

PM 3/78 (2) Consignment inspection of seed and grain of cereals

Specific scope: This Standard describes the procedure by which consignments of seed and grain of cereals are subjected to import control, including sampling and detection. This Standard covers seed and grain from wheat (*Triticum aestivum*, *Triticum durum*), triticale (*Triticosecale*), rice (*Oryza sativa*), rye (*Secale cereale*), oat (*Avena sativa*), barley (*Hordeum vulgare*), maize (*Zea mays*) and millets (*Sorghum* spp. and others).

Contamination of seed lots by invasive alien plants is not considered in this Standard. This Standard does not cover small consignments imported for trials or breeding purposes.

Specific approval: First approved in 2015-09. Revision approved in 2021-09.

Authors and contributors are given in the Acknowledgements section.

1 | INTRODUCTION

There are many important cereal crops in the EPPO region. The cereals covered in this Standard are produced in the EPPO region but are also imported from other parts of the world (e.g. Argentina, Australia, Canada, India, USA). Imported consignments may carry regulated pests which may be specific to one cereal or may affect different species. These pests are listed in the EPPO A1 or A2 Lists of pests recommended for regulation as quarantine pests or regulated by specific EPPO countries. Depending on the cereal and its origin, many EPPO member countries require that the seed and/or grain should already have been inspected in the field during the growing season. For other cereals, many EPPO member countries require that they are inspected or tested in representative samples and found free from the relevant organisms.

For example, the requirements of the European Union for imports of wheat and triticale from countries where *Tilletia indica* occurs consist of area freedom of the pest for seed and area or place of production freedom for grain. For grain, place of production freedom is evaluated by inspection of the growing crop as well as testing of samples of grain at harvest and pre-shipment and finding them free from the pathogen.

Some EPPO countries have similar requirements for *Tilletia controversa* and *Xanthomonas translucens* pv. *translucens* (both of which occur in many EPPO countries). Some EPPO countries also require consignment freedom for *Listronotus bonariensis* (absent from the EPPO region).

2 | PHYTOSANITARY INSPECTIONS

ISPM no. 5 *Glossary of Phytosanitary Terms* (IPPC, 2016) defines inspection as 'Official visual examination of plants, plant products or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations'. General background information on phytosanitary inspection of consignments is given in EPPO Standard PM 3/72 (2) *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification* (EPPO, 2009). Because inspection of seed and grain of cereals is usually not appropriate for most seed-borne pests, the collecting of samples for laboratory testing should be included in import inspection procedures. It is often necessary to take representative samples for laboratory testing for detection and identification of the pathogen of concern.

Inspection should also be carried out for the detection of organisms for which the phytosanitary risk has not yet been determined.

When an unfamiliar pest or a pest from the EPPO Alert List is detected, the procedures specified in EPPO Standard PM 5/2 (2) *Pest risk analysis on detection of a pest in an imported consignment* (EPPO, 2002) should be followed to allow the NPPO to make a decision as to what phytosanitary action to take.

The procedures described in this Standard are mainly specific to consignment inspection in an EPPO importing country, but they may also be applicable for export inspection (when the requirements of the importing country are similar, e.g. the same listed pests are covered). The general elements of this inspection procedure apply to inspection in both the exporting and the importing country.

Inspections of consignments of seed and grain of cereals in the importing country are usually performed at the point of entry although they can be carried out at

inland locations at the place of unloading. One of the advantages of inspections at points of entry is that it can reduce the risk of a pest being spread during transport or consignments being distributed to a number of locations. In addition, re-export of consignments is a more viable option for consignments at ports than those inland. An advantage of inland inspection is that it may be easier to take a representative sample throughout the consignment compared to the point of entry, where it may only be possible to take samples from the entrance of shipping containers. When a sample for laboratory testing has been taken from the consignment it should remain under official control until the final laboratory result confirms the absence of the relevant listed pests. Seeds should not be sown or grain processed until absence is confirmed.

3 | COMMODITIES CONCERNED

Seed and grain of cereals are usually traded in either bags or bulk. Seeds are intended for planting and are usually imported in smaller quantities than grain. Grain is intended for consumption and/or processing for animal feed or for processing for human consumption.

4 | REGULATED PESTS LIKELY TO BE CARRIED IN CONSIGNMENTS OF SEEDS AND GRAIN OF CEREALS IN THE EPPO REGION

This Standard mainly relates to the EPPO A1 and A2 listed pests recognized as important for the different cereals considered, as well as pests regulated in EPPO member countries. The phytosanitary procedures described in the Standard are primarily aimed at preventing the introduction of these specific pests into the EPPO region via imported consignments of seed and grain. They could also be used to detect other non-regulated pests or exotic pests of economic relevance for seed and grain.

Soil-borne pests are not included in the Standard as the risk of their presence on seed and grain cereals can be considered to be very low and any presence is incidental.

