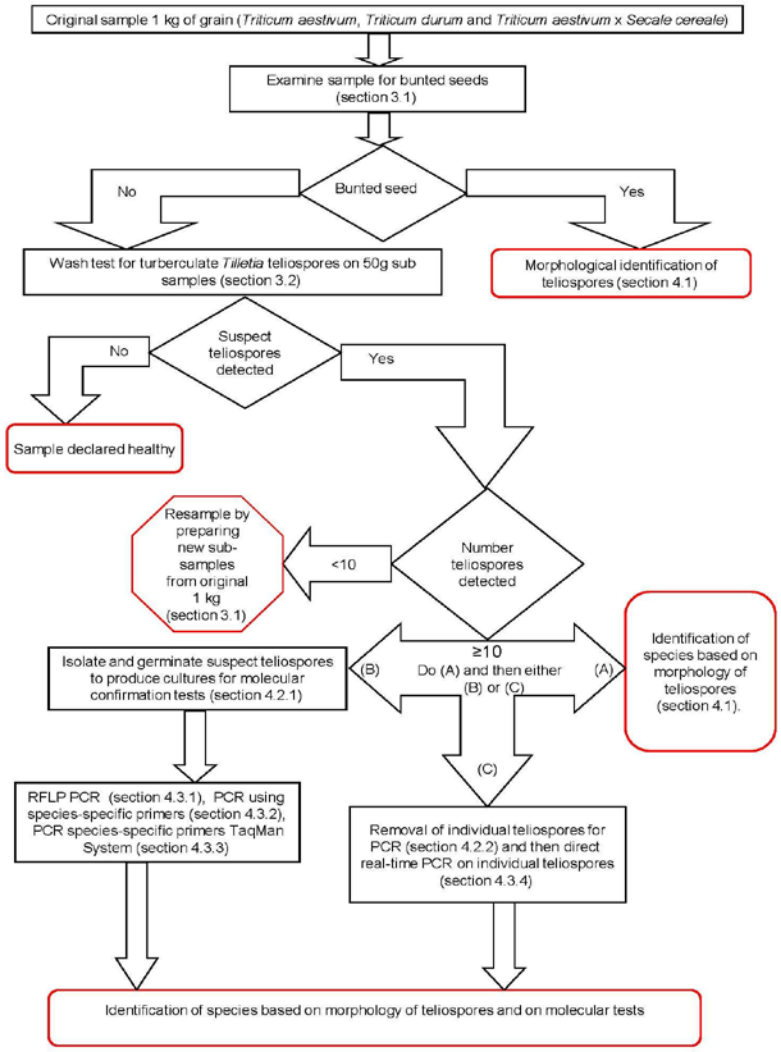
			Primer pairs (sequence 5′-3′)	**Probes** **(modifications 5′, 3′)**	**Channel**	**Target**		
			KB-DL-For: CTTCGGAAGAGTCTCCTT (nt. 64–81[a]) KB-DL- Rev: CCGGACAGGTACTCAG (nt. 127–142)	ACGGAAGGAACGAGGC (nt. 105–120) (6-FAM, BHQ1)	Green	*T. indica*		
			~~ACGGAAGGAACAAGGC(nt. 67–82[b])(JOE, BHQ1)~~	~~Yellow~~	~~*T. walkeri*~~			
			~~Hor-DL-For: GGCCAATCTTCTCTACTATC(nt. 40–59[c])Hor-DL-Rev: CCGGACAGGATCACTA (nt. 87–102)~~	~~CAACCCAGACTACGGAGGGTGA (nt. 60–81)~~ ~~(CAL Fluor Red 610, BHQ2)~~	~~Orange~~	~~*T. horrida*~~		
			~~Tri-DL-For: ATTGCCGTACTTCTCTTC(nt. 56–73[d])~~ ~~Tri-DL-Rev: GTAGTCTTGTGTTTGGATAATAG (nt. 99–112)~~	~~AGAGGTCGGCTCTAATCCCATCA (nt. 75–97)(Quasar 670, BHQ2)~~	~~Red~~	~~Broad range[1]~~		
			~~Ehr-DL-For: CGCATTCTTATGCTTCTTG(nt. 72–90[e])Ehr-DL-Rev: GTTAGGAACCAAAGCCATC (nt. 128–146)~~	~~CAGAGTCATTGGTTCTTCGGAGC (nt. 104–126)(Quasar 705, BHQ2)~~	~~Crimson~~	~~*T. ehrhartae*~~		
154.	*107*	Editorial	Notes: GenBank No. [a]AF398434, [b]AF310180, [c]AF310171, [d]AF398447, [e]AY770433. The list of the reference material used and place of origin is in Tan *et al.* (2009) and material is held at EMAI in Australia (refer to contacts list, section 6 of this diagnostic protocol). nt. = nucleotide.	Table 3 (paragraphs [105] to [108]) should be located immediately after paragraph [102] where it is referred to.	English	EPPO
