2013-003: Draft Annex to ISPM 27 – Anguina spp.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
1.	G	Substantive	I support the document as it is and I have no comments		PPPO, Kenya, Guyana	Noted.
2.	G	Substantive		In addition to the information presented here, it might be nice to also have a small discussion about ways to detect/separating out nematode galls from healthy seed. It might be useful for the reader to know that this can be done visually and by floating the nematode galls out.	United States of America	INCORPORATED: draft protocol revised consequently. A new paragraph was added in the section 3.2.1.
3.	G	Technical	consider the use of a simulated RFLP digestion rather than an actual gel RFLP digestion? Something similar is in the ~~attached~~ paper deWaard et al 2010 whereby the COI region is sequenced then analysis is done on the actual sequence (in Geneious or related analysis program) using the enzyme cutting sites to generate a simulated RFLP assay.	Reference: De Waard, JR, Mitchell, A, Keena, MA, Gopurenko, D, Boykin, L, Armstrong, KF, Pogue, MG, Lima, J, Floyd, R, Hanner, RH & Humble, LM 2010, 'Towards a Global Barcode Library for Lymantria (Lepidoptera: Lymantriinae) Tussock Moths of Biosecurity Concern' PLoS One, vol 5, no. 12, pp. e14280	Australia	CONSIDERED BUT NOT INCORPORATED: the current protocol proposes alternative tests to RFLP. Additionally, there is no comprehensive methodology and specific barcode to be used for a reliable identification of the targeted species.
4.	G	Technical	Some brand names are not linked to Footnote 1 and should be linked. This is the case for brand names mentioned in paragraphs 204 and 218	Footnotes and brand names (based on SC decision and according to TPDP instruction to authors). If in the DP there is more than one mention to a brand name, the second mention (and the subsequential mentions) to a brand name shall be associated with	Uruguay, Argentina	INCORPORATED. Disclaimer adjusted to the standard text

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
				the footnote number with the full text (e.g. If the first mention to a brand name is "footnote 1", the subsequent mentions to brand names should be accompanied by the same footnote number, i.e., "footnote number 1"		in all diagnostic protocols.
5.	1	Substantive	Draft Annex to ISPM 27 – *Anguina* spp. (2013-003) The title of this standard is not pertinent. Add the identification method of the juveniles of Anguina spp.	The title of this standard is “Anguina spp. Nematodes", but only three species be introduced. It is suggested to consider whether the subject is accurate. Basically, the nematodes of Anguina intercepted in port are second-stage juveniles and it is difficult to identify by morphological method.	China	CONSIDERED, BUT NOT INCORPORATED: The scope and title of the DP are defined by the SC. The title is in line with the scope, as for other adopted DPs (i.e. at genus level and then some species specific). Regarding the identification of juveniles of Anguina spp., the protocol already provides information on J2 morphological identification in tables 2 and 3. It is not clear which additional information is expected to be included in the protocol.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
6.	10	Substantive	Three *Anguina* species, *A. tritici* (Steinbuch, 1799) Filipjev, 1936, *A. agrostis* **(**Steinbuch, 1799) Filipjev, 1936 and *A. funesta* Price, Fisher & Kerr, 1979 are considered of economic importance as agricultural and quarantine pests in various countries (Chizhov and Subbotin, 1990; Krall, 1991). Other species with importance in a limited geographic range include: The reason for not introducing other species is not appropriate.	This paragraph lists the Anguina spp. nematodes with economic significance, which may implies that other species are not economically significant. It is suggested to eliminate the possibility of such imply.	China	CONSIDERED , BUT NOT INCORPORATED : The IPPC diagnostic protocols aim at contributing to secure coordinated, effective action to prevent and to control the introduction and spread of pests of plants and plant products. In this respect, all species of Anguina genus are not relevant to be included in such protocol. The scope of this DP is to detect and identify the three species: *A. tritici, A. agrostis* and *A. funesta.*
7.	20	Technical	A. *pacificae* Cid del Prado Vera & Maggenti, 1984 (Ferris, 2013).	The species Anguina paludicola Bertozzi & Davies should also be included here. This is an economically important species. Anguina paludicola occurs in Australia and is associated with the livestock disease flood plain staggers. Livestock become ill after consuming R. toxicus bacteria within A. paludicola nematode galls in the apical meristems of the grasses Polypogon monspeliensis and Agrostis avenacea.	United States of America	CONSIDERED BUT NOT INCORPORATED: Tracking back to the molecular work of Subbotin (ITS phylogeny) and Powers (RFLP), it is only a single bp

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
						change that separates A. paludicola from another Anguina sp. One would hesitate to rely on such a small amount of variation that could be easily mistaken. This species fits in the category of “other” in comparison to the ones that are covered by name. The scope of this DP as agreed by the SC is to detect and identify the three species: *A. tritici, A. agrostis* and *A. funesta.*
8.	21	Editorial	*A. tritici* (wheat gall nematode, bunted wheat) has been recorded in major wheat growing areas on all continents (Southey, 1972) and is widely distributed (EPPO, 2015). This species can cause severe crop losses to *Secale cereale* L. (rye) (35–65%) and *Triticum aestivum* L. (wheat) (20–50%) (Leukel, 1929, 1957; Anwar *et al*., 2001). However, the use of modern seed cleaning methods that separate galls from healthy grains has almost eliminated this species from commercial wheat production in developed countries. For example, recent surveys for *A. tritici* in stored grain harvested from states of the United States with records of this nematode did not provide any evidence that it was still present in the country (CABI, 2001, 2014b).	In the sentence 'Recorded hosts are T. aestivum, Triticum monococcum L. (emmer), Triticum dicoccum Schrank, Triticum durum Desf., Triticum ventricosum Ces, S. cereale (rye) and Triticum spelta L . (spelt). Hordeum vulgare L. (barley) is a very poor host' present hosts in alphabetical order. Delete 's' to oats	EPPO, European Union	INCORPORATED

