International Rules for Seed Testing 2021

Validated Seed Health Testing Methods

7-003: Detection of *Botrytis cinerea* in *Helianthus annuus* (sunflower) seed

Including changes and editorial corrections adopted at the online Ordinary General Meeting 2020

Effective from 1 January 2021

Validation reports

See References. Copies are available by e-mail from the ISTA Secretariat at ista.office@ista.ch.

Please send comments, suggestions or reports of problems relating to this method to the ISTA Seed Health Committee, c/o ISTA Secretariat.

Disclaimer

Whilst ISTA has taken care to ensure the accuracy of the methods and information described in this method description, ISTA shall not be liable for any loss or damage, etc. resulting from the use of this method.

Safety precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during weighing out of ingredients. It is assumed that persons carrying out this test are in a laboratory suitable for carrying out microbiological procedures and familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic techniques. Dispose of all waste materials in an appropriate way (e.g. autoclaving, disinfection) and in accordance with local health, environmental and safety regulations.

Note on the use of the translations

The electronic version of the International Rules for Seed Testing includes the English, French, German and Spanish versions. If there are any questions on interpretation of the ISTA Rules, the English version is the definitive version.

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7-003: Detection of *Botrytis cinerea* in *Helianthus annuus* (sunflower) seed

Host: *Helianthus annuus* L.
Pathogen(s): *Botrytis cinerea* Pers. ex Pers. (Perfect state *Botryotinia fuckeliana* (de Bary) Whetzel, syn. *Sclerotinia fuckeliana* (de Bary) Fuckel.)

Prepared by: ISTA Seed Health Committee, Method Validation Sub-committee

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Revision history

Version 1.0, 2001-02-26
Revised 2001-02-26 J. Sheppard, V. Cockerell
Reprinted 2003
Version 1.1, 2008-01-01: Treated seed revised; Reporting results revised
Version 2.0, 2010-11-01: Modification of method
Version 2.1, 2014-01-01: Addition of positive control
Version 2.2, 2017-01-01: Reporting results revised
Version 2.3, 2021-01-01: Sample preparation changed to Sample size and paragraph revised

Background

This method was originally published in the *ISTA Handbook of Seed Health Testing* in 1981 as Working Sheet No. 44 prepared by C. Anselme & R. Champion, La Minière, France. The method was incorporated into the newly revised *Annexe to Chapter 7* in 2002 from the 1999 edition of the ISTA Rules. The method was reviewed by the ISTA-Seed Health Committee in 2006 (Cockerell & Koenraadt, 2007) with the recommendation to accept for a further five years.

An ISTA Proficiency test for Method 7-003 highlighted problems with both over- and underestimation of *Botrytis cinerea* by laboratories. Confusion with saprophytes may have caused overestimation by some laboratories, while differences in the criteria as to when a seed is infected (presence of one conidiophore versus soft rot on roots) led to underestimation of *B. cinerea* by some laboratories. The ISTA SHC agreed that an experiment be carried out to establish whether the use of a malt solution exacerbates the proliferation of saprophytes, leading to incorrect assessments by laboratories. The results showed when malt solutions of 1 % and 3 % were used, *B. cinerea* levels were significantly higher than the true value after 9 days' incubation, and also after 7 days with 3 % malt. The malt solution was also shown to increase the saprophyte count compared to no malt.

New morphological criteria was described for the determination of infected seed during the SHC Workshop in South Africa, 2008, and finally agreed at the SHC workshop in SNES, France, 2–5 March 2010.

As a result of this work the following changes have been made:
- removal of malt solution;
- blotters now soaked with distilled/deionised water;
- incubation reduced to 7 days, with examinations made at 5 and 7 days.

Treated seed

This method has not been validated for the determination of *Botrytis cinerea* on treated seed. Seed treatments may affect the performance of the method. (Definition of treatment: any process, physical, biological or chemical, to which a seed lot is subjected, including seed coatings. See 7.2.3.)

Sample size

The sample (total number of seeds tested) size to be tested depends on the desired tolerance standard (maximum acceptable percentage of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). The minimum sample size should be 400 seeds.