155.	*107*	Editorial	Notes: GenBank No. [a]AF398434, [b]AF310180, [c]AF310171, [d]AF398447, [e]AY770433. The list of the reference material used and place of origin is in Tan *et al.* (2009) and material is held at EMAI in Australia (refer to contacts list, section 6 of this diagnostic protocol). nt. = nucleotide.	Table 3 (paragraphs [105] to [108]) should be located immediately after paragraph [102] where it is referred to.	English	Georgia, Russian Federation, Netherlands, European Union
156.	*108*	Editorial	[1]Includes *T. caries, T. laevis, T. contro~~a~~versa, T. fusca, T. bromi, T. goloskokovii*.	Although the fungus is widely spelt as both "controversa" and "contraversa", it is recommended to keep consistency in this protocol.	English	New Zealand
157.	*108*	Editorial	[1]Includes *T. caries, T. laevis, T. contro~~a~~versa, T. fusca, T. bromi, T. goloskokovii*.	Typo	English	OIRSA
158.	*108*	Substantive	[1]Includes *T. caries, T. laevis, T. contra~~o~~versa, T. fusca, T. bromi, T. goloskokovii*.	The correct name is T. controversa	English	Mexico
159.	*116*	Editorial	Laboratory of Plant Inspection and Quarantine, Shenzhen Entry-Exit Inspection and Quarantine		English	China

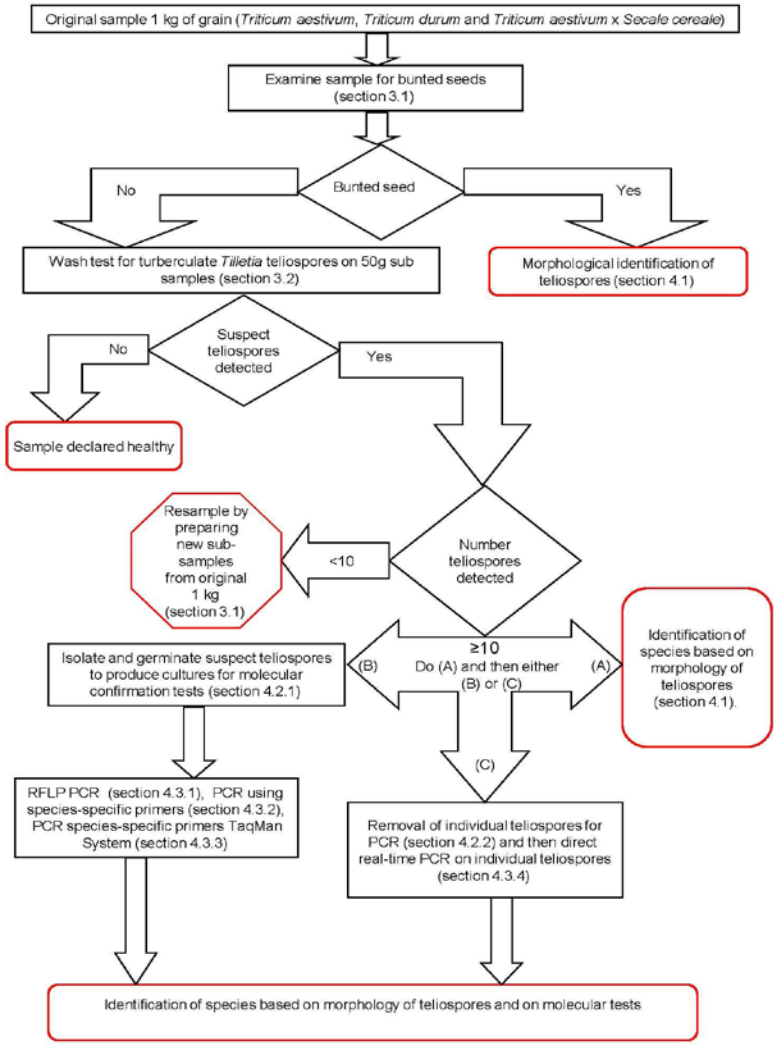
			Bureau, Shenzhen ~~City~~, 518045 Guangdong Province, China (Dr Guiming Zhang; email: zgm2001cn@yahoo.com.cn; Tel: +86 755 8211 1148; Fax: +86 755 2558 8630).			