EPPO Datasheets and EPPO Diagnostic Protocols are available via EPPO (2021). For additional up-to-date information the respective scientific literature should be used.

EPPO A1 and A2 Lists of pests recommended for regulation as quarantine pests are subject to additions and deletions. The present list will therefore need to be revised whenever relevant new pests are listed.

Specific pests of seed and grain of cereals

Wheat and triticale

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
Fungi <i>Tilletia indica</i>	Bacteria <i>Xanthomonas translucens</i> pv. <i>translucens</i>	Fungi <i>Tilletia controversa</i> (Tilletiaceae) (Azerbaijan A1 2007, Israel A1 2009, Tunisia Quarantine pest 2012)
Insects <i>Listronotus bonariensis</i>		

Rye

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
Fungi <i>Tilletia indica</i>	Bacteria <i>Xanthomonas translucens</i> pv. <i>translucens</i>	
Insects <i>Listronotus bonariensis</i>		

Rice

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
Insects <i>Listronotus bonariensis</i>	Nematodes <i>Aphelenchoides besseyi</i>	
Bacteria <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> <i>Xanthomonas oryzae</i> pv. <i>oryzicola</i>		

Barley

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
Insects <i>Listronotus bonariensis</i>	Bacteria <i>Xanthomonas translucens</i> pv. <i>translucens</i>	Viruses <i>Barley stripe mosaic virus</i> (Israel Quarantine pest 2009, Jordan A1 2013, Georgia A1 2018, Turkey A1 2016, Uzbekistan A1 2008, Turkey A1 2016)

Oats and millets

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
Insects <i>Listronotus bonariensis</i>		

Maize

A1 pests	A2 pests	Other pests regulated by specific EPPO member countries
Insects <i>Listronotus bonariensis</i>	Bacteria <i>Pantoea stewartii</i>	Fungi <i>Cochliobolus carbonum</i> (Azerbaijan A1 2007, Israel Quarantine pest 2009, Kazakhstan A1 2017)
	Fungi <i>Stenocarpella macrospora</i> <i>Stenocarpella maydis</i>	

5 | LOT IDENTIFICATION

General background information on lot identification is given in EPPO Standard PM 3/72 (1) *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification* (EPPO, 2009). A lot represents a homogenous part of a consignment. In the case of cereals, criteria for lot identification could be commodity, variety or place of production.

a. Consignments in bags

Common criteria for lot identification can be applied for consignments arriving in bags, such as commodity (e.g. wheat, barley), variety or place of production.

b. Consignments in bulk

For consignments arriving in bulk, it is difficult to identify homogeneous lots, as the consignments are a mixture from different areas of production. As a result, an adequate sampling procedure is of great importance to ensure representative inspection results. The lots should therefore be defined in accordance with the chosen sampling procedure. Lots should be defined as the smallest quantity which can be handled separately (e.g. a compartment or container).

6 | SAMPLING

This section contains guidance on sampling for inspection and laboratory testing of consignments of seeds or grains of cereals. In general sampling should be performed following the guidelines from the International Seed Testing Association (ISTA). Inspection may reveal contaminating insect pests, which are usually lighter in weight than grain, but this is not an appropriate procedure for pathogens or nematodes. To detect pathogens, sampling combined with laboratory testing should be used. As the most important listed pests for cereals are

pathogens or nematodes, this section focuses on guidance on sampling for laboratory testing.

Phytosanitary inspections are usually done after checking the documents associated with the consignment (in particular the Phytosanitary Certificate) and the integrity of the consignment. The general background for carrying out import inspections is included in ISPM no. 20 *Guidelines for a phytosanitary import regulatory system* (IPPC, 2017) and ISPM no. 23 *Guidelines for inspection* (IPPC, 2005a).

6.1 | Sampling for inspection and laboratory testing (general aspects)

The minimum size of the primary samples (size of sample) has to be chosen so that the composite sample (aggregated primary samples) contains at least the minimum amount of seed required for the requested laboratory test. Table 3 or 4 in ISPM no. 31 *Methodologies for sampling of consignments* (IPPC, 2005b) can be used to determine the sample size needed to detect a specified level of infection in a lot of seed or grain. For seed, a more stringent level should be chosen than for grain. If 2995 seeds are inspected from a lot of grain this provides a 95% confidence of detecting an infection present in 0.1% of the seeds. For seed, a sample size of 4603 seeds would provide a 99% confidence of detecting an infection present in 0.1% of the seeds. Depending on the 1000-seed weight of the cereal, the requirements will be met with minimal sample sizes between 0.5 and 2.5 kg.

