DOI: 10.1111/epp.12773

EPPO STANDARD - PHYTOSANITARY PROCEDURES

PM 3/80 (2) Consignment inspection of seed of *Solanum lycopersicum* and its hybrids

Specific scope: This Standard describes the procedure by which consignments of tomato seed (*Solanum lycopersicum* and its hybrids) should be subjected to phytosanitary import inspection including sampling and identification.

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

Authors and contributors are given in the Acknowledgements section.

1 | INTRODUCTION

Tomato seed is an important pathway for the introduction of pests to a new area. Moreover, there is worldwide commerce in this seed. Consignments of tomato seed may carry pests included in the EPPO A1 or A2 Lists of pests recommended for regulation as quarantine pests or otherwise regulated by EPPO countries.

At import, consignment freedom from pests, in particular those recommended for regulation as quarantine pests and regulated pests, is usually verified by laboratory testing before release of the consignment. Similar procedures may be applied in the exporting country before forwarding the consignment if the importing country requires consignment freedom of specific pests or as a verification of the efficacy of other phytosanitary measures (e.g. treatment). Place of production inspection of the mother plants is usually required for seed.

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 *Elements common to inspection of places of production, area-wide surveillance, inspection of consignments and lot identification* (EPPO, 2009).

The procedures described in this Standard are mostly 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. The general elements of this inspection procedure apply to inspection in both the exporting and the importing country.

For pests recommended for regulation as quarantine pests and regulated pests, it is important to maximize the chance of detection by targeting the consignments most likely to carry the pests (e.g. the most susceptible varieties, place of origin of the seeds, instances of non-compliance of consignments of certain origins or from certain producers).

Inspection should 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 *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.

In the exporting country, inspections and/or sampling for testing should preferably be done at the premises of the producer or exporter at a stage where the whole consignment is still accessible, i.e. before packaging or loading. Field inspection and sampling in the field should be done at the most appropriate time according to EPPO (2009).

Phytosanitary inspection of consignments of tomato seed in the importing country may be carried out at the point of entry or at the point of destination, depending on the possibility for carrying out an efficient inspection and provided that the seed remains under official control.

When a lot has been selected for inspection it should be kept in mind that because visual examination of tomato seeds is usually not appropriate for detecting seed-borne pests, the collecting of samples for laboratory testing should be included in import inspection procedures.

Laboratory testing of consignments is in most cases based on sampling because the consignments are too big for testing in full and many laboratory tests are destructive. Only very small consignments may be tested in full if non-destructive tests are available. The sampling

procedures and intensity laid down in the International Seed Testing Association (ISTA) rules for making a representative sample for quality aspects can be used to obtain a sample for regulated pests (ISTA, 2021). The size of the sample tested (working sample) in the laboratory will depend on the target pest and the test used.

Following sampling, the imported consignment should remain in detention (under official control and not be given an entry permit) until the laboratory test has confirmed absence of the relevant pests from the submitted sample.

3 | COMMODITIES CONCERNED

Seeds of *Solanum lycopersicum* are traded in very different sized lots, ranging from a few grams to tonnes of seed.

Seeds are transported in packages, boxes or containers or more seldom in bulk. Many seed lots have been treated with pesticides or may be pelleted.

Seeds of *S. lycopersicum* may be infested or contaminated by different pests described in this Standard. The origin of the seed should be considered when assessing the pest risk. Only very few pests are seed transmitted; treatments such as acid extraction are commonly practised to clean the seed and will kill most surface-contaminating pests.

4 | PESTS OF SEED OF *S. LYCOPERSICUM*

This Standard mainly relates to the EPPO A2 List pests recommended for regulation and recognized to be of primary importance as seed-transmitted pests of tomato. It also covers those pests listed by some EPPO member countries but not included in the EPPO lists.

The phytosanitary procedures described in the Standard are aimed at preventing the introduction and spread of these pests in the EPPO region via imported consignments of tomato seed. They could also be used to detect other non-regulated pests, exotic pests of economic relevance for tomato and contamination, for example by soil.

For species registered on the A1 or A2 Lists, a datasheet or diagnostic protocol is available (see the EPPO website http://www.eppo.int/ or the EPPO global database https://gd.eppo.int/; EPPO, 2004, 2013a, 2013b, 2016, 2021). The relevant scientific literature should be consulted for additional up-to-date information.