160.	*117*	Technical	USDA ARS NAA, Fort Detrick MD 21702 USA (Mr Gary Peterson; email: gary.peterson@ars.usda.gov). USDA APHIS, Riverdale, MD, USA (Dr. Mary Palm, Mary.E.Palm@aphis.usda.gov) USDA APHIS, Beltsville, MD, USA (Dr. John McKemy, John.M.McKemy@aphis.usda.gov)	Contacts for USDA APHIS.	English	United States of America
161.	*121*	Editorial	The protocol has been enhanced by D.G. Wright, Department of Agriculture and Food, Western Australia, Australia; K.J.D Hughes, Food and Environment Agency, Sand Hutton, York, United Kingdom; and Guiming Zhang, Laboratory of Plant Inspection and Quarantine,Shenzhen ~~City~~, China. V. Cockerell, Science and Advice for Scottish Agriculture, Edinburgh (United Kingdom) reviewed the protocol.		English	China

162.	*129*	Editorial	 Figure 3. Flow diagram showing the process to be used for the detection and identification of Tilletia indica in grain samples.	1. First line: "grain" should be replaced by "seeds and grain" (comes before see [146]) 2. "Resample by preparing new sub-samples form original 1 kg (section 3.1)": "section 3.1" should be replaced by "section 3.2" (see [20] and [43]). 3. "Isolate and germinate suspect teliospores to produce cultures for molecular confirmation tests (section 4.2.1.)" should be replaced by "Isolate and germinate suspect teliospores (section 4.2.1) to produce cultures for molecular tests (sections 4.3.1.-4.3.3)" (see [20] line 8). 4. "Removal of individual teliospores for PCR (section 4.2.2)..." should be replaced by "Removal of individual teliospores for PCR (section 4.2.3)..." (see [20] line 9 and sentence added by the Secretariat at the end of [130]). 5. ">=10 Do (A) and then either (B) or (C)" is not very clear. Moreover, according to [38], (B) or (C) is not always necessary after (A). So could be modified as following: Number teliospores detected * < 10 -> ... * > 10 -> Identification of species based on morphology of teliospores (section 4.1) -> bunted kernels found in the larger sample ** Yes -> Identification of species based on morphology of teliospores found in the wash test and on the bunted kernels of the larger sample ** No -> OR (B)...OR (C)... -> Identification of species based on morphology of teliospores and on molecular tests	English	EPPO, Georgia, Russian Federation, Netherlands, European Union