a. Consignments in bags up to 100 kg

For consignments in bags, phytosanitary inspections should start with an overall examination of the packaging and means of conveyance to obtain indications of adverse conditions during transport (e.g. temperature, high moisture content), to check the physical condition of the seeds or grain and to look for live or dead insects. If adverse conditions are identified, this should be taken into account during sampling to target bags with a higher risk of infestation. An adequate proportion of seeds or grains from each lot should then be subjected to a systematic examination to detect the presence or signs of listed pests on the seed and grain of cereals as listed above. To ensure that a representative sample of a lot is inspected and/or tested, the minimal sampling intensity (number of primary samples) has to be chosen as indicated by the ISTA requirements (see Table 1) (ISTA, 2021). There is no difference between sampling for inspection or for laboratory testing. A sampling procedure needs to be followed for both.

b. Consignments in bulk or in bags over 100 kg

For consignments in bulk or in bags over 100 kg, phytosanitary inspections should start with an overall examination of the container and/or means of conveyance

TABLE 1 Minimum sampling intensity for seed lots in bags up to 100 kg based on ISTA requirements

Number of containers/bags	Minimum number of primary samples to be taken
1–4	3 primary samples from each container/bag
5–8	2 primary samples from each container/bag
9–15	1 primary sample from each container/bag
16–30	15 primary samples from the lot
31–59	20 primary samples from the lot
60–100	30 primary samples from the lot

TABLE 2 Minimum sampling intensity for seed lots in bulk or in bags of over 100 kg according to the ISTA requirements

Seed lot size (to the nearest kg)	Minimum number of primary samples to be taken
100–500	At least 5 primary samples
501–3000	1 primary sample for each 300 kg but not less than 5
3001–20 000	1 primary sample for each 500 kg but not less than 10
More than 20 001	1 primary sample for each 700 kg but not less than 40

to obtain indications of adverse conditions during transport (e.g. temperature, high moisture content), to check the physical condition of the cereal seeds or grain and to look for live or dead insects. If adverse conditions are identified, this should be taken into account during sampling and the areas targeted for sampling. An adequate proportion of seeds or grains from each lot should then be subjected to a systematic examination to detect the presence or signs of listed pests on the seed and grain of cereals as listed above. To ensure that a representative sample of a lot is inspected and/or tested, the minimum sampling intensity (number of primary samples) has to be chosen as indicated by the ISTA (see Table 2) (ISTA, 2021) and the ISO requirements (see Table 3) (ISO 24333:2009) for bigger lots. For static seed lots, particular attention must be paid to ensure a homogeneous distribution of the primary samples throughout the pile (Figure 1).

6.2 | Sampling for inspection and laboratory testing (specific aspects)

a. Consignments in bags

Samples should preferably be taken when the bags are unloaded and placed so that sampling can be performed. Primary samples from bags are preferably taken using sampling sticks or spears (e.g. a ‘stick

TABLE 3 Minimum sampling intensity for seed lots in bulk up to 1500 tonnes based on ISO Norm 24333:2009 (mass of a primary sample between 0.4 and 3 kg)

Size of the lot (L) in tonnes	Minimum number of primary samples to be taken
L < 15 tonnes	3 primary samples per lot
15 < L < 30 tonnes	8 primary samples per lot
30 < L < 45 tonnes	11 primary samples per lot
45 < L < 100 tonnes	15 primary samples per lot
100 < L < 300 tonnes	18 primary samples per lot
300 < L < 500 tonnes	20 primary samples per lot
500 < L < 1500 tonnes	25 primary samples per lot
L = 1500 tonnes	25 primary samples per lot

TABLE 4 Simplified quantities of cereals to be sampled to meet statistical and analytical requirements

Cereal	Sample weight (g) for grain/seed
Wheat/triticale	300/500
Rye	300/500
Rice	300/500
Barley	300/500
Oats	300/500
Maize	1500/2500
Millets	300/500

Note: The weights indicate the minimum size of the working sample if a 95% (for grain) or 99% (for seed) confidence level for detecting a 0.1% infection present in a sampled lot.

trier’, ‘spiral trier’ or ‘Nobbe trier’). The trier used has to correspond to the type of seed to be sampled. The width of the trier opening has to be about double the seed length and the length of the trier opening should be two to five times the width of the trier opening. Its length should penetrate half of the width of the sampled bags. The bags from which primary samples are taken must be randomly selected and samples should be taken from the top, middle and bottom of bags. The primary samples are aggregated to form a composite sample, which has to be reduced into smaller samples for inspection and/or laboratory testing (working samples). It is usual practice that a divider or riffle (Figure 2) is used to separate the composite sample into smaller more homogeneous samples.

The collected samples should be handled carefully, packed tightly into muslin sample bags and sealed to reduce movement of the seed within the bag. Each muslin bag should be packaged in a separate polypropylene bag to prevent the possible escape of harmful organisms.

If samples for quality checks have been taken from cargo agents according to ISTA rules, the phytosanitary inspection can be performed on a subsample of the agent's original composite sample for quality checks.