The EPPO A1 and A2 Lists of pests recommended for regulation as well as the regulations of EPPO member countries are subject to additions and deletions. The present list will therefore need to be revised whenever relevant new pests are listed (Table 1).

Other pospiviroids of relevance for tomato include *Tomato chlorotic dwarf viroid*, *Tomato planta macho viroid* and *Columnea latent viroid*. For an indication of the status of these pests consult the EPPO Global Database (EPPO, 2021).

5 | LOT IDENTIFICATION

General background information on lot identification is given in EPPO (2009).

For tomato seed, lot identification should be made on the basis of the following specifications:

- Country of origin.
- Place of production: marks on boxes or bags may give a unique indication referring to the place of production (labels on packaging or boxes may indicate producer numbers, packing station number).
- Varieties (several varieties of the same commodity may be present in the same consignment but only one variety per lot). Varieties included may not be mentioned on the Phytosanitary Certificate but are usually included in the invoice or indicated on the boxes. Varieties may show different susceptibility to pests and it is important to target inspection on the most susceptible varieties.
- Date of harvest, if available.

TABLE 1 Seed-transmitted pests of tomato

EPPO A2 pests	Other pests regulated by specific EPPO member countries
Bacteria (including phytoplasmas)	
Clavibacter michiganensis subsp. *michiganensis*	
Xanthomonas euvesicatoria pv. *euvesicatoria*	
Xanthomonas hortorum pv. *gardneri*	
Xanthomonas euvesicatoria pv. *perforans*	
Xanthomonas vesicatoria	
Ralstonia pseudosolanacearum	
Ralstonia solanacearum	
Viruses and viroids	**Viruses and viroids**
Chrysanthemum stunt viroid	*Tomato apical stunt viroid* (TASVd) [Jordan ('A1 List'), Tunisia ('quarantine pest')]
Potato spindle tuber viroid	
Tomato ringspot virus	*Tomato black ring virus* (*Nepovirus*) [Norway ('quarantine pest'), Turkey ('A2 List'), Israel ('quarantine pest') EU (RNQP)]
Pepino mosaic virus	
Tomato brown rugose fruit virus	

6 | INSPECTION AND SAMPLING FOR LABORATORY TESTING

This section contains guidance on visual checks of consignments of tomato seed and on sampling for laboratory testing. Visual checks 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, 2004) and ISPM no. 23 *Guidelines for inspection* (IPPC, 2005).

6.1 | Hygiene measures

In order not to spread and increase infections, adequate precautions should be taken during inspections and sampling, such as protective clothes (coat, overshoes, gloves, etc.). Gloves should be disposable and changed between sampling of separate lots. Equipment and tools used during sampling and inspection should be cleaned and disinfected between sampling of separate lots. EPPO (2020) provides detailed guidance on hygiene measures for *Tomato brown rugose fruit virus*, which may also be suitable for other viruses.

6.2 | Inspection

Phytosanitary inspections should start with an overall examination of the consignment, container, packaging and means of conveyance to obtain indications of adverse conditions during transport (e.g. temperature, moisture content), to check the physical condition of the tomato seed, to look for live or dead insects or contamination (e.g. with soil) and for planning seed sampling.

Visual examination of imported consignments of tomato seed alone is not considered to be sufficient to prove the absence of pests as this will only reveal visually detectable pests such as insects. Therefore, no additional details on sampling for inspection are given in this Standard.

6.3 | Sampling for laboratory testing (general aspects)

As seed infestations generally do not produce visible symptoms on tomato seed, representative samples from consignments should be taken to the laboratory for detection of infestation and for identification. Lots for testing should be selected on a risk basis based on their origin, size, records of previous test results, varietal susceptibility and recent interception records.

A consignment may consist of one or more lots, and sampling may be carried out at consignment or lot level. As the result of the testing will have regulatory impact on all the lots covered by the submitted sample, the inspector should consider how many lots should be covered by the sample. Sampling plans should be formulated to determine the frequency of submission of samples for laboratory testing [ISPM no. 31 *Methodologies for sampling of consignments* (IPPC, 2008)].

The procedures of taking representative samples from the consignment and handling the submitted sample in the laboratory both contribute to the validity of the test result. ISPM no. 31 (IPPC, 2008) provides general information on procedures for the sampling of lots. Specific methodologies for representative sampling of consignments consisting of seed as well as specific equipment for seed sampling are provided in the ISTA rules (see Figure 1).