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			Recorded hosts are *T. aestivum*, *Triticum monococcum* L. (emmer), *Triticum dicoccum* Schrank, *Triticum durum* Desf., *Triticum ventricosum* Ces, *S. cereale* (rye) and *Triticum spelta* L. (spelt). *Hordeum vulgare* L. (barley) is a very poor host. There is little evidence that this nematode reproduces on *Avena sativa* L. (oats) and other grasses; although there are some reports of damage to oat~~s~~ at seedling stage by second-stage juveniles, no galls have been observed.			
9.	*21*	Technical	*A. tritici* (wheat gall nematode, bunted wheat) has been recorded in major wheat growing areas on all continents (Southey, 1972) and ~~is~~was historically widely distributed (EPPO, 2015). This species can cause severe crop losses to *Secale cereale* L. (rye) (35–65%) and *Triticum aestivum* L. (wheat) (20–50%) (Leukel, 1929, 1957; Anwar *et al.*, 2001). However, the use of modern seed cleaning methods that separate galls from healthy grains has almost eliminated this species from commercial wheat production in developed countries. For example, recent surveys for *A. tritici* in stored grain harvested from states of the United States with records of this nematode did not provide any evidence that it was still present in the country (CABI, 2001, 2014b). Recorded hosts are *T. aestivum*, *Triticum monococcum* L. (emmer), *Triticum dicoccum* Schrank, *Triticum durum* Desf., *Triticum ventricosum* Ces, *S. cereale* (rye) and *Triticum spelta* L. (spelt). *Hordeum vulgare* L. (barley) is a very poor host. There is little evidence that this nematode reproduces on *Avena sativa* L. (oats) and other grasses; although there are some reports of damage to oats at seedling stage by second-stage juveniles, no galls have been observed on this host.	Is there a reference for the host information?	United States of America	INCORPORATED: text modified according to changes proposed and reference added.
10.	*22*	Editorial	*Clavibacter tritici*, the bacterium which is the causal agent of yellow ear rot or "tundu" of *T. aestivum*, is associated with the presence of *A. tritici*. Freshly harvested infected wheat cockles containing the bacterium are toxic to cattle and sheep (Anwar *et al.*, 2001). *A. tritici* has been shown to vector *Rathayibacter toxicus* (toxic yellow slime bacterium) under experimental conditions (Riley and McKay, 1990).	Editorial	United States of America	INCORPORATED

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
11.	24	Technical	*A. agrostis* has been shown to vector *R. toxicus* under experimental conditions (Riley and McKay, 1990). Several older references (e.g. Goodey, 1960) to this species being the causal agent of disease in livestock relate to galls on *Festuca* spp., ~~and records are therefore uncertain~~ may actually refer to the species *A. funesta* (Southey, 1973).	More technically correct	United States of America	INCORPORATED
12.	26	Technical	This species is recorded as a vector of the bacterium *R. toxicus*, which causes the disease annual ryegrass toxicity when consumed by livestock. Annual ryegrass toxicity is responsible for severe losses in the livestock industry in Australia (Price *et al*., 1979). Rangeland infested by the nematode and bacterium is unusable for grazing (Figures 1 to 3).	For clarity	United States of America	INCORPORATED
13.	36	Substantive	**Name:** *Anguina tritici* (Steinbuch, 1799) ~~Filipjev, 1936~~Chitwood, 1935	Chitwood was cited as the second revising author as indicated in CABI, 2016 and CIH Descriptions of plant parasitic nematodes Set 1, No 13, Anguina triciti.	Singapore	INCORPORATED
14.	67	Editorial	**3.1.1 *Anguina tritici* (After Southey, 1972; Krall, 1991.)** *A. tritici* incites seed galls (ear cockles) in cereals. Invasive juveniles emerge from the seed galls in the soil and attack newly germinated seedlings. They establish infection on the tissues of young leaves near the growing point where they feed as an ectoparasite causing leaf distortion and crinkling. Infected hosts become stunted and exhibit shorter and deformed stems and leaves. Severely infected plants do not form ears or form only stunted ears on stunted stems. A diseased ear is much wider and shorter than a normal ear and has short deformed awns (Figure 4). The second-stage juveniles (J2) stimulate the formation of galls in floral tissues in place of seed development. Galls vary from light and dark brown to almost black (Figure 5). They are smaller than healthy grains (Figure 5(A)). The nematodes can survive in a quiescent stage in seeds (Figure 6). Slight elevations occur on the	Figure 4 and Figure 5 have A and B parts, therefore clarity is requested as to which figure is being reffered to in the text	South Africa	INCORPORATED: reference to relevant images was included.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			upper leaf surface with indentations on the lower side. Other leaf symptoms include wrinkling, twisting, curling of the margins towards the midrib, distortion, buckling, swelling and bulging. A tight spiral coil evolves, and dwarfing, loss of colour or development of a mottled yellowed appearance, and stem bending may also occur. In severe infection, the entire above-ground plant is distorted to some degree and therefore the disease is usually obvious (CABI, 2015).			
15.	67	Editorial	**3.1.1 *Anguina tritici* (After Southey, 1972; Krall, 1991.)** *A. tritici* incites seed galls (ear cockles) in cereals. Invasive juveniles emerge from the seed galls in the soil and attack newly germinated seedlings. They establish infection on the tissues of young leaves near the growing point where they feed as an ectoparasite causing leaf distortion and crinkling. Infected hosts become stunted and exhibit shorter and deformed stems and leaves. Severely infected plants do not form ears or form only stunted ears on stunted stems. A diseased ear is much wider and shorter than a normal ear and has short deformed awns (Figure 4). The second-stage juveniles (J2) stimulate the formation of galls in floral tissues in place of seed development. Galls vary from light and dark brown to almost black (Figure 5). They are smaller than healthy grains (Figure 5(A)). The nematodes can survive in a quiescent stage in seed galls (Figure 6). Slight elevations occur on the upper leaf surface with indentations on the lower side. Other leaf symptoms include wrinkling, twisting, curling of the margins towards the midrib, distortion, buckling, swelling and bulging. A tight spiral coil evolves, and dwarfing, loss of colour or development of a mottled yellowed appearance, and stem bending may also occur. In severe infection, the entire above-ground plant is distorted to some degree and therefore the disease is usually obvious (CABI, 2015).	Maybe move this information about gall formation to the end of this paragraph. We think that would keep all of the symptom information together and be a bit clearer for the reader to follow.	United States of America	INCORPORATED