Materials

Reference material: reference cultures or other appropriate material
Media: blotters (filter paper), e.g. Whatman No. 1 or equivalent
Petri dishes: when sowing density is given by a number of seeds per Petri dish, a diameter of 90 mm is assumed.
Incubator: capable of operating in the range 20 ±2 °C

Methods

1. Pretreatment: None.
2. Plating

2.1 Place two pieces of blotter (88 mm in diameter) in each 90 mm Petri dish (bottom) and soak with distilled/deionised water. Drain away excess distilled/deionised water. Place 5 seeds in each Petri dish.

2.2 Positive control (reference material): Aseptically place seeds in an appropriate number of plates to obtain the reference culture, or plate a reference culture on media. The number of plates required will depend on the level of contamination of the positive control seed lot.

3. Incubation: 7 days at 20 °C in darkness
4. Examination: Examination is carried out after 5 and 7 days. A contaminated seed could present several criteria; one of these criteria is sufficient for the seed to be recorded as infected. Compare with positive control.

Examination by naked eye

- A soft rot, covered by an abundant grey mycelium (Fig. 1); the presence of mycelium with sporulation is needed, since soft rots can also be due to saprophytes.

Examination by high-power microscope (magnification ×150–200)

- Tape-like hyphae producing bunches of branching conidiophores (Figs. 2 and 3).
- Isolated conidiophore on teguments, cotyledons or the root (Fig. 4). In doubtful cases, confirmation may be made by examining the mycelium under the microscope (×150) for tape-like hyphae and ovoid, hyaline one-celled conidia 8–11 × 6–19 µm (Fig. 5).
- Non-sporulated mycelium of *Botrytis cinerea* on teguments, cotyledons or the root, recognisable by tape-like hyphae (Fig. 6).

General methods

Checking tolerances: Tolerances provide a means of assessing whether or not the variation in results within or between tests are sufficiently wide as to raise doubts about the accuracy of the results. Suitable tolerances, which can be applied to most direct seed health tests, can be found in Table 5B Part 1 of Chapter 5 of the ISTA Rules, or Table G1 in Miles (1963).

Reporting results: The result of a seed health test should indicate the scientific name of the pathogen detected and the test method used. When reported on an ISTA Certificate, results are entered under ‘Other Determinations’.

The report must indicate the number of seeds tested.

In the case of a negative result (pathogen not detected), the results must be reported as ‘not detected’.

In the case of a positive result, the report must indicate the percentage of infected seeds.

Quality assurance

Critical control points (CCP)

None listed.

Figure 1. Soft rot of the root with abundant grey mycelium of *Botrytis cinerea*.

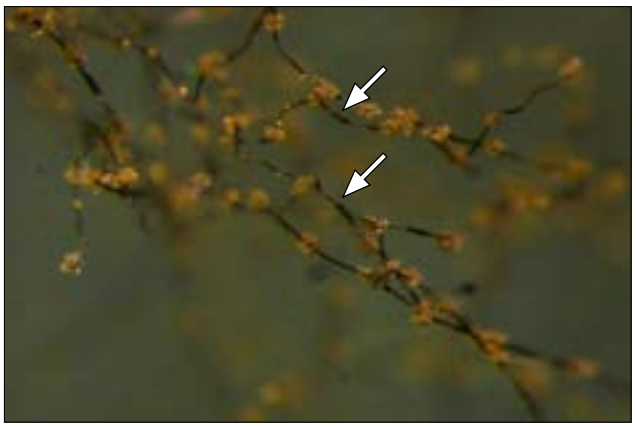

Figure 2. Sporulated mycelium with tape-like hyphae (arrows) of *Botrytis cinerea*.