6.4 | Sampling in the consignment

The number of primary samples drawn in the consignment will vary according to the size and composition of the consignment. The size of the submitted sample and the number of seeds in a working sample for laboratory testing are independent of the size of the consignment when representative sampling in the consignment is carried out according to the ISTA rules. The number of seeds that need to be tested will depend on the target pest(s) and the test(s). The sample may not be tested in one go but the working sample may be subdivided into (much) smaller groups which will then be tested individually. The number of subgroups that any working sample may be subdivided into depends on the practical work input needed for the chosen method and the sensitivity of the method.

Representative sampling in the consignment should be done in such a way as to provide a high probability for any target organism present in the consignment to become included in the composite sample in the same proportion as in the consignment. Targeted sampling may not result in a composite sample having the same pest proportion as in the consignment, but in a more concentrated level of pest in the composite sample. A proper sampling technique in the consignment is essential considering that the individual seed lots may not be homogeneous and distribution of infected seeds in the seed lot may be clustered or aggregated [ISPM 31, 5.2 (IPPC, 2008)]. The higher the sampling intensity the greater the chance of including the target organism(s) in the composite sample if it is present in the consignment.

The practical sampling procedure for creation of a representative submitted sample is described in the ISTA rules. The procedures involve drawing of the primary samples, a composite sample and finally a submitted sample for the laboratory, as outlined in Figure 1. The

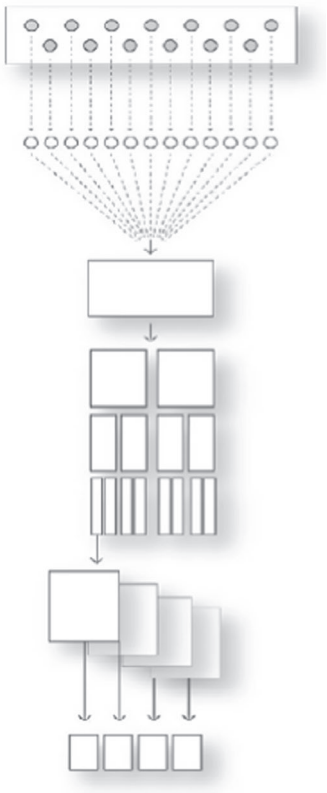

FIGURE 1 Schematic overview of sampling for laboratory testing based on ISTA rules

number of primary samples taken will be determined by the size of the seed lot, and samples are taken using seed lot sampling techniques as described in the ISTA rules. The ISTA rules should be consulted for guidance on the techniques relevant to the consignment in question. The resulting composite seed sample should be thoroughly mixed and reduced in size in the way described in the ISTA rules to ensure that the submitted sample remains representative of the seed lot.

6.5 | Sample submitted for laboratory analysis

The sample submitted to the laboratory should be of sufficient size for testing for all target pests. Only if the samples are considered representative of the seed lot may the laboratory result apply to the lot and not only to the samples. It is possible to submit a single sample to the laboratory because it is then possible to make the submitted sample homogeneous and divide it into representative subsamples and working samples using the techniques described in the ISTA rules.

6.6 | Statistical considerations

Statistical considerations on the number of seeds to be tested and minimum infestation levels to be detected focus on the submitted sample and are not related to the lot. Only if the working sample is considered to be a representative sample of the seed lot may the laboratory result apply to the lot and not only to the submitted sample or working sample. The number of seeds which may be tested together depends on the methodology available. Some tests may allow testing of 1 kg of seed all together (plating for bacteria after soaking the seed), while other tests may allow testing of a few seeds together (polymerase chain reaction, enzyme-linked immunosorbent assay, plating and microscopy of seeds). In the latter case, large amounts of seed may be tested by repeating the process several times.

If 2972 seeds from a correctly mixed representative sample are tested, the test result will provide a 95% confidence of detecting a target pest which is present on 0.1% of the seeds. The 1000 seed weight for the lot should be determined. As an indication, approximately 9 g of tomato seed will generally contain at least 3000 seeds.

If 597 seeds are tested, the test result will provide a 95% confidence of detecting a target pest which is present on 0.5% of the seeds.

Some working samples can be tested for more than one pest if the same test is used for the different pests (e.g. pospiviroids and *Pepino mosaic virus* can be tested on the same working sample), whereas some other tests require separate working samples for each species.