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
16.	69	Editorial	*A. agrostis* is considered to be an economically important nematode pest of bentgrass. In grasses, seed galls are difficult to detect as they are covered by lemmas and paleae. A small scarifier can be used to remove lemmas and paleae without damage to seeds or galls. This allows visual identification of galls (Alderman *et al.*, 2003). Galled flowers have glumes of two or three times the normal length, lemmas five to eight times the normal length, projecting beyond the glumes as a sharp point, and paleae developing to about four times the normal length. Galls are at first greenish, and later become dark purple–brown. They reach 4–5 mm long (Figures 7 and 8). Lodicules, stamens and sometimes other flower parts are suppressed in parasitized flowers. Symptoms of the inflorescence also include elongated flower galls, which are modified ovaries, that look greenish or purple and may be 4–5 mm long. Seed galls containing the nematodes are dark brown. They may look similar to normal seeds but are lighter and hence can be separated mechanically from them.	Grammar check	Singapore	**Noted.** The DP will be edited after consultation period and before notification period.
17.	69	Editorial	*A. agrostis* is considered to be a economically important nematode pest of bentgrass. In grasses, seed galls are difficult to detect as they are covered by lemmas and paleae. A small scarifier can be used to remove lemmas and paleae without damage to seeds or galls. This allows visual identification of galls (Alderman *et al.*, 2003). Galled flowers have glumes of two or three times the normal length, lemmas five to eight times the normal length, projecting beyond the glumes as a sharp point, and paleae developing to about four times the normal length. Galls are at first greenish, and later become dark purple–brown. They reach 4–5 mm long (Figures 7 and 8). Lodicules, stamens and sometimes other flower parts are suppressed in parasitized flowers. Symptoms of the inflorescence also include elongated flower galls, which are modified ovaries, that look greenish or purple and may be 4–5 mm long. Seed galls containing the nematodes are dark brown. They	Deletion of "a" and addition on "an" for gramaticall correctness	South Africa	INCORPORATED

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			may look similar to normal seeds but are lighter and hence can be separated mechanically from them.			
18.	*69*	Editorial	*A. agrostis* is considered to be an economically important nematode pest of bentgrass. In grasses, seed galls are difficult to detect as they are covered by lemmas and paleae. A small scarifier can be used to remove lemmas and paleae without damage to seeds or galls. This allows visual identification of galls (Alderman *et al*., 2003). Galled flowers have glumes of two or three times the normal length, lemmas five to eight times the normal length, projecting beyond the glumes as a sharp point, and paleae developing to about four times the normal length. Galls are at first greenish, and later become dark purple–brown. They reach 4–5 mm long (Figures 7 and 8). Lodicules, stamens and sometimes other flower parts are suppressed in parasitized flowers. Symptoms of the inflorescence also include elongated flower galls, which are modified ovaries, that look greenish or purple and may be 4–5 mm long. Seed galls containing the nematodes are dark brown. They may look similar to normal seeds but are lighter and hence can be separated mechanically from them.	grammar	Philippines	INCORPORATED : see answer to comment 17
19.	*69*	Editorial	*A. agrostis* is considered to be an economically important nematode pest of bentgrass. In grasses, seed galls are difficult to detect as they are covered by lemmas and paleae. A small scarifier can be used to remove lemmas and paleae without damage to seeds or galls. This allows visual identification of galls (Alderman *et al*., 2003). Galled flowers have glumes of two or three times the normal length, lemmas five to eight times the normal length that, projecting beyond the glumes as a sharp point, and paleae developing to about four times the normal length. Galls are at first greenish, and later become dark purple–brown. They reach 4–5 mm long (Figures 7 and 8). Lodicules, stamens and sometimes other flower parts are suppressed in parasitized flowers. Symptoms of the inflorescence also include elongated flower galls, which are modified ovaries, that look greenish or purple and	We think the using both the terms “seed gall” and “flower gall” here is a bit confusing for the reader. Maybe just chose one of these terms. “Seed gall” is used in the paragraph above.	United States of America	INCORPORATED : see answer to comment 17

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			may be 4–5 mm long. Seed galls containing the nematodes are dark brown. They may look similar to normal seeds but are lighter and hence can be separated mechanically from them.			
20.	*71*	Substantive	The life cycle of *A. funesta* is similar to that outlined for *A. agrostis*. Information on the biology of this species can be found in Price *et al.* (1979). Symptoms of infestation are shown in Figures 1 to 3. include detailed symptoms for A. funesta	Since detailed symptoms were provided for A. agrostis and A. tritici we suggest to include detailed symptoms for A. funesta instead of just reffering to the figures 1 to 3.	Philippines	INCORPORATED
21.	*71*	Technical	The life cycle of *A. funesta* is similar to that outlined for *A. agrostis*. Information on the biology of this species can be found in Price *et al.* (1979). Symptoms of infestation are shown in Figures 1 to 3.	It's okay to say the life cycles of both species are similar, but it might be helpful for the reader if the symptoms of A. funesta infestation are described here in detail.	United States of America	INCORPORATED
22.	*72*	Technical	**3.2 Nematode extraction** Add the panning method for galls to nematode extraction	In actual testing, it also needs some skills to detect galls from seeds and this is difficult, necessary introduction is needed.	China	CONSIDERED BUT NOT INCORPORATED: no description of the panning method is currently available in the references available. If provided, the drafting team would be please to include it.
23.	*76*	Editorial	Detailed descriptions of extraction equipment and procedures can be found in the EPPO standard on nematode extraction (EPPO, 2013a).	Suggest that EPPO be written in full for this is the first time it is being used in the text	South Africa	CONSIDERED BUT NOT INCORPORATED: this is not part of the formatting rules for the IPPC protocol.
24.	*77*	Substantive	All stages of anguinid nematodes can be extracted from plant tissue, and infective juveniles can also be isolated from soil or growing medium, using the Baermann funnel technique, the modified Baermann tray method	Why is it necessary to detect the degree of infestation/infection? Since we are dealing with a regulated pest is it necessary to know the level of infestation? because the mere presence of the	Philippines	CONSIDERED BUT NOT INCORPORATED:

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			(Hooper and Evans, 1993), an adapted sugar flotation method (Coolen and D'Herde, 1972) or the mistifier technique (Hooper *et al.*, 2005). These extraction methods should be conducted for 48 h at room temperature ~~to detect low levels of infestation~~. Any plant material to be tested should be cut into pieces or sliced before extracting for increased efficacy of extraction. The number of infective juveniles that may be recovered from soil depends on soil type, sampling depth, host plant and seasonal factors (Hooper, 1986). A large amount of fresh organic matter in the soil sample (e.g. plant residue after harvest) can influence nematode numbers because of its decomposition process, which might be toxic to nematodes or increase the number of saprophytic nematodes, or because the organic matter hampers extraction by clogging sieves or contaminating the supernatant obtained in density-based methods.	regulated pest be it low or high level should be addressed accordingly.		the protocol doesn't instruct the NPPO, but the protocol may be used in different situations with different purposes. The protocol instructs the operator how to conduct the nematodes extraction to get the best recovery rate.
25.	77	Technical	All stages of anguinid nematodes can be extracted from plant tissue, and infective juveniles can also be isolated from soil or growing medium, using the Baermann funnel technique, the modified Baermann tray method (Hooper and Evans, 1993), an adapted sugar flotation method (Coolen and D'Herde, 1972) or the mistifier technique (Hooper *et al.*, 2005). These extraction methods should be conducted for 48 h at room temperature to detect low levels of infestation. Any plant material to be tested should be cut into pieces or sliced before extracting for increased efficacy of extraction. The number of infective juveniles that may be recovered from soil depends on soil type, sampling depth, host plant and seasonal factors (Hooper, 1986). A large amount of fresh organic matter in the soil sample (e.g. plant residue after harvest) can influence nematode numbers because of its decomposition process, which might be toxic to nematodes or increase the number of saprophytic nematodes, or because the organic matter hampers extraction by clogging sieves or contaminating the supernatant obtained in density-based methods.	Is soil sampling often done for anguid nematodes? We would think seed samples would be more efficient. You may want to state when soil sampling would be used.	United States of America	CONSIDERED BUT NOT INCORPORATED: DP should not instruct the NPPOs. Soil sampling is effective when screening for Anguinids such as *A. pacificae* or other species infecting vegetative plant tissue. The Cobbs extraction method is quite efficient. Soil sampling and analysis also allow detection from growing media

