



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



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

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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.

Crop: *Helianthus annuus* (Sunflower)

Pathogen: *Botrytis cinerea* Pers. ex Pers. Perfect state: *Sclerotinia fuckeliana* (de Bary) Fuckel.

Prepared by: ISTA-PDC Method Validation Sub-committee

Revision History: Version 1.0 February 26, 2001
Revised 26.02.2001 J. Sheppard, V. Cockerell
Reprinted 2003
Version 1.1 2008-01-01
“Treated seed” revised; “Reporting results” revised

Submitted by: ISTA-PDC Method Validation Sub-committee

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.

Safety Precautions

Ensure 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 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. autoclave, disinfect) 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 when ever possible. |
| Media | - Blotters (filter paper), e.g. Whatman No 1 or equivalent
3% malt solution |
| 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. Blotter

Place two pieces (88 mm in diameter) of filter paper in each of 90 mm-diameter Petri dishes, and add 5 mL of a solution of 3% malt extract. Pour off the excess liquid and plant 5 seeds in each Petri dish.

3. Incubation

9 days at 20 °C in darkness.

4. Examination

After 5, 7 and 9 days examine the seeds by naked eye for roots showing a soft rot and covered by an abundant grey mycelium (Fig. 1). These seeds are recorded as infected. Examination is repeated after 7 and 9 days incubation. New infections are marked on

the blotter, and the sum of the three counts gives the total infection.

In doubtful cases confirmation may be made by examining the mycelium at $\times 200$ magnification for septae, ribbon-like hyphae and tufts of branching conidiophores (Fig. 3).

General Methods (common to many test procedures)

1. 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 the *Handbook of Tolerances and Measures of Precision for Seed Testing* by S.R. Miles (*Proceedings of the International Seed Testing Association* 28 (1963) No 3, p 644).

2. 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 International Seed Analysis Certificate*, results are entered under Other Determinations.

Quality Assurance

Critical Control Points

None Listed

References

The following references are extracted from the ISTA Handbook on Seed Health Testing, Working Sheet No. 44, C. Anselme and R. Champion, 1981.

Anselme, C. et 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.

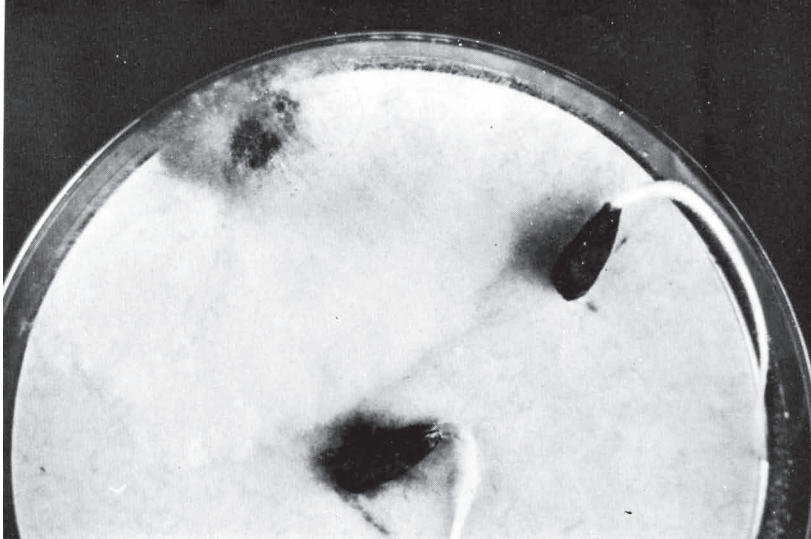


Fig. 1. Sunflower seeds with colonies of abundant ash grey mycelium of *Botrytis cinerea* after 9 days incubation on blotters.

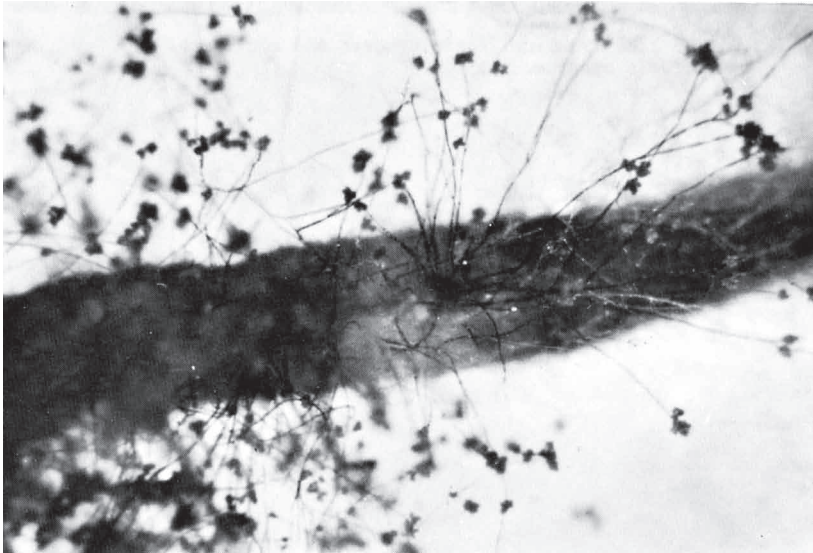


Fig. 2. Colonies of *Botrytis cinerea* on rootlets. x 18.

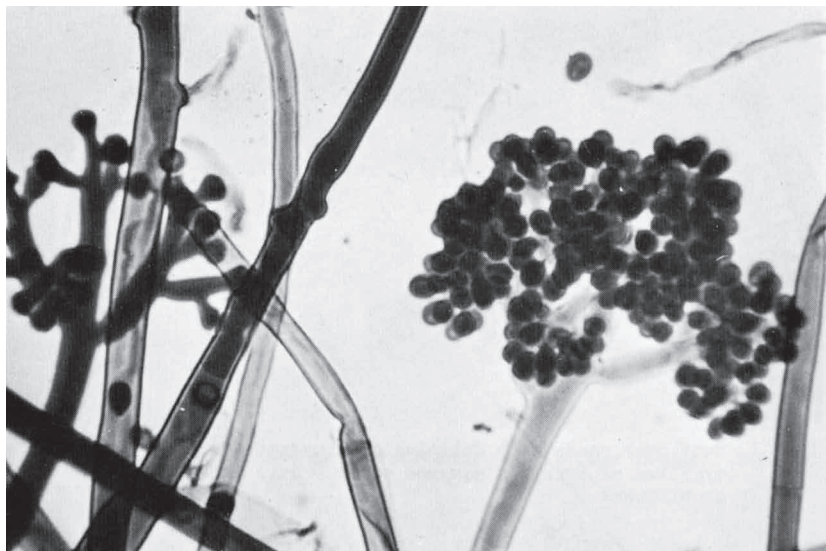


Fig. 3. Tape-like mycelium, conidiophores and conidia of *Botrytis cinerea*.
150 x.