Appendix 1 provides information on the minimum size of the laboratory working sample when 95% or 99% confidence levels are required.

ACKNOWLEDGMENTS

This Standard was first drafted by Ms L. Stenstrup, from Landbrugsstyrelsen (DK), and later updated in 2021. It was reviewed by the Panel on Phytosanitary Inspections.

REFERENCES

EPPO (2002) EPPO Standards PM 5/2 (2) Pest risk analysis on detection of a pest in an imported consignment. *Bulletin OEPP/EPPO Bulletin* 32, 231–233.

EPPO (2004) EPPO Standards PM 7/33 (1) *Potato spindle tuber pospiviroid*. *Bulletin OEPP/EPPO Bulletin* 34, 257–269.

EPPO (2009) EPPO Standards PM 3/72 (2) 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 (2013a) EPPO Standards PM 7/110 (1) *Xanthomonas* spp. (*Xanthomonas euvesicatoria, Xanthomonas gardneri, Xanthomonas perforans, Xanthomonas vesicatoria*) causing bacterial spot of tomato and sweet pepper. *Bulletin OEPP/EPPO Bulletin* 43, 7–20.

EPPO (2013b) EPPO Standards PM 7/113 (1) *Pepino mosaic virus*. *Bulletin OEPP/EPPO Bulletin* 43, 94–104.

EPPO (2016) EPPO Standards PM 7/42 (3) *Clavibacter michiganensis* subsp. *michiganensis*. *Bulletin OEPP/EPPO Bulletin* 46, 202–225.

EPPO (2020) *Pest risk analysis for tomato brown rugose fruit virus*. EPPO, Paris. Available at https://gd.eppo.int/TOBRFV/documents

EPPO (2021) EPPO Global Database on quarantine pests (available online). https://gd.eppo.int/

IPPC (2004) *Guidelines for a phytosanitary import regulatory system*. ISPM no. 20. IPPC, FAO, 1–20. https://www.ippc.int/static/media/files/publications/en/2014/05/07/ispm_20_2004_en_2013-05-07.pdf [accessed 15 August 2015].

IPPC (2005) *Guidelines for inspection*. ISPM no. 23, IPPC Secretariat, FAO, 1–9. https://www.ippc.int/static/media/files/publications/en/2013/10/09/ispm_23_2005_en_2013-08-26.pdf [accessed on 15 August 2015].

IPPC (2008) *Methodologies for sampling of consignments*. ISPM no. 31, IPPC Secretariat, FAO, 1–19. https://www.ippc.int/static/media/files/publications/en/1323947615_ISPM_31_2008_En_2011-11-29_Refor.pdf [accessed on 15 August 2015].

IPPC (2016) ISPM no. 5 *Glossary of Phytosanitary Terms*. IPPC Secretariat, Rome (IT).

ISTA (2021) International Rules for Seed Testing, Vol. 2021, Chapter 2, i-2-44 (52) https://doi.org/10.15258/istarules.2021.02 [accessed on 12 May 2021].

APPENDIX 1 – MINIMUM SIZE OF THE LABORATORY WORKING SAMPLE NEEDED TO ACHIEVE 95% AND 99% CONFIDENCE LEVELS

Column 1 of Table A1 lists a range of maximum infestation levels which a NPPO may decide to accept in a consignment of tomato seed. The respective minimum size of the laboratory working sample is shown in column 2 if the *95% confidence level* is selected.

The number of seeds that need to be tested (column 2) may not be tested in one go, but the working sample may be subdivided into (much) smaller groups which will then be tested individually. The number of subgroups of any working sample may be subdivided, depending on the practical work input needed for the chosen method and the sensitivity of the method.

Column 1 of Table A2 lists a range of maximum infestation levels which a NPPO may decide to accept in a consignment of tomato seed. The respective minimum size of the laboratory working sample is shown in column 2 if the *99% confidence level* is selected.

The number of seeds that need to be tested (column 2) may not be tested in one go, but the working sample may be subdivided into (much) smaller groups which will then be tested individually. The number of subgroups of any working sample may be subdivided, depending on the practical work input needed for the chosen method and the sensitivity of the method.