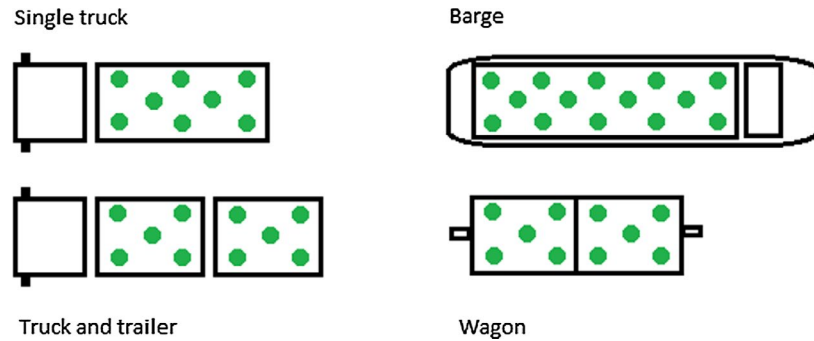


FIGURE 1 Example of sampling pattern for different transport types [Colour figure can be viewed at wileyonlinelibrary.com]



FIGURE 2 Example of a riffle sampler [Colour figure can be viewed at wileyonlinelibrary.com]

b. Consignments in bulk

Sampling consignments in bulk depends strongly on the practical limitations or possibilities at the point of unloading or inspection. Samples should preferably be taken when the grain is being unloaded by the importer's cargo agent for quality checks. The samples should always be taken using sampling techniques adapted to the situation encountered (e.g. automatic or manual sampling from the seed stream, using a cargo or bulk sampler) or sampling from a conveyor unloading system. To ensure a representative composite sample, primary samples must be taken at regular intervals as long as grain is flowing.

In the case of static grain still in the truck, trailer, wagon, barge or already been unloaded in a temporary store, the inspector should collect primary samples evenly distributed from the bulk (see example in Figure 1) to constitute the composite sample. To ensure a homogeneous sampling, it is important to take into account the depth of the receptacle or the height of the pile. Up to a depth of 2 m, sampling can be conducted manually. Up to a depth of 2.5 m, mechanical sampling techniques should be used. Between 2.5 and 9 m, suction sampling devices should be used. When sampling is conducted on a bulk with a considerable height, sampling from every 2 m corresponds to

a primary sample. However, when the depth is more than 9 m, sampling should be done during the loading/unloading, when the grain is flowing, or a primary sample must be taken from each bucket if a bucket loader is used.

The composite sample should be subdivided into smaller samples for inspection and/or laboratory testing (working samples). A divider should be used to separate the composite sample into smaller more homogeneous samples.

The collected samples should then be handled carefully, packed tightly into muslin sample bags and sealed to reduce movement of the seed within the bag. Each muslin bag should be packaged in a separate polypropylene bag to prevent the possible escape of harmful organisms from the muslin bag.

ACKNOWLEDGEMENTS

This Standard was first drafted and later updated by Mr A. Von Felten from the Swiss Federal Plant Protection Service (CH). It was reviewed by the Panel on Phytosanitary Inspections.

REFERENCES

- CABI (2014a) *Invasive Species Compendium*, datasheet: *Tilletia controversa*. In *Invasive Species Compendium*. CAB International, Wallingford (GB). www.cabi.org/isc.
- CABI (2014b) *Invasive Species Compendium*, datasheet: *Aphelenchoides besseyi*. In *Invasive Species Compendium*. CAB International, Wallingford (GB). www.cabi.org/isc.
- CABI (2014c) *Crop Protection Compendium*, datasheet: *Pantoea stewartii*. In *Invasive Species Compendium*. CAB International, Wallingford (GB). www.cabi.org/isc.
- EPPO/CABI (1997) *Quarantine Pests for Europe and EPPO Datasheets*, 2nd edn. CAB International, Wallingford (GB).
- EPPO (2021) EPPO Global Database. Available at: <https://gd.eppo.int/>
- EPPO (1991) EPPO Standard PM 3/34 *Barley stripe mosaic hordeivirus: Inspection and Test Method for Barley seeds*. *Bulletin OEPP/EPPO Bulletin* 21, 257–259.
- EPPO (2017) PM 7/39(2) *Aphelenchoides besseyi*. *Bulletin OEPP/EPPO Bulletin* 47, 384–400.
- EPPO (2016) EPPO Standard PM 7/60(2) *Pantoea stewartii* subsp. *stewartii*. *Bulletin OEPP/EPPO Bulletin* 46, 226–236.
- EPPO (2018) PM 7/29(3) *Tilletia indica*, EPPO, Paris. *Bulletin OEPP/EPPO Bulletin* 48, 7–31.
- EPPO (2007) EPPO Standard PM 7/80 *Xanthomonas oryzae*. *Bulletin OEPP/EPPO Bulletin* 37, 543–553.