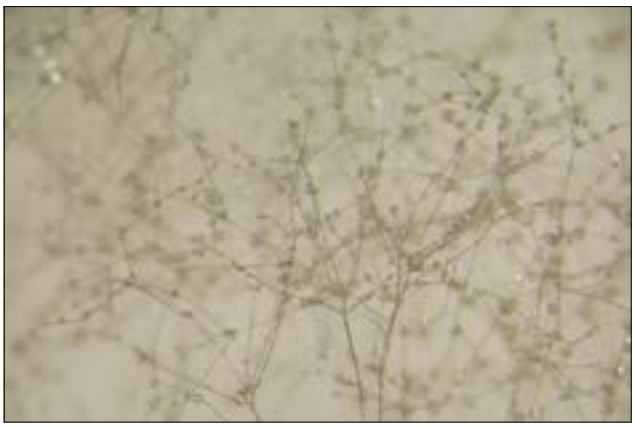

Figure 3. Sporulated mycelium of *Botrytis cinerea*.

Figure 4. Isolated conidiophores of *Botrytis cinerea*.

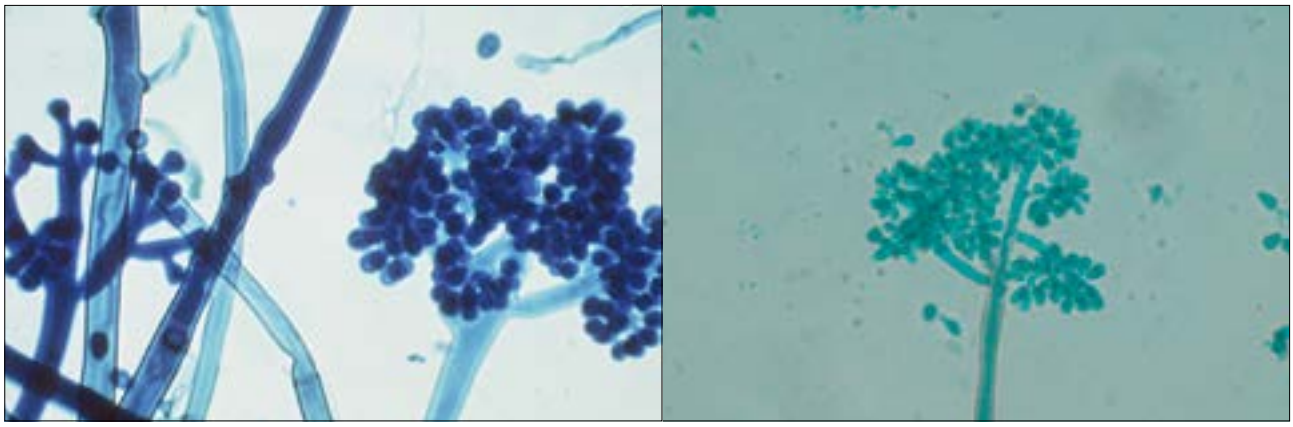

Figure 5. Conidiophores and conidia of *Botrytis cinerea*.

Figure 6. Non-sporulated mycelium of *Botrytis cinerea* with tape-like hyphae (arrow).

References

Note: The first two references are extracted from the *ISTA Handbook of Seed Health Testing*, Working Sheet No. 44, C. Anselme & R. Champion, 1981.

Anselme, C. & Champion R. (1975). Etude de la transmission du *Botrytis cinerea* par les semences de Tournesol (*Helianthus annuus*). *Seed Science and Technology*, **3**, 711–717.

Champion, R. (1969). Quelques parasites importants transmis par les semences. Identification au laboratoire. *Agriculture*, **322**, 3–8.

Cockerell, V. & Koenraadt, H. (2007). Five Year Review of Official Methods Introduced in 2001 to Chapter 7, ISTA International Rules for Seed Testing, ISTA Seed Health Committee Report. *Seed Testing International*, **133**, April 2007. International Seed Testing Association, Basserdorf, Switzerland.

Miles, S. R. (1963). Handbook of tolerances and of measures of precision for seed testing. *Proceedings of the International Seed Testing Association*, **28** (3), 525–686.

Validation references

ISTA (2011). Validation report for revised 7-003: Detection of *Botrytis cinerea* on *Helianthus annuus* method. *Method Validation Reports*. International Seed Testing Association, Bassersdorf, Switzerland.