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
						during import/export activities.
26.	*78*	Editorial	The Baermann funnel technique (and modifications of it, such as the tray method, or Seinhorst mistifier, described by Hooper (1986)) is a reference technique for extraction of nematodes from soil and plant material. A piece of rubber tubing is attached to the stem of a glass funnel (with a recommended slope of approximately 30 degrees) and is closed by a spring or screw clip. The funnel is placed in a support and almost filled with tap water. A plastic sieve or wire basket with a large enough aperture size to allow nematodes to actively pass through is placed just inside the rim of the funnel. Plant tissue cut into small pieces or soil is placed either directly onto the mesh or onto a single-ply tissue supported by the mesh, and the water level is adjusted so the substrate is only just submerged. Active nematodes pass through the mesh and sink to the bottom of the funnel stem. Alternatively, funnels made of plastic or stainless steel and/or tubing made of silicone can be used. However, regarding the latter, diffusion of oxygen into water is lower than for polyethylene (Stoller, 1957), which can lead to slow asphyxiation of the nematodes. Depending on the plant tissue, most (50–80%) of the motile nematodes present will be recovered within 24 h; however, samples can be left on the funnel for up to 72 h to increase the recovery rate. For longer extraction periods, regular tapping of the funnel and addition of fresh water increases nematode motility, compensates for evaporation and lack of oxygen and therefore improves the recovery rate. The efficacy of extraction can also be improved by adding 1–3% hydrogen peroxide (H_2O_2) for oxygen supply (Tarjan, 1967, 1972). Following the extraction period, a small quantity of water containing the nematodes is run off and observed under a stereomicroscope (Flegg and Hooper, 1970).	Editorial	United States of America	INCORPORATED
27.	*79*	Substantive	Motile and immotile nematodes can be extracted from plant material by the sugar flotation method (Coolen	suggest specifying rotor speed in "g" (graviational force), since "rpm" will differ between centrifuges,	South Africa	INCORPORATED

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			and D'Herde, 1972). The plant material is washed, cut into pieces of about 0.5 cm, and 5 g portions are macerated in 50 ml tap water in a domestic blender at the lowest mixing speed for 2 min. The suspension of nematodes and tissue fragments are washed through a 750 µm sieve placed on top of a 45 µm sieve. The residue on the 45 µm sieve is collected and poured into two 50 ml centrifuge tubes. About 1 ml kaolin is added to each tube, the mixture is thoroughly stirred and then it is centrifuged at 1500g. for 5 min. The supernatant is decanted and sucrose solution (density 1.13 g/cm^3) is added to the tubes. The mixture is thoroughly stirred and centrifuged at 1750 r.p.m. for 4 min. The supernatant is washed through a 45 µm sieve, the residue is collected and the nematodes are studied under a stereomicroscope. Instead of sucrose, zinc sulphate ($ZnSO_4$), magnesium sulphate ($MgSO_4$) or colloidal silica can be used.	depending on the radius of the rotor to obtain the same "g"		
28.	*79*	Technical	Motile and immotile nematodes can be extracted from plant material by the sugar flotation method (Coolen and D'Herde, 1972). The plant material is washed, cut into pieces of about 0.5 cm, and 5 g portions are macerated in 50 ml tap water in a domestic blender at the lowest mixing speed for 2 min. The suspension of nematodes and tissue fragments are washed through a 750 µm sieve placed on top of a 45 µm sieve. The residue on the 45 µm sieve is collected and poured into two 50 ml centrifuge tubes. About 1 ml kaolin is added to each tube, the mixture is thoroughly stirred and then it is centrifuged at 1500g. for 5 min. The supernatant is decanted and sucrose solution (density 1.13 g/cm^3) is added to the tubes. The mixture is thoroughly stirred and centrifuged at 1750 r.p.m. for 4 min. The supernatant is washed through a 45 µm sieve, the residue is collected and the nematodes are studied under a stereomicroscope. Instead of sucrose, zinc sulphate ($ZnSO_4$), magnesium sulphate ($MgSO_4$) or colloidal silica can be used.	Van Bezooijen (2006) states that this extraction method (sugar flotation method) can distort the nematodes and make them hard to visually identify. You may want to indicate when this extraction method would be appropriate, and when it would not be the best method to use. Reference: van Bezooijen, J. 2006. Methods and techniques for nematology. Wageningen University, Wageningen, The Netherlands. 112 pp.	United States of America	CONSIDERED BUT NOT INCORPORATED: Van Bezooijen explained that this was a problem only if worms were left in the extraction fluid for an extended time. Prompt extraction and cleaning in water should resolve the problem.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
29.	82	Substantive	For the sieve blend method, which is described as the most effective in Griesbach *et al.*, (1999), the seeds are first soaked in water and aerated with an air stone and aquarium pump for 24 h. The seeds are then collected on a 25 µm pore size sieve and back-washed into a blender with 400 ml water. The mixture is homogenized for 30 s. One litre of tap water is added progressively as the mixture is passed through 850 µm pore size (20 mesh) and 25 µm pore size sieves. Residue from the 25 µm sieve is back-washed into dishes, and the nematodes are observed.	The most effective extraction method, as mentioned by Griesbach et al. (1999), is not the sieve blend method as indicated in the IPPC protocol, it is the blender-funnel-host stimulant method (method 4 in the article).	EPPO, European Union	INCORPORATED
30.	82	Technical	For the sieve blend method, which is described as the most effective in Griesbach *et al.*, (1999), the seeds (1) are first soaked in water and aerated with an air stone and aquarium pump for 24 h. The seeds are then collected on a 25 µm pore size sieve and back-washed into a blender with 400 ml water(2). The mixture is homogenized for 30 s(2). One litre of tap water is added progressively as the mixture is passed through 850 µm pore size (20 mesh) and 25 µm pore size sieves. Residue from the 25 µm sieve is back-washed into dishes, and the nematodes are observed.	(1) It is not clear what the scope of this extraction method is. Does it apply to any type of seed, as the original paper from Griesbach et al. (1999) only refers to Agrostis seeds. (2) Additionnally, the paragraph refers to specific volume of water and duration of homogeneization. As the size of the sample may change, it might be more valuable for the operator to have the objective of each step of the extraction process with specific timing as an exemplae for a sample of xx grams of seeds (examples of objectives: soaking seeds to soften galls, use of blender to mix the seeds or to crush them)..	EPPO, European Union	INCORPORATED
31.	84	Editorial	The scope of this protocol is to facilitate identification of *Anguina* to genus. Both morphological (section 4.1) and molecular (section 4.2) approaches are presented.	Addition of the wording:" level" for clarity purposes and to make the sentence consistent with paragraph 112	South Africa	INCORPORATED
32.	86	Editorial	Information for morphological identification of valid genera within the Anguinidae is provided in section 4.1.2.1. Species of Anguinidae are probably one of the most variable groups among the Tylenchin~~d~~a regarding morphological characters (Brzeski, 1998). Identification to species can be unreliable if morphological characters are used in isolation; information regarding biology, host plant and symptoms of infection should also be taken into consideration. Often, only juveniles are found in seed galls, which can further complicate identification as important morphological features in adult specimens cannot be observed. Morphological information for the three species of economic importance is provided in	The classification system used in Para.[34] of the standard was "…Rhabditida, Tylenchina,…".	China	INCORPORATED