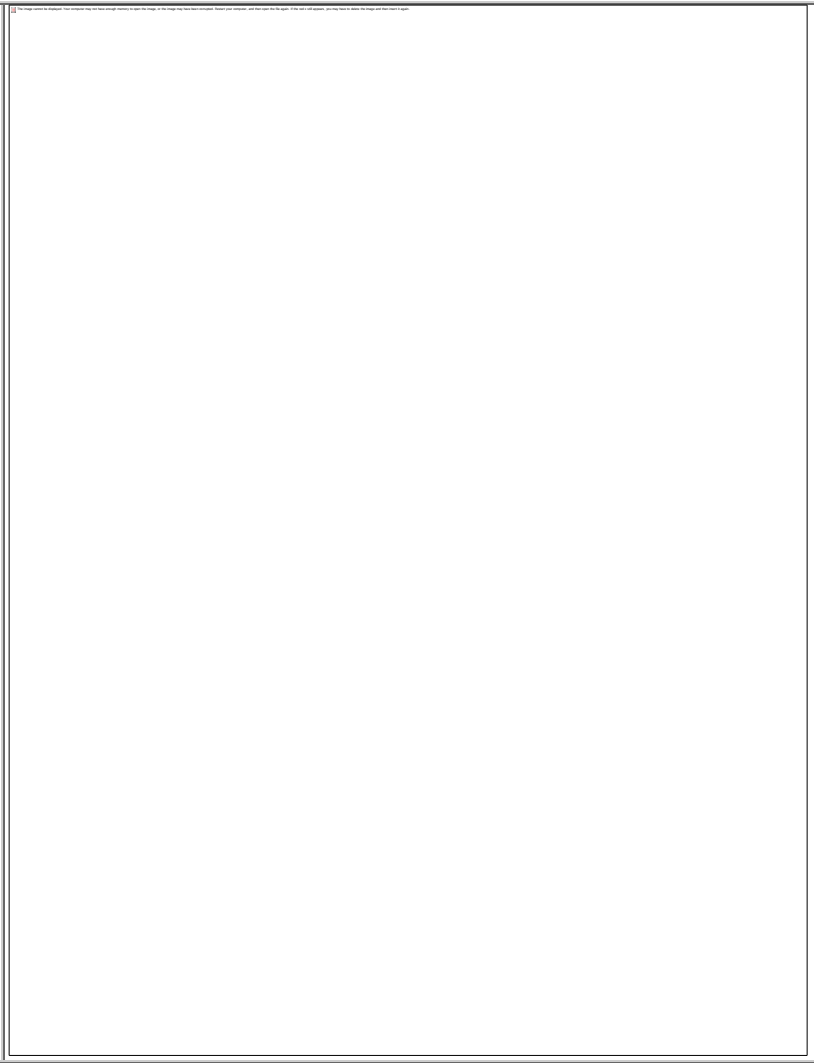
163.	*129*	Substantive	 Figure 3. Flow diagram showing the process to be used for the detection and identification of Tilletia indica in grain samples.	Delete "Resample by preparing new sub-samples from original 1 Kg. (section 3.1)". The arrow leading to it (	English	United States of America

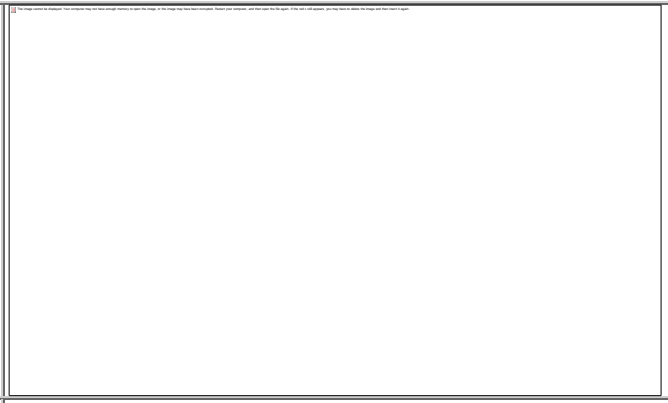
164.	*129*	Technical	 Figure 3. Flow diagram showing the process to be used for the detection and identification of Tilletia indica in grain samples.	It is not clear what to do if less than 10 teliospores were detected after repeated resampling. Insert the words "three replicates of" to the fourth step on the left "Wash test for turberculate Tilletia teliospores on three replicates of 50g subsamples (section 3.2) Please clarify whether all three tests 4.3.1 to 4.3.3 are required or only one of these tests is required at the lower left corner of the flow chart. Suggest to add an optional step to confirm results by DNA sequence analysis.	English	New Zealand

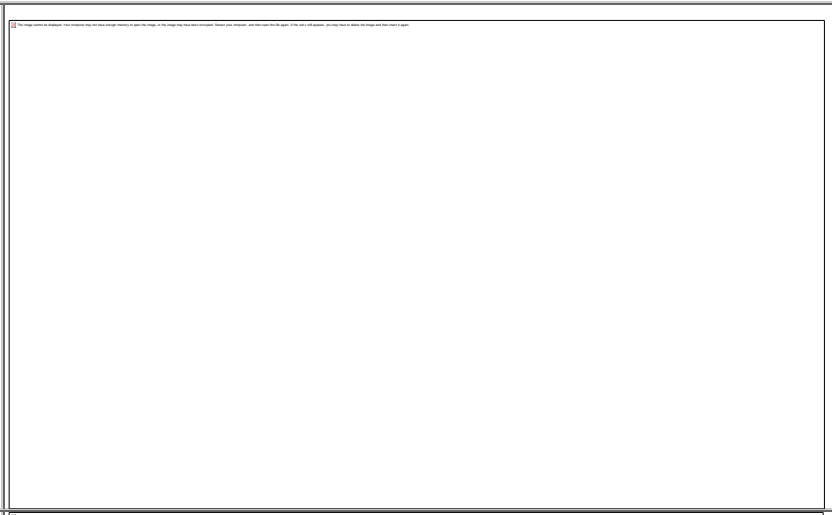
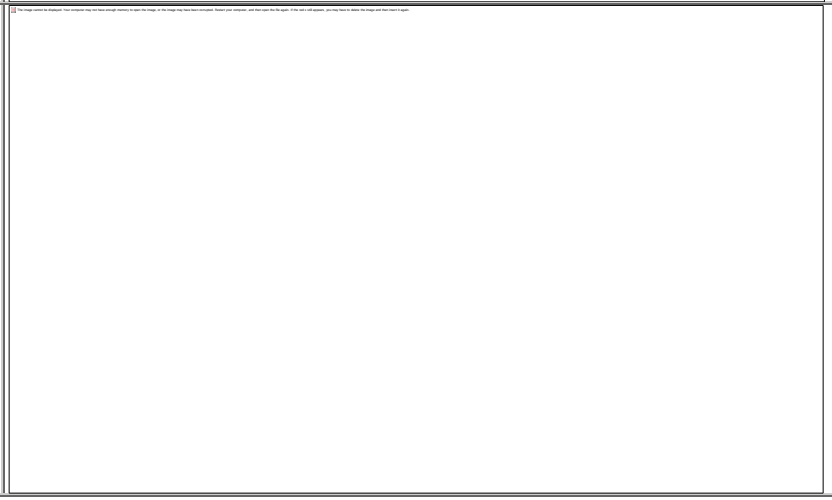
165.	*129*	Technical	 Figure 3. Flow diagram showing the process to be used for the detection and identification of Tilletia indica in grain samples. To modify in the top of flowchart “firstly check samples sterile gall seed” to" firstly washing sample to check whether the fishy Ustilago spores, if any, and then check the have a sterile gall seed ”. at the middle of flowchart:“If the Tilletia spores number is less than 10, sampling and washed the sample once more in order to increase the number of spores”. While the Spore number is greater than 10, increasing the arrow to: (A) morphological identification, (B) separating germinated spores	This is consistent with the testing procedures in the actual work, as well as consistent with EPPO India fishy black spikestandards (Reference: Diagnostic protocols for regulated pests , 2004 OEPP/EPPO, Bulletin OEPP/EPPO Bulletin 34, 155 –157) The figure did not say how to proceed after sampling and washing the sample once more of the samples washed check in Flowchart. Because the spores collected from a sample is not the same species, there may be two or more types spores, the measured data may be biased, such as: the spores T. indica spores 