



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



7-006: Detection of *Colletotrichum lindemuthianum* on *Phaseolus vulgaris* (Bean)

Published by: International Seed Testing Association (ISTA), Bassersdorf, Switzerland
2008

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: *Phaseolus vulgaris* (Bean)

Pathogen: *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi et Cav.

Prepared by: ISTA-PDC Method Validation Sub-committee

Revision History: Version 1.0 November 19, 2001
Revised 19.11.2001 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. 45 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.

Studied in ISTA Comparative Tests: 1962

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 *Colletotrichum lindemuthianum* 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 | - Paper towelling. |
| Sodium hypochlorite solution | - (1% available chlorine) for seed disinfection. |
| 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

Seeds are submerged in a solution of 1% (available chlorine) sodium hypochlorite for 10 min and allowed to drain.

2. Between Paper (BP)

Spread the seeds in replicates of 50 on double sheets of paper towelling 350 x 450 mm which have been soaked in water. Cover seeds with one sheet of paper towelling soaked in water. Fold the paper twice lengthways and cover it with a sheet of polythene to maintain the moisture during incubation.

3. Incubation

7 days at 20 °C in darkness

4. Examination

After 7 days remove the seed coats and examine the cotyledons by naked eye for black depressed areas with well delimited outlines (Fig. 2). Check each spot for the presence of acervuli with or without dark brown setae using $\times 25$ magnification (Fig. 3). The septate setae measure approx. $6 \mu\text{m} \times 100 \mu\text{m}$. The pale orange acervuli contain cylindrical, hyaline conidia with rounded ends containing one or two guttulae. Conidia measure

2.5–5.5 µm × 11–20 µm (Mordue, 1971; Kulshrestha, Mathur, and Neergaard, 1976). The use of a high-power microscope (magnification ×200) is sometimes necessary (Fig. 3). Small spots may require longer incubation for development of acervuli.

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.

Preparation of Media and Solutions

1. Sodium Hypochlorite Solution

Sodium hypochlorite for pretreatment of seed can be prepared from commercial bleach diluted to 1% available chlorine. The concentration of chlorine in commercial bleach varies considerably. Use the formula $V_{\text{stock}} = V_{\text{final}} \times C_{\text{final}} / C_{\text{stock}}$ (where V = volume and C = % available chlorine) to calculate the volume of commercial bleach stock solution required to prepare sodium hypochlorite solutions for use in seed pretreatment.

To prepare a 1 L solution of sodium hypochlorite containing 1% chlorine from a stock of commercial bleach containing 12% available chlorine:

$$V_{\text{stock}} = V_{\text{final}} \times C_{\text{final}} / C_{\text{stock}} \qquad V_{\text{stock}} = 1 \times 1/12 = 0.083$$

Thus add 83 mL of the 12% stock to 917 mL water.

Quality Assurance

Critical Control Points

None listed

References

The following references are extracted from the ISTA Handbook on Seed Health Testing, Working Sheet No. 45, C. Anselme and R. Champion, 1981.

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

Kulshrestha, D.D., Mathur, S.B. and Neergaard, P. (1976). Identification of seed borne species of *Colletotrichum*. Friesia 11, 116-125.

Mordue, J.E.M. (1971). C.M.I. Description of pathogenic fungi and bacteria No. 316. Commonwealth Mycological Institute, Kew.

International Seed Testing Association, Committee on Plant Diseases (1963) Report on the Fifth International Conference (workshop) on seed pathology sponsored by the International Seed Testing Association convened by the ISTA Committee on Plant Diseases. Bundesanstalt für Pflanzenbau und Samenprüfung in Wien 17th to 23rd September, 1962, p. 23. Copenhagen. Mimeographed.



Fig. 1. Dry, white bean seeds showing symptoms

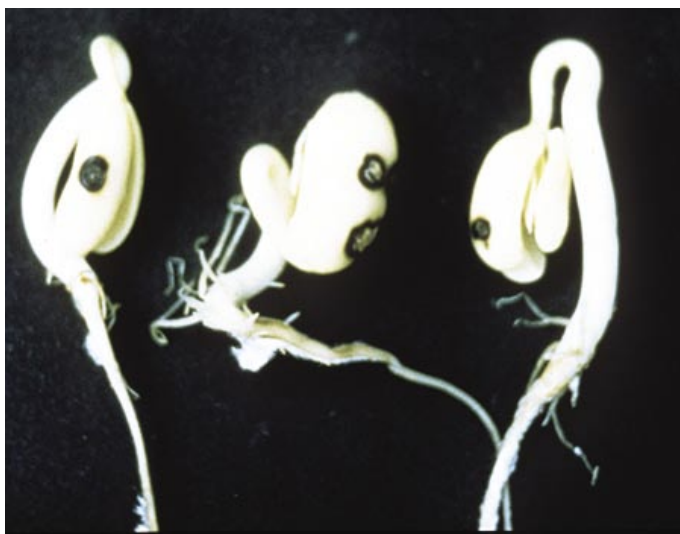


Fig. 2. Cotyledons of seedlings after 7 days incubation with seed coats removed, showing black areas with well defined outline.

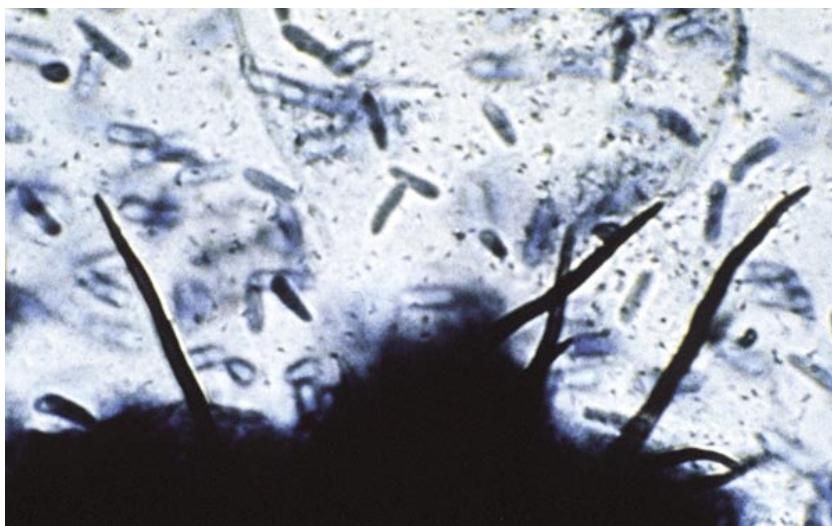


Fig. 3. Acervuli with cylindric, hyaline conidia and dark brown septate setae. x 150.