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			section 4.1.2.2; however, this information should be used in combination with other sources to confirm diagnosis. Keys to species have been provided by Krall (1991) (10 species) and Brzeski (1998) (four species recorded from temperate Europe).			
33.	*89*	Substantive	However, T a temporary microscope slide preparations can be made quickly for instant examination but such slides may remain usable for only several weeks.	We suggest that paragraph no. 89 be paragraph 90	Philippines	INCORPORATED
34.	*90*	Substantive	If possible, pPermanent slides should be prepared for future reference and deposited in nematode reference collections. Methods of preparing permanent slide mounts of nematodes have been described in detail elsewhere (Seinhorst, 1962; Hooper, 1986). The slow evaporation method as described by Hooper (1986) is outlined in section 4.1.1.2 so as to preserve the structures and characteristics of the nematodes.	We suggest that paragraph 90 be paragraph 89 since permanent slide preparation is the ideal procedure	Philippines	MODIFIED. The change of order of the paragraphs was done, but as this preparation of slides may not be possible all the time, the term “if possible” is kept.
35.	*93*	Editorial	A glass slide, free of dust, is placed on the side of the microscope stage. A small drop of single strength triethanolamine and formalin (TAF) fixative (10% of 35% formaldehydein, 1% triethanolamine and 89% distilled water) or another appropriate fixative is put in the centre of the slide and an appropriate amount of paraffin wax shavings is positioned around the drop (the wax will help support the coverslip and seal it to the slide).	There is 35%-40% formaldehyde in formalin. Thus, there is only 0.012%-0.014% formaldehyde in TAF including 10% of 35% formalin in Para. 93. And there was no report confirming that this concentration of formaldehyde could fix nematodes.	China	CONSIDERED AND MODIFIED. A footnote was added to explain that formalin comprises 35–40% formaldehyde in water.
36.	*93*	Substantive	A glass slide, free of dust, is placed on the side of the microscope stage. A small drop of single strength triethanolamine and formalin (TAF) fixative (10% of 35-40% formalin, 1% triethanolamine and 89% distilled water) or another appropriate fixative is put in the centre of the slide and an appropriate amount of paraffin wax shavings is positioned around the drop (the wax will help support the coverslip and seal it to the slide).	To be consistent with the reagents used, suggest to use percentage concentration as the basis of formulation as some formalin have ranges of 37 - 40% w/v concentration.	Singapore	MODIFIED: the text has been amended and a footnote added for clarity.
37.	*101*	Substantive	The nematodes are transferred to an embryo dish or suitable watch glass half full of single strength TAF	To be consistent with the reagents used. Suggest to use percentage concentration as the basis of	Singapore	MODIFIED: the text has been amended

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			~~fixative (7 ml formalin (40% formaldehyde), 2 ml triethanolamine, 91 ml distilled water~~) triethanolamine and formalin (TAF) fixative (10% of 35 - 40% formalin, 1% triethanolamine and 89% distilled water). The dish is covered and left to fix for a minimum of one week.	formulation as some formalin have ranges of 37-40% w/v concentration.		and a footnote added for clarity.
38.	*111*	Technical	**4.1.2 Morphological identification at genus level and selected species** Add the morphological classification keys for morphological identification of Anguina spp. species.	Add the morphological classification keys for nematodes of the genus Anguina spp. to facilitate morphological identification.	China	CONSIDERED BUT NOT INCORPORATED: see answer to comment 41.
39.	*113*	Editorial	Comparative morphology of genera assigned to the Anguininae is presented in Table 1. Definitions of terminology used in the following sections can be found in the European and Mediterranean Plant Protection Organization's *Diagnostic protocols for regulated pests: Pictorial glossary of morphological terms in nematology* (EPPO, 2013b). Key characters for identification are shown in bold.	Suggest deletion of the full name of EPPO if the suggested changes in paragraph 76 are accepted	South Africa	MODIFIED Full name of EPPO is deleted.
40.	*117*	Editorial	**4.1.2.2 Selected Anguina species**	Suggest that "Anguina" be written in italic to be consistent with paragraph 140	South Africa	INCORPORATED
41.	*117*	Technical	**4.1.2.2 Selected Anguina species**	The addition of a comment to reiterate the scope of the protocol is proposed along the following lines 'There has been little recent morphological work on the genus and there are no reliable up-to-date morphological keys to species. The drafting team therefore decided to facilitate identification to genus with some summary information only on important pest species.'	EPPO, European Union	INCORPORATED
42.	*140*	Editorial	This section provides information on molecular tests that allow the identification of isolated nematodes of~~to~~ the major *Anguina* species. The tests are generally performed following a morphological examination in order to confirm the results obtained.	Editorial	United States of America	INCORPORATED
43.	*140*	Substantive	This section provides information on molecular tests that allow the identification of isolated nematodes to the major *Anguina* species. The tests are generally	If the species is already accurately identified based on the morphological examination we do not need to proceed with the molecular test.	Philippines	CONSIDERED BUT NOT INCORPORATED:

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			performed if ~~following~~ a morphological examination is indefinite in order to confirm the results obtained.			the term "generally" is included to give the necessary flexibility to each country to systematically include a molecular confirmation or not. It doesn't formally instruct what to do. Molecular tests can be performed following morphological examination in order to confirm the results obtained
44.	141	Substantive	Molecular diagnosis of *Anguina* spp. is based on polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) (Powers *et al*., 2001), real-time PCR (Ma *et al*., 2011; Li *et al*., 2015) or sequencing of the internal transcribed spacer (ITS) region of ribosomal (r)RNA (Subbotin *et al*., 2004). The choice of test depends on whether identification requires confirmation of both the presence and the absence of particular species, and on the availability of species standards for controls. include ANNEX for this The method described by Ma *et al.* (2011) is limited to positive identification of *A. funesta,* while the other methods are able to simultaneously distinguish multiple species within the same test. PCR combined with analysis of RFLP is the most common way in which to simultaneously distinguish a range of *Anguina* species from each other (Powers *et al*., 2001).	We think that we need to mention in this paragraph or create an annex f to where thespecies standards can be obtained	Philippines	CONSIDERED BUT NOT INCORPORATED: this protocol is already an annex of ISPM27. So it is not possible to create an annex to an annex. Furthermore this paragraph is part of the introduction to molecular identification.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
45.	*141*	Technical	Molecular diagnosis of *Anguina* spp. is based on polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) (Powers *et al.*, 2001), real-time PCR (Ma *et al.*, 2011; Li *et al.*, 2015) or sequencing of the internal transcribed spacer (ITS) region of ribosomal (r)RNA (Subbotin *et al.*, 2004). The choice of test depends on whether identification requires confirmation of both the presence and the absence of particular species, and on the availability of species standards for controls. The method described by Ma *et al.* (2011) is limited to positive identification of *A. agrostisfunesta*, while the other methods are able to simultaneously distinguish multiple species within the same test. PCR combined with analysis of RFLP is the most common way in which to simultaneously distinguish a range of *Anguina* species from each other (Powers *et al.*, 2001).	The original paper of Ma et al. (2001) refers to A. agrostis.	EPPO, European Union	INCORPORATED
46.	*144*	Technical	In this diagnostic protocol, methods (including reference to brand names) are described as published, as these defined the original level of sensitivity, specificity and/or reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	Text deleted because it is included in Footnote 1 in paragraph 149, for consistency with adopted DP	Uruguay, Argentina	MODIFIED: according to instructions to authors and agreement by the SC (and according to the recent adopted DPs), this disclaimer is in the main text and in footnotes (if a brand name in the protocol).
47.	*145*	Substantive	**4.2.1 DNA extraction** This paragraph should not provide the trade names for specific products for detecting.	It is not proper that information on products of specific brand appears in this standard.	China	Considered, but not incorporated: paragraph n°144 explains that brand names are given as they contribute to

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
						describe the conditions in which the performance of the test was obtained. This is commonly done in other IPPC diagnostic protocols.
48.	*147*	Substantive	The nematode pieces in 8 µl double distilled sterile water are transferred to a microcentrifuge tube containing nematode extraction buffer - volume (10 mM Tris, pH 8.2; 2.5 mM $MgCl_2$; 50 mM KCl; 0.45% Tween 20; 0.05% gelatin; 60 µg/ml proteinase-K) (Thomas *et al.*, 1997) and frozen at –70 °C for 15 min or until needed. The extract is thawed and incubated at 60 °C for 60 min then the proteinase-K is denatured by heating at 95 °C for 15 min. The protocol by Ma *et al.* (2011) cuts the nematode in 8 µl double distilled water and transfers this suspension to a tube containing 1 µl PCR buffer (components) with 1 µl proteinase-K (1 µg/ml), with freezing as described above and incubation at 65 °C for 1 h followed by 95 °C for 10 min.	Please specify the nematode extraction buffer volume. Specify PCR buffer components	Philippines	CONSIDERED BUT NOT INCORPORATED: an example of volume has been added, but it can be adjusted depending of the needs. For PCR buffer, it depends of the TAQ enzyme that is used for the PCR and its associated PCR buffer. So no information was added for this point
49.	*149*	Editorial	~~[1] In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may~~	the same as of paragraph 144	Philippines	CONSIDERED BUT NOT INCLUDED: paragraph 149 is the footnote itself, and not in the main text. See answer to comment 46.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			~~be adjusted to the standards of individual laboratories, provided that they are adequately validated.~~			
50.	149	Editorial	[1] In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and reproducibility achieved. The use of names of reagents, chemicals or equipment in these diagnostic protocols implies no approval of them to the exclusion of others that may also be suitable. Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated.	This wording is repeated in line 144. It is probably only needed in one section of the document.	United States of America	CONSIDERED BUT NOT INCLUDED: paragraph 149 is the footnote itself, and not in the main text. See answer to comment 46.
51.	149	Technical	[1] ~~In this diagnostic protocol, methods (including reference to brand names) are described as published, as these define the original level of sensitivity, specificity and reproducibility achieved~~. The use of names of reagents, chemicals or equipment in ~~these~~ this diagnostic protocol~~s~~ implies no approval of them to the exclusion of others that may also be suitable. ~~Laboratory procedures presented in the protocols may be adjusted to the standards of individual laboratories, provided that they are adequately validated~~. This information is given for the convenience of users of this protocol and does not constitute an end orsment by the CPM of the chemical, reagent and/or eq uipment named. Equivalent products may be used if the y can be shown to lead to the same results.	Text deleted, because is already mentioned in paragraph 144. Text added according text of footnotes agreed.	Uruguay, Argentina	CONSIDERED BUT NOT INCLUDED: paragraph 149 is the footnote itself, and not in the main text. See answer to comment 46.
52.	154	Editorial	The PCR reaction and cycling conditions ~~is conducted~~ as described by Szalanski *et al.* (1997) is presented in (Table 4). Alternatively, the amplification can be conducted according to Meng *et al.* (2012) (Table 5). After PCR, 5 µl of the product is analysed electrophoretically on a 1.5% agarose gel in Tris-acetate-ethylenediaminetetraacetic acid (EDTA)	Grammar	Philippines	INCORPORATED