10% and 90% of the T. horrida spores, measurement results may be close to T. horrida, such as to increase the molecular identification can increase the accuracy of identification results; Thus it is suggested that: If the average diameter of the spores in the sample is greater than or equal to 35μm,they were judged to be T. indica; such as spores average diameteris less than 35μm, and each spore diameter less than 35μm, they were judged not to contain T. indica,; if few spore diameter greater than or equal to 35μm, it required separating the spores and make molecular identification.	English	China

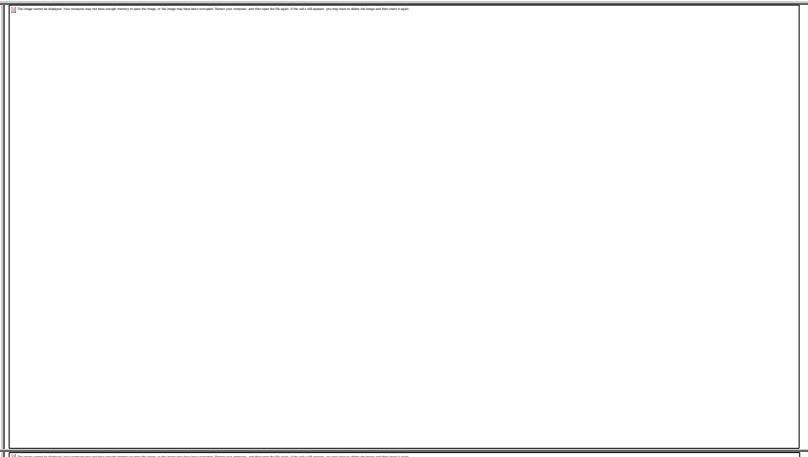
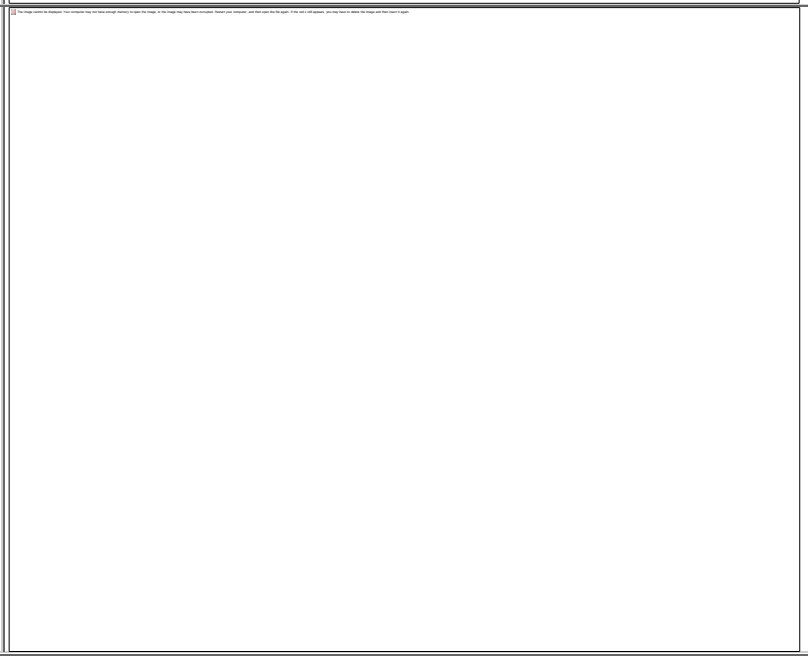
166.	*129*	Technical	 Figure 3. Flow diagram showing the process to be used for the detection and identification of Tilletia indica in grain samples.	As per comment in paragraph 20, the text in the second red box on the left should read "Sample delared non-infested with Tilletia indica". The term "healthy" is quite vague and is not sufficiently specific. In addition , the flow diagram requires molecular confirmation tests or PCR when more than 10 teliospores are detected for identification to species based on morphology and molecular tests. Molecular confirmation should be optional and not mandatory. As such, the arrow in the middle of the diagram pointing at A is an acceptable stopping point if the laboratory is confident with their identification. Under this diagram, it should reas A) and then either (B) or (C) if required.	English	Canada

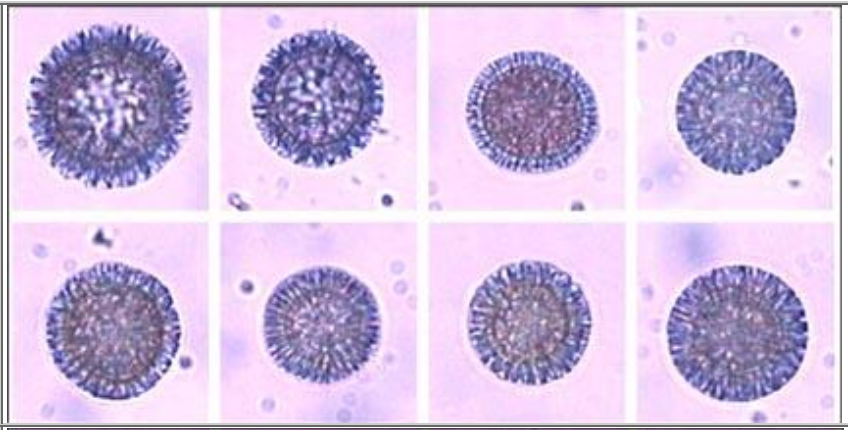
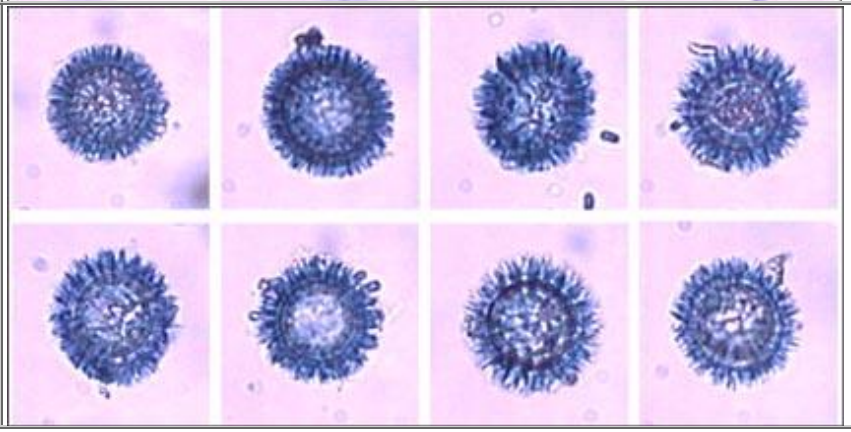
167.	*130*	Editorial	**Figure 3.** Flow diagram showing the process to be used for the detection and identification of *Tilletia indica* in ~~grain~~seed and grain samples. ***[Secretariat notes that the reference to section 4.2.2 in the lower right-hand box should be 4.2.3; this will be modified before being sent for member consultation]***	The flow diagram is referred to before the footnote, so should be clear that it applies to both seed and grain.	English	EPPO, Georgia, Russian Federation, Netherlands, European Union

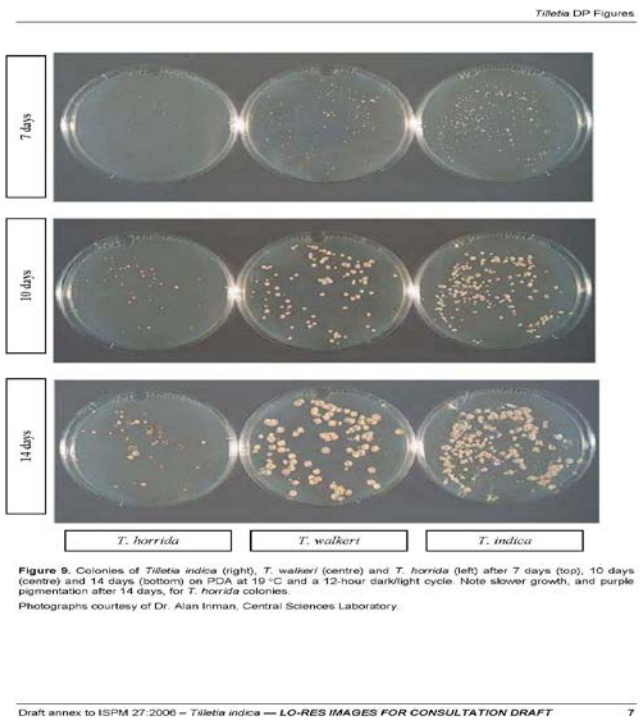