- EPPO (2009) EPPO Standard PM 3/72 *Elements common to inspection of places of production, area wide surveillance, inspection of consignments and lot identification*. *Bulletin OEPP/EPPO Bulletin* 39, 260–262.
- EPPO (2002) EPPO Standard PM 5/2 (2) *Pest risk analysis on detection of a pest in an imported consignment*. *EPPO Bulletin* 32, 235–239.
- Ferro DN (1976) *New Zealand insect pests*. Lincoln University College of Agriculture, New Zealand.
- IPPC (2017) IPPC Standards ISPM no. 20: *Guidelines for a phytosanitary import regulatory system*, 2004. IPPC Secretariat, Rome (IT).
- IPPC (2016) ISPM no. 5 *Glossary of Phytosanitary Terms*. IPPC Secretariat, Rome (IT).
- IPPC (2005a) IPPC Standard ISPM no. 23: *Guidelines for inspection*, 2005. IPPC Secretariat, Rome (IT).
- IPPC (2005b) IPPC Standard ISPM no. 31 *Methodologies for sampling of consignments*, 2005. IPPC Secretariat, Rome (IT).
- ISO 24333 (2009) Cereals and cereal products- sampling. Available at: <https://www.iso.org/standard/42165.html#:~:text=ISO%2024333%3A2009%20specifies%20requirements,of%20their%20quality%20and%20condition>.
- ISTA (2021) *International Rules for Seed Testing 2021, Chapter 2: Sampling*. ISTA, Zurich (CH).
- Mathur SB & Cunfer BM (1993) *Seed-Borne Diseases and Seed Health Testing of Wheat*. pp. 31–43. Danish Government Institute of Seed Pathology for Developing Countries, Copenhagen (DK).
- McGee DC (1994) Seed assays for Stewarts wilt and other seed-borne diseases of corn. In: *Proc. Annu. Corn Sorghum Res. Conf.* 48, pp. 161–168. American Seed Trade Association, Washington (US).
- Murray GM & Wright DG (2007) *National Contingency Plan for Tilletia controversa, the Cause of Dwarf Bunt of Wheat*. Part I: Pest Risk Assessment'. Plant Health Australia, Canberra, ACT, Australia. vi +16 pp.

APPENDIX 1 - SYMPTOMS, SAMPLING AND IDENTIFICATION OF PESTS OF CEREALS PRESENT ON THE EPPO A1 AND A2 LISTS OF PESTS OR OTHERWISE LISTED PESTS

For each of the pests mentioned below basic information on host range, biology, detection and identification can be found in Quarantine Pests for Europe, 2nd edition (EPPO/CABI, 1997), as well as in EPPO Datasheets and EPPO Diagnostic Standards. Illustrations are available on the EPPO website (<http://www.eppo.int>). When an EPPO Diagnostic Standard exists, it is mentioned in the text. The fact that there is no EPPO Diagnostic Standard does not mean that there is no diagnostic method available in the scientific literature.

(A) Insects

(1) *Listronotus bonariensis* (EPPO A1 List)

Can be present on seed of wheat, triticale, rye, rice, barley, oat, millets and maize.

Symptom description

Listronotus bonariensis is a polyphagous pest of pasture grasses and cereals native to South America. The major symptoms of adults on host plants are rectangular holes near the leaf tips, which look like windows. In addition, adults produce fibrous frass deposits on leaves. Larvae feed in the lower parts of stems and can cause yellowing of young leaves. More details of symptoms on host plants are described in the relevant EPPO Datasheet. In seed consignments it is mainly the adult stage which is found.

Description of adults

Colour variable from light grey-brown to dark brown or black, about 3 mm long and 1.5 mm wide. They are compact, hard bodied and have a pronounced snout and characteristic white pale stripes. The body is covered with numerous hairs and white wax-like scales which tend to hold dust, producing a dirty grey appearance (Ferro, 1976).

Sampling and identification

Sampling should be performed as described above. Of the sample selected for inspection, small quantities of about 50–100 g of the seeds should be spread on a white tray. Any adult beetles found should then be examined using a hand lens or dissecting microscope. If no final diagnosis can be made at the point of inspection a subsample of the suspicious adult insects should be sent to a laboratory for confirmation.

(B) Fungi

(1) *Tilletia indica* (EPPO A1 List)

Can be present on wheat, rye and triticale.

Symptom description

Seeds are usually only partially colonized, showing various degrees of infection. Point infections are most common, but infection may also spread down the axial groove and, in severe cases, the whole grain may appear bunted (a picture of grain infected with *T. indica* (Karnal bunt) can be viewed in EPPO Standard PM 7/29(3) *Tilletia indica*). The bunted grains are therefore the main criteria for visual identification of *T. indica* in seed or grain consignments. They are an indication, but not sufficient for identification, of *T. indica*. A distinct identification can only be made for *T. indica* based on the morphology of the characteristic teliospores (see EPPO Standard PM 7/29(3) *Tilletia indica*).