TABLE A1 Minimum size of the laboratory working sample if a 95% confidence level is selected

Maximum infestation level the NPPO decides to accept in consignments of tomato seed (%)	Calculated minimum working sample size (number of seeds) to be tested in the laboratory	Approximate weight of working sample (approx. 350 tomato seeds/g) (g)
0.001	299 572	856
0.01	29 956	86
0.02	14 977	43
0.04	7488	21
0.1	2972	8.5
0.5	597	1.7
1.0	298	0.9

TABLE A2 Minimum size of the laboratory working sample if a 99% confidence level is selected

Maximum infestation level the NPPO decides to accept in consignments of tomato seed (%)	Calculated minimum working sample size (number of seeds) to be tested in the laboratory	Approximate weight of working sample (approx. 350 tomato seeds/g) (g)
0.001	460 515	1316
0.01	46 049	132
0.02	23 024	66
0.04	11 511	33
0.1	4603	13.2
0.5	919	2.6
1.0	458	1.3

APPENDIX 2 – SHORT PROCEDURE FOR INSPECTORS

General

The visual examination should begin with an overall examination of the consignment. Visual examination of the container, packaging and means of conveyance can provide indications of adverse conditions during transport (e.g. signs of damp or wetness) which may affect the physical condition of the seed.

Hygiene measures

In order not to spread and increase infections, adequate precautions should be taken during inspections and sampling, such as protective clothes [coat, overshoes, gloves (gloves should be disposable and changed between sampling of separate lots, etc.]. Equipment and tools used during sampling and inspection should be cleaned and disinfected between sampling of separate lots.

Inspection

Visual examination of imported consignments of tomato seed alone is not considered to be sufficient to prove the absence of pests, as this will only reveal visually detectable pests such as insects.

Sampling for laboratory testing (general aspects)

As seed infestations generally do not produce visible symptoms on tomato seed, representative samples from consignments should be taken to the laboratory for detection of infections and for identification. Lots for testing should be selected on a risk basis based on their origin, size, records of previous test results, varietal susceptibility and recent interception records.

A consignment may consist of one or more lots, and sampling may be carried out at consignment or lot level. As the result of the testing will have regulatory impact on all the lots covered by the submitted sample, the inspector should consider how many lots should be covered by the sample. Sampling plans should be formulated to determine the frequency of submission of samples for laboratory testing (ISPM no. 31 *Methodologies for sampling of consignments*).

The procedures for taking representative samples from the consignment and handling the submitted sample in the laboratory both contribute to the validity of the test result. ISPM no. 31 provides general information on procedures for the sampling of lots. Specific methodologies for representative sampling of consignments consisting of seed as well as specific equipment for seed sampling are provided in the ISTA rules (see Figure 1 in this Standard).

Sampling in the consignment

The number of primary samples taken from the consignment will vary according to the size and composition of the consignment. The size of the submitted sample and the number of seeds in a working sample for laboratory testing are independent of the size of the consignment when representative sampling in the consignment is carried out according to the ISTA rules. The number of seeds that need to be tested will depend on the target pest(s) and the test(s). The sample may not be tested in one go but the working sample may be subdivided into (much) smaller subsamples which will then be tested individually. The number of subgroups that any working sample may be subdivided into depends on the practical work input needed for the chosen test and the sensitivity of the test.

Representative sampling in the consignment should be done in such a way as to provide a high probability for any target organism present in the consignment to become included in the composite sample in the same proportion as in the consignment. Targeted sampling may not result in a composite sample having the same pest proportion as in the consignment, but in a more concentrated level of pest in the composite sample. A proper sampling technique in the consignment is essential considering that the individual seed lots may not be homogeneous and distribution of infected seeds in the seed lot may be clustered or aggregated (ISPM no. 31, 5.2). The higher the sampling intensity, the greater the chance of including the target organism(s) in the composite sample if it is present in the consignment.

The practical sampling procedure for creation of a representative submitted sample is described in the ISTA rules. The procedures involve drawing of the primary

samples, a composite sample and finally a submitted sample for the laboratory, as outlined in Figure 1. The number of primary samples taken will be determined by the size of the seed lot, and samples are taken using seed lot sampling techniques as described in the ISTA rules. The ISTA rules should be consulted for guidance on the techniques relevant to the consignment in question. The resulting composite seed sample should be thoroughly mixed and reduced in size in the way described in the ISTA rules to ensure that the submitted sample remains representative of the seed lot.

Appendix 1 provides details on the minimum size of the laboratory working sample needed to achieve 95% and 99% confidence levels.