168.	*131*	Substantive		Figures and/or SEM picture images should be added to clarify the differences between T. indica and other species. It is difficult to understand the differences between T. indica and other species. (Even though the original figures may be clearer than those in this protocol.)	English	Japan

169.	*133*	Substantive		Figures and/or SEM picture images should be added to clarify the differences between T. indica and other species. It is difficult to understand the differences between T. indica and other species. (Even though the original figures may be clearer than those in this protocol.)	English	Japan
170.	*134*	Editorial		Paragraph to be deleted because the photographs are the same as in [133].	English	EPPO

171.	*134*	Editorial		Paragraph to be deleted because the photographs are the same as in [133].	English	Georgia ,Russian Federation ,Netherlands ,European Union
172.	*134*	Substantive		Figures and/or SEM picture images should be added to clarify the differences between T. indica and other species. It is difficult to understand the differences between T. indica and other species. (Even though the original figures may be clearer than those in this protocol.)	English	Japan

173.	*136*	Substantive			Figures and/or SEM picture images should be added to clarify the differences between T. indica and other species. It is difficult to understand the differences between T. indica and other species. (Even though the original figures may be clearer than those in this protocol.)	English	Japan
174.	*138*	Substantive			Figures and/or SEM picture images should be added to clarify the differences between T. indica and other species. It is difficult to understand the differences between T. indica and other species. (Even though the original figures may be clearer than those in this protocol.)	English	Japan

175.	*140*	Substantive		Figures and/or SEM picture images should be added to clarify the differences between T. indica and other species. It is difficult to understand the differences between T. indica and other species. (Even though the original figures may be clearer than those in this protocol.)	English	Japan
176.	*142*	Substantive		Figures and/or SEM picture images should be added to clarify the differences between T. indica and other species. It is difficult to understand the differences between T. indica and other species. (Even though the original figures may be clearer than those in this protocol.)	English	Japan