Sampling and identification

Seed and grain lots should be sampled as described above. The resulting composite sample should be reduced to a working sample of about 1 kg for inspection and laboratory testing. On seeds with severe *T. indica* infestation, teliospores may be seen on the surface with the naked eye and/or low-power microscopy (910–970 magnification) (Mathur & Cunfer, 1993). In any case the working sample should be sent for laboratory testing according to EPPO Standard PM 7/29(3) *Tilletia indica* (EPPO, 2018).

(2) *Tilletia controversa*

Can be present on wheat and triticale.

Symptom description

Tilletia controversa creates bunt balls on the florets that look superficially like grey-brown or black seeds. The fragile cover (pericarps) of the bunt balls remains intact initially (covered smut) but is easily broken at harvest. At high levels of infection, the harvested seed or grain appears grey and mixed with black bunt balls (Murray & Wright, 2007).

Teliospores are yellow-brown to red-brown (mature spores are mostly much darker), globose or subglobose, mostly 19–24 µm (17–32 µm) in diameter. Mature spores are typically surrounded by a hyaline gelatinous sheath 1.5–5.5 µm thick. In median view, the exospore is reticulate, with relatively large, regular, polygonal areolae, 1.5–3 µm high and 3.5 µm in diameter (CABI, 2014a). As the spore morphology of *T. controversa* is very similar to that of *Tilletia caries*, the identification of spores by morphology is almost impossible.

Sampling and identification

Seed and grain lots should be sampled as described above. The resulting composite sample should be reduced to a 1 kg working sample for inspection and laboratory testing. Severe infestations can be seen with the naked eye. The identification of the *Tilletia* spp. can only be done by laboratory testing. According to the Diagnostic Protocol of the Australian National Contingency Plan for *Tilletia*

controversa (Murray & Wright, 2007), the identification of *T. controversa* is only possible by detailed morphological studies combined with multigene sequencing of the *efl*, *actin* and *rpb2* genes.

(3) *Cochliobolus carbonum*

Can be present on maize.

Symptom description

No characteristic symptoms are visible on seeds. The only indication is that the seeds become mouldy.

Sampling and identification

Seed and grain lots should be sampled as described above. Visual identification is not an accurate method for the identification of *C. carbonum*, therefore the sample should be sent directly for laboratory testing. The most common used method for diagnostics is the Cf2.1 ISU Freezing Blotter Method (McGee, 1994).

(4) *Stenocarpella macrospora* and *Stenocarpella maydis* (EPPO A2 List)

Can be present on maize.

Symptom description

Seeds infested with the two *Stenocarpella* species show discoloration and are shrivelled, mouldy and may be rotten when heavily infected. These symptoms are not diagnostic as many fungal infestations show similar symptoms. Seeds may be infested asymptotically. Visual identification is not an appropriate method for the identification of *S. macrospora* or *S. maydis*, therefore the samples should be sent directly for laboratory testing.

Sampling and identification

Information on diagnostics is provided in the EPPO Datasheet on *S. macrospora* and *S. maydis*.

(C) Bacteria

(1) *Xanthomonas translucens* pv. *translucens* (EPPO A2 List)

Can be present on wheat, triticale, rye and barley.

Symptom description

Symptoms on grain and seeds are not easy to detect. Kernels may become shrunken at their base and can show a purple-black discoloration of the surface. Symptoms may be misidentified as physiological disorders which can produce similar symptoms.

Sampling and identification

Seed and grain lots should be sampled as described above. Visual identification is not an appropriate method for the identification of *X. translucens* pv. *translucens*, therefore the sample should be sent directly for laboratory testing. Information on diagnostics is provided in

the Datasheet on *X. translucens* pv. *translucens* (EPPO/CABI, 1997).

(2) *Xanthomonas oryzae* pv. *oryzae* and *Xanthomonas oryzae* pv. *oryzicola* (EPPO A1 List)

Can be present on rice.

Symptom description

The two *X. oryzae* pathovars do not produce any specific symptoms on seeds.

Sampling and identification

Seed and grain lots should be sampled as described above. Visual identification is not an appropriate method as no characteristic symptoms of the two *X. oryzae* pathovars are visible on seeds, therefore samples should be sent directly for laboratory testing. Details on the identification of the two *X. oryzae* pathovars can be found in the EPPO Diagnostic Protocol PM 7/80 *Xanthomonas oryzae* (EPPO, 2007).

(3) *Pantoea stewartii* subsp. *stewartii* (EPPO A2 List)

Can be present on maize.

Symptom description

No characteristic symptoms are visible on seeds. Harvested seeds which are severely infected are deformed, shrunken and discoloured (CABI, 2014c).

Sampling and identification

Seed and grain lots should be sampled as described above. Visual identification is not an accurate method as no characteristic symptoms are visible on seeds, therefore samples should be sent directly for laboratory testing. Details on the identification of *P. stewartii* can be found in the EPPO Diagnostic Protocol PM 7/60(2) *Pantoea stewartii* subsp. *stewartii* (EPPO, 2016).

(D) Nematodes

(1) *Aphelenchoides besseyi* (EPPO A2 List)

Can be present on rice.

Symptom description

On rice seed *A. besseyi* can cause a reduction in seed size and sometimes visual discolouration, lesions or empty seeds (CABI, 2014b). However, there are frequently no symptoms of rice seed infestation to be seen.

Sampling and identification

Seed and grain lots should be sampled as described above. Isolation and identification of *A. besseyi* can be done from seeds, chaff or hulls of the sample taken for laboratory testing. Details on identification of *A. besseyi* can be found in the ISTA rules annexed to chapter 7, *Seed Health Testing Methods: 7-025: Detection of A. besseyi on Oryza sativa* and in the EPPO Diagnostic protocol PM 7/39 *Aphelenchoides besseyi* (EPPO, 2017).

(E) Viruses**(1) Barley stripe mosaic virus**

Can be present on barley.

Symptom description

No characteristic symptoms are visible on seeds, although seeds are small and shrivelled.

Sampling and identification

Seed and grain lots should be sampled as described above. Visual identification is not an appropriate method as no characteristic symptoms are visible on seeds, therefore samples should be sent directly for laboratory testing. Details on the identification of *Barley stripe mosaic virus* can be found in EPPO Standard PM 3/34 Barley stripe mosaic hordeivirus (EPPO, 1991).

APPENDIX 2 - SHORT PROCEDURE FOR INSPECTION OF CONSIGNMENTS OF SEED AND GRAIN OF CEREALS TO BE USED BY INSPECTORS

This short procedure includes the main elements for practical work of an inspector when carrying out phytosanitary inspections of consignments of seed and grain of cereals at the point of entry. It is considered that documentary checks and identity checks should already have been carried out. A general outline of the inspection procedure is included in Figure 3.

1. Lots to be inspected should be identified, preferably based on variety, origin or category. For large

consignments in bulk, the lots should be defined as the smallest quantity which can be handled separately. In practice, this means a container or compartment of a specific cultivar represents a lot.

2. To get a representative sample of the lot, the sampling intensity (amount of primary samples) should be as indicated in Tables 1, 2 or 3 of the present Standard. If samples for quality checks are taken from cargo agents respecting similar rules, the phytosanitary inspection can be performed on a subsample from the composite sample taken for the quality checks.
3. An appropriate quantity of seeds or grain (working sample) from the representative composite sample should be selected for inspection, taking into account statistical requirements (see Table 1 or Table 2 of ISPM no. 31 *Methodologies for sampling of consignments*) and minimum amounts needed for laboratory testing. Table 4 of the present Standard shows simplified seed and grain quantities for each cereal in order that the requirements are met.

Appropriate equipment for carrying out the inspections and for sampling should be available at the point of inspection.

Inspection

Inspection should be performed on a working sample from the composite sample using the minimum weights indicated in Table 2. The visual examination of seed and grain should be performed on the following small grain cereals for the relevant harmful organisms:

Commodity	Pest	Visual symptom
Wheat/triticale	<i>Tilletia indica</i>	Bunted kernels
	<i>Tilletia controversa</i>	Bunt balls
	<i>Listronotus bonariensis</i>	Adult beetles
	<i>Xanthomonas translucens</i> pv. <i>translucens</i>	No characteristic symptoms
Rye	<i>Listronotus bonariensis</i>	Adult beetles
	<i>Xanthomonas translucens</i> pv. <i>translucens</i>	No characteristic symptoms
	<i>Tilletia indica</i>	Bunted kernels
	<i>Tilletia controversa</i>	Bunt balls
Rice	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	No characteristic symptoms
	<i>Xanthomonas oryzae</i> pv. <i>oryzicola</i>	No characteristic symptoms
	<i>Aphelenchoides besseyi</i>	Visual discoloration, lesions or empty seeds, but frequently no symptoms are observed
	<i>Listronotus bonariensis</i>	Adult beetles
Barley	<i>Listronotus bonariensis</i>	Adult beetles
	<i>Xanthomonas translucens</i> pv. <i>translucens</i>	No characteristic symptoms
	<i>Barley stripe mosaic virus</i>	No characteristic symptoms
Oat	<i>Listronotus bonariensis</i>	Adult beetles
Maize	<i>Listronotus bonariensis</i>	Adult beetles
	<i>Pantoea stewartii</i>	No characteristic symptoms
	<i>Cochliobolus carbonum</i>	No characteristic symptoms
	<i>Stenocarpella macrospora</i>	No characteristic symptoms
	<i>Stenocarpella maydis</i>	No characteristic symptoms
Millets	<i>Listronotus bonariensis</i>	Adult beetles

Sampling for laboratory testing

With the exception of *L. bonariensis*, laboratory analysis is always needed to determine if seeds from the above-mentioned cereals are free from the relevant harmful organisms. They can be infested without characteristic symptoms or with no visual symptoms. This is different for *L. bonariensis*, as the visual symptoms are obvious and a sample for laboratory testing should only be sent for analysis if adults are found during inspection.