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			(TAE) buffer. The gel is stained with ethidium bromide and photographed using a gel imaging system with an ultraviolet light filter.			
53.	*154*	Technical	The reaction is conducted as described by Szalanski *et al.* (1997) (Table 4). Alternatively, the amplification can be conducted according to Meng *et al.* (2012) (Table 5). After PCR, 5 µl of the product is analysed electrophoretically on a 1.5% agarose gel in Tris-acetate-ethylenediaminetetraacetic acid (EDTA) (TAE) buffer. The gel is stained with gel red ~~ethidium bromide~~ and photographed using a gel imaging system with an ultraviolet light filter.	ethidium bromide is very toxic/carcinogenic, we recomment to use other stain e.g. gel red	Philippines	MODIFIED. Noted and thank you for providing alternatives to ethidium bromide. Even though the DP recommends ethidium bromide (it can be used), it is up to each country to decide which stain to use. However, it is known that ethidium bromide provide more sensitivity for the electrophoresis. If a lab has experience with other stain less toxic, it can be use as long as it gives the same level of confidence of the results.
54.	*166*	Technical	**Table 7.** Restriction fragment sizes for *Anguina* species and associated restriction fragment length polymorphism (RFLP) patterns (after Powers *et al.*, 2001)	The test results will be more intuitive.	China	CONSIDERED BUT NOT INCORPORATED. There is no specific reference to brand

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			It is not proper that information on products of specific brand appears in this standard.			name in this paragraph. See answer to comment 47.
55.	180	Substantive	Li *et al.* (2015) designed a TaqMan real-time PCR to identify *A. funesta, A. agrostis, A. tritici* and *A. pacificae.* This test includes forward and reverse genus-specific primers combined with a fluorescent probe (modified with TET dye and BHQ-2 Black Hole Quencher1). This primer and probe set was designed to serve as an internal control for confirming the presence of *Anguina* spp. as well as the integrity of the PCR components and user performance. The test also includes primer and probe sets specifically designed for detection of each of the target species mentioned above and is intended for identification of single juveniles. Species-specific probes were modified with 6-FAM and BHQ-1 and were simultaneously detectable on a different fluorescent channel in duplex PCRs. Sensitivity of the test was demonstrated through construction of standard curves from reactions using serially diluted nematode DNA: the test was able to detect as little as 1.25 copies of the ITS rDNA. The specificity of each primer and probe set was demonstrated in singleplex and duplex reactions (i.e. the species-specific and genus-specific primer sets) tested against all of the target species as well as several non-target nematodes including *Anguina* spp., *Meloidogyne* spp., *Pratylenchus* spp. and *Ditylenchus* spp.	Are there reference materials, collections, depository where we can request/obtain or purchase standards for certain nematode species	Philippines	CONSIDERED BUT NOT INCORPORATED. Unfortunately this information is not currently available and may change in time, it is not included. The contact points in section 6 may help to find specific contacts for such needs.
56.	180	Technical	Li *et al.* (2015) designed a TaqMan real-time PCR to identify *A. funesta, A. agrostis, A. tritici* and *A. pacificae.* This test includes forward and reverse genus-specific primers combined with a fluorescent probe (modified with TET dye and BHQ-2 Black Hole Quencher1). This primer and probe set was designed to serve as an internal control for confirming the presence of *Anguina* spp. as well as the integrity of the PCR components and user performance. The test also includes primer	The explanation of what duplex PCRs are, should be introduced at their first mention to help understanding. Otherwise it might be misleading, as one may understand that two species can be detected simultaneously.	EPPO, European Union	INCORPORATED

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			and probe sets specifically designed for detection of each of the target species mentioned above and is intended for identification of single juveniles. Species-specific probes were modified with 6-FAM and BHQ-1 and were simultaneously detectable on a different fluorescent channel in duplex PCRs (i.e the species-specific and genus-specific primer sets). Sensitivity of the test was demonstrated through construction of standard curves from reactions using serially diluted nematode DNA: the test was able to detect as little as 1.25 copies of the ITS rDNA. The specificity of each primer and probe set was demonstrated in singleplex and duplex reactions (i.e. the species-specific and genus-specific primer sets) tested against all of the target species as well as several non-target nematodes including *Anguina* spp., *Meloidogyne* spp., *Pratylenchus* spp. and *Ditylenchus* spp.			
57.	*204*	Technical	**Reagent** / **Final concentration** PCR-grade water / –† PCR buffer (Including $MgCl_2$) / 1× $MgCl_2$ / 6.0 mM dNTPs / 0.24 mM Species-specific primer (forward) / 240 nM Species-specific primer (reverse) / 240 nM Species-specific probe / 120 nM ASf internal control primer (forward) / 160 nM ASr internal control primer (reverse) / 160 nM ASp internal control probe / 120 nM DNA polymerase (Platinum Taq (Invitrogen)) / 1.0 U DNA (volume) / 1 µl **Cycling parameters** Initial denaturation / 95 °C for 20 s Number of cycles / 40	The final concentration of dNTPs is not correct. The original paper of Li et al. (2015) indicates 0.24 mM for each dNTPs.	EPPO, European Union	INCORPORATED

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
			Denaturation: 95 °C for 1 s; Optics OFF Annealing: 60 °C for 40 s; Optics ON Ramp: 5 °C per s **Expected amplicons** Size: 74–85 bp			
58.	222	Substantive	*Positive amplification control.* This control is used to monitor the efficiency of the amplification test (apart from the extraction). Pre-prepared (stored) nucleic acid of target nematode may be used.	Better to use standard of a particular nematode species. Question is it available?	Philippines	CONSIDERED BUT NOT INCLUDED: as mentioned, it concerns the nematode species targeted by the PCR. But no standard positive material is currently and largely available. Better contact the contact points of section 6 to get up-to-date information on this point.
59.	224	Editorial	*Negative amplification control (no template control).* This control is necessary for conventional ...	To merge para 224 & 225 to one paragraph to complete the paragraph.	Singapore	INCORPORATED.
60.	224	Editorial	*Negative amplification control (no template control).* This control is necessary for co	merge paragraph 224 and 225	Philippines	INCORPORATED see answer to comment 59
61.	224	Editorial	*Negative amplification control (no template control).* This control is necessary for co	It looks like 224 and 225 need to be combined.	United States of America	INCORPORATED see answer to comment 59

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
62.	*225*	Editorial	nventional PCR to rule out false positives due to contamination during preparation of the reaction mixture. PCR-grade water that was used to prepare the reaction mixture is added at the amplification stage.	merging of paragraph 225 and 224 proposed to rectify the typing error ("nventional") and provide clarity to the sentence	South Africa	INCORPORATED see answer to comment 59
63.	*240*	Substantive	Records and evidence should be retained as described in section 2.5 of ISPM 27 (*Diagnostic protocols for regulated pests*).	Thus, it is ideal to do permanent preparations as commented on paragraph 90	Philippines	MODIFIED: order between paragraph 89 and 90 has been change to give more emphasis to permanent preparations. But "if possible" has been kept to allow flexibility when needed.
64.	*249*	Editorial	A request for a revision to a diagnostic protocol may be submitted by N~~n~~ational P~~p~~lant P~~p~~rotection O~~o~~rganizations (NPPOs), R~~r~~egional P~~p~~lant P~~p~~rotection O~~o~~rganizations (RPPOs) or Commission on Phytosanitary Measures (CPM) subsidiary bodies through the IPPC Secretariat (ippc@fao.org), which will in turn forward it to the Technical Panel on Diagnostic Protocols (TPDP).	For consistency usage of acronyms.	Singapore	Considered but not incorporated. The text was revised to follow the IPPC style guide for ISPMs (the Style guide can be obtained at: https://www.ippc.int/en/core-activities/standards-setting/).
65.	*307*	Editorial	**9. Figures** Improve the image information so as to meet the requirements.	There are no scales for some images, and no links are provided for some network images.	China	CONSIDERED BUT NOT INCORPORATED: The scale bar is provided for each image where relevant (i.e. figures 9 to 13, drawings of

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
						nematodes). All images are given with their photo credit. These have not been obtained from "network of images".
66.	309	Substantive	**Figure ~~1~~ 6.** Healthy *Lolium rigidum* seed (left), *Anguina funesta* gall (centre) and nematode gall colonized by *Rathayibacter toxicus* (right).	Figure 1 should be figure 6 as per discussion on symptoms on paragraph 71	Philippines	CONSIDERED BUT NOT INCORPORATED: the figure is already called in paragraph 26. So the numbering is correct.
67.	312	Editorial	**Figure 2.** Healthy *Lolium rigidum* seed (left), *Anguina funesta*-infested ~~infected~~ nematode gall (centre) and *Anguina funesta*-*infested*~~infected~~ bacterial gall (right).	Infected should be replaced by infested. The use of the term is in addition not consistent across the figures	EPPO, European Union	INCORPORATED
68.	312	Substantive	**Figure ~~2~~7.** Healthy *Lolium rigidum* seed (left), *Anguina funesta*-infected nematode gall (centre) and *Anguina funesta*-infected bacterial gall (right).	Figure 2 should be figure 7 as per discussion on symptoms on paragraph 71	Philippines	CONSIDERED BUT NOT INCORPORATED: the figure is already called in paragraph 26. So the numbering is correct.
69.	315	Substantive	**Figure ~~3~~ 8.** Gumming disease of *Lolium* due to *Rathayibacter toxicus.*	Figure 3 should be figure 8 as per discussion on symptoms on paragraph 71	Philippines	CONSIDERED BUT NOT INCORPORATED: the figure is already called in paragraph 26. So the numbering is correct.

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
70.	*315*	Technical	**Figure 3.** Gumming disease of *Lolium* due to *Rathayibacter toxicus*.	Gumming disease is not caused by Anguina therefore should not be included in this figures	Philippines	CONSIDERED BUT NOT INCLUDED: The figure with gumming disease's symptoms may help when perform diagnostic especially for the first time. It may avoid confusion. In section 1 "Pest Information" it says that *A. tritici* has been shown to be a vector of *Rathayibacter toxicus,* thus, the figure is presented to avoid confusion.
71.	*318*	Substantive	**Figure ~~4~~1.** (A) Healthy *Triticum aestivum* ears (left) and those infested with *Anguina tritici* (right).	Figure 4 should be figure 1 as per discussion on symptoms on paragraph 67	Philippines	CONSIDERED BUT NOT INCORPORATED: the figure is called for the first time in paragraph 67 (whereas the 3 previous ones were called in paragraph 26). So the numbering is correct.
72.	*325*	Editorial	**Figure 5.** (A) Healthy *Triticum aestivum* seeds (left) and those infested~~infected~~ with *Anguina tritici* (right).	Same terminology issue as for Figure 2	EPPO, European Union	INCORPORATED
73.	*325*	Substantive	**Figure ~~5~~ 2.** (A) Healthy *Triticum aestivum* seeds (left) and those infected with *Anguina tritici* (right).	Figure 5 should be figure 2 as per discussion on symptoms on paragraph 67	Philippines	CONSIDERED BUT NOT INCORPORATED: the figure is called for the

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
						first time in paragraph 67 (whereas the 3 first ones were called in paragraph 26). So the numbering is correct.
74.	331	Editorial	**Figure 6.** Invasive juveniles of *Anguina tritici* survive in a quiescent state within a seed gall, emerging to infest~~infect~~ germinated seedlings.	Same terminology issue than for other figures	EPPO, European Union	INCORPORATED
75.	331	Substantive	**Figure ~~6.~~ 3** Invasive juveniles of *Anguina tritici* survive in a quiescent state within a seed gall, emerging to infect germinated seedlings.	Figure 6 should be figure 3 as per discussion on symptoms on paragraph 67	Philippines	CONSIDERED BUT NOT INCORPORATED: the figure is called for the first time in paragraph 67 (whereas the 3 first ones were called in paragraph 26). So the numbering is correct.
76.	334	Editorial	**Figure 7.** *Agrostis* plants infested~~infected~~ with *Anguina agrostis*.	Same terminology issue than for other figures	EPPO, European Union	INCORPORATED
77.	334	Substantive	**Figure ~~7.~~4** *Agrostis* plants infected with *Anguina agrostis*.	Figure 7 should be figure 4 as per discussion on symptoms on paragraph 69	Philippines	CONSIDERED BUT NOT INCORPORATED: the figure is called for the first time in paragraph 69 (whereas the 3 first ones were called in paragraph 26). So the numbering is correct.
78.	337	Editorial	**Figure 8.** Agrostis plants infested~~infected~~ with Anguina agrostis.	Same terminology issue than for other figures	EPPO, European Union	INCORPORATED

Comm. no.	Para. no.	Comment type	Comment	Explanation	Country	SC response
79.	*337*	Substantive	**Figure ~~8~~. 5** Agrostis plants infected with Anguina agrostis.	Figure 8 should be figure 5 as per discussion on symptoms on paragraph 69	Philippines	CONSIDERED BUT NOT INCORPORATED: the figure is called for the first time in paragraph 69 (whereas the 3 first ones were called in paragraph 26). So the numbering is correct
80.	*351*	Editorial	**Figure 12.** *Anguina agrostis* juvenile: (A) whole nematode; (B) tail; and (C) head. Source: University of Nebraska-Lincoln (http://nematode.unl.edu/aagrost.htm*).* Photo © Peter Mullin, 2001.	Some of these pictures seem a bit blurry. Are there higher resolution versions available?	United States of America	CONSIDERED BUT NOT INCLUDED: Noted. The pictures are not blurry because the resolution is low. They're blurry because it is magnified so much (x1000). The DP drafting group or TPDP does not have better pictures at that magnification.