177.	*145*	Editorial		The resolution of this image can be improved. The shape of the image should not be changed. Please remove the header, the legend, and the footer from the image.	English	New Zealand
178.	*147*	Editorial	[Footnote 1] The term “seeds” is used in the rest of the protocol but it also represents “grain” in case a sample of a commodity class for seeds intended for processing or consumption is involved.	The text regarding footnote 1 should be placed at the bottom of the page where footnote 1 is used rather than at the end of diagnotic protocol.	English	Canada
179.	*147*	Editorial	[Footnote 1]The term “seeds” is used in the rest of the protocol but it also represents “grain” in case a sample of ~~a~~the commodity class ~~for~~ "seeds intended for processing or consumption" is involved.	Clearer wording.	English	European Union
180.	*147*	Substantive	~~[Footnote 1]The term “seeds” is used in the rest of the protocol but it also represents “grain” in case a sample of a commodity class for seeds intended for processing or consumption is involved~~.	See explanation in paragraph 7	English	COSAVE, Paraguay, Chile, Brazil
181.	*147*	Substantive	~~[Footnote 1]The term “seeds” is used in the rest of the protocol but it also represents “grain” in case a sample of a commodity class for seeds intended for processing or consumption is involved~~.	See explanation in paragraph 7	English	Uruguay
182.	*147*	Substantive	~~[Footnote 1]The term “seeds” is used in the rest of the protocol but it also represents “grain” in case a sample of a commodity class for seeds intended for processing or consumption is involved~~.	See explanation in paragraph 7	English	Argentina
183.	*148*	Editorial	[Footnote 2] The use of products of the brand Applied Biosystems in this diagnostic protocol implies no approval of them to the exclusion of others that may also be suitable. This information is	The text regarding footnote 2 should be placed at the bottom of the page	English	Canada

			given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.	where footnote 2 is used rather than at the end of diagnotic protocol.		
