



International Rules for Seed Testing
Annexe to Chapter 7: Seed Health Testing Methods



7-003: Detection of *Botrytis cinerea* on *Helianthus annuus* (Sunflower)

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 damage etc., resulting from the use of this method.

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

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7-003: *Botrytis cinerea* on *Helianthus annuus***Crop:** *Helianthus annuus* (Sunflower)**Pathogen:** *Botrytis cinerea* Pers. ex Pers. Perfect state: *Sclerotinia fuckeliana* (de Bary) Fuckel.**Prepared by:** V. Grimault, I. Serandat, C. Poisblaud, Q. Brunelle, C. Brochard
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E-mail: valerie.grimault@geves.fr**Sponsored by:** ISTA Seed Health Committee**Submitted by:** ISTA-PDC Method Validation Sub-committee**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

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 and 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 & Koenraad, 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 under-estimation 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/de-ionised water;
- incubation reduced to 7 days, with examinations made at 5 and 7 days.

Validation studies: Grimault, V *et al.* (2011).Copies are available by e-mail from ista.office@ista.ch, or by mail from the ISTA Secretariat.

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Safety precautions

Ensure that you are familiar with hazard data and take appropriate safety precautions, especially during preparation of media, autoclaving and weighing out of ingredients. It is assumed that this procedure is being carried out in a microbiological laboratory by persons familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic technique. Dispose of all waste materials in an appropriate way (e.g. autoclaving or disinfection) and in accordance with local safety regulations.

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)

Materials

Reference material: the use of reference cultures or other appropriate material is recommended whenever possible.

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.

Sample preparation

The test is carried out on a working sample of 400 seeds as described in Section 7.4.1 of the ISTA Rules.

Method

1. Pretreatment: none.
2. Plating: Place two pieces of blotter (88 mm in diameter) in each 90 mm Petri dish and soak with distilled/de-ionized water. Drain away excess distilled/de-ionized water. Place 5 seeds in each Petri dish.
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.

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.

7-003: *Botrytis cinerea* on *Helianthus annuus***Examination by high-power microscope (magnification $\times 150$ – 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 ($\times 150$) for tape-like hyphae and ovoid, hyaline one-celled conidia $8\text{--}11 \times 6\text{--}19 \mu\text{m}$ (Fig. 5).
- Non-sporulated mycelium of *Botrytis cinerea* on teguments, cotyledons or the root, recognizable by tape-like hyphae (Fig. 6).

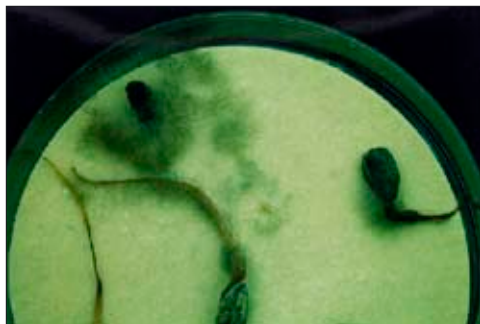


Figure 1. Soft rot of the root with abundant grey mycelium of *Botrytis cinerea*.

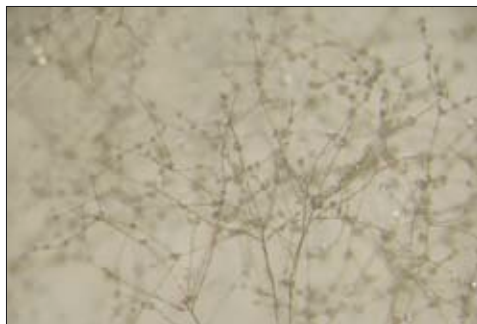


Figure 2. Sporulated mycelium with tape-like hyphae (arrows) of *Botrytis cinerea*.

Figure 3. Sporulated mycelium of *Botrytis cinerea*.

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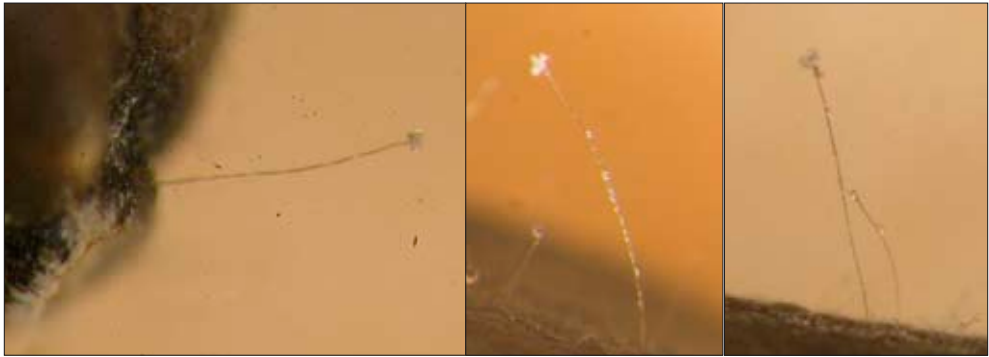


Figure 4. Isolated conidiophores of *Botrytis cinerea*.

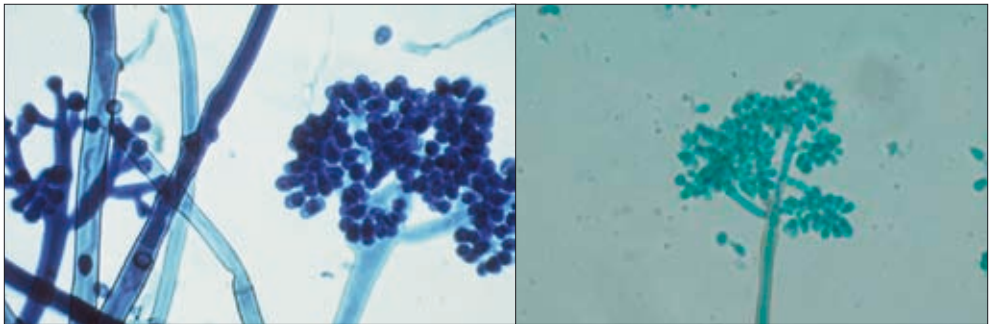


Figure 5. Conidiophores and conidia of *Botrytis cinerea*.



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

7-003: *Botrytis cinerea* on *Helianthus annuus***General methods (common to many test procedures)****Checking tolerances**

Tolerances provide a means of assessing whether or not the variation in result within or between tests is 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 Tables 5B of Chapter 5 of the ISTA Rules, or 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.

Quality assurance**Critical control points**

None listed.

References

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

Anselme, C. and 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. and Koenraad, 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.

Grimault, V., Serandat, I., Poisblaud, C., Brunelle, Q. and Brochard, C. (2011). Validation report for revised 7-003: Detection of *Botrytis cinerea* on *Helianthus annuus* method, ISTA Method Validation Report 2011. 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), 644.