The size of the working sample sent to the laboratory should be as indicated in Table 4. If only one working sample can be selected from the composite sample, the working sample has to be used for inspection and for laboratory testing. For laboratory testing the working sample should be packed tightly into a muslin sample bag and sealed to reduce movement of the seed within the bag. Each muslin bag should then be packaged in a separate polypropylene bag.

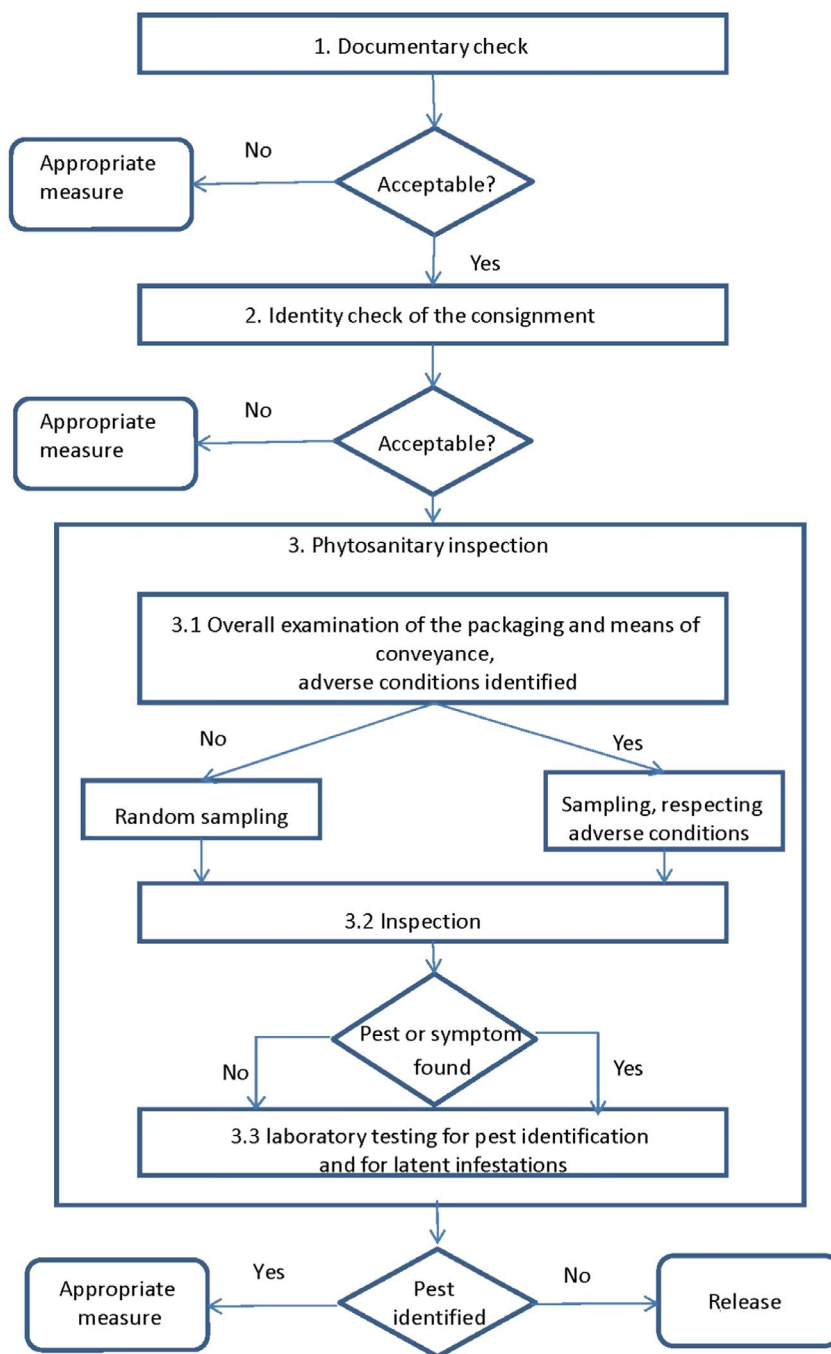


FIGURE 3 General outline of the inspection procedure [Colour figure can be viewed at wileyonlinelibrary.com]