184.	*149*	Editorial	[Footnote 3] The use of products of the brand Promega in this diagnostic protocol implies no approval of them to the exclusion of others that may also be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.	The text regarding footnote 3 should be placed at the bottom of the page where footnote 3 is used rather than at the end of diagnotic protocol.	English	Canada
185.	*150*	Editorial	[Footnote4]The use of products of the brand Invitrogen in this diagnostic protocol implies no approval of them to the exclusion of others that may also be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.	The text regarding footnote 4 should be placed at the bottom of the page where footnote 4 is used rather than at the end of diagnotic protocol.	English	Canada
186.	*151*	Editorial	[Footnote 5] The use of products of the brand QIAGEN in this diagnostic protocol implies no approval of them to the exclusion of others that may also be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.	The text regarding footnote 5 should be placed at the bottom of the page where footnote 5 is used rather than at the end of diagnotic protocol.	English	Canada
187.	*152*	Editorial	[Footnote 6] The use of products of the brand Bioline in this diagnostic protocol implies no approval of them to the exclusion of others that may also be suitable. This information is given for the convenience of users of this protocol and does not constitute an endorsement by the CPM of the chemical, reagent and/or equipment named. Equivalent products may be used if they can be shown to lead to the same results.	The text regarding footnote 6 should be placed at the bottom of the page where footnote 6 is used rather than at the end of diagnotic protocol.	English	Canada
188.	*153*	Editorial	[Footnote 7] A. Radova, State Phytosanitary Administration, Olomouc (CZ); I. Vloutoglou, Benaki Phytopathological Institute, Athens (GR); A. Porta-Puglia, Istituto Sperimentale per la Patologia Vegetale, Rome (IT); C. Montuschi, Servizio Fitosanitario Regionale, Bologna (IT); I. van Brouwershaven, NPPO, Wageningen (NL); M. de Jesus Gomes, E. Diogo & M.R. Malheiros, Direcção-Geral de Protecção das Culturas, Lisboa (PT); V. Cockerell, Science and Advice for Scottish Agriculture, Roddinglaw, Edinburgh (GB); A. Barnes, FERA, Sand Hutton, York (GB).	The text regarding footnote 7 should be placed at the bottom of the page where footnote 7 is used rather than at the end of diagnotic protocol.	English	Canada