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

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

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Crop: *Phaseolus vulgaris* (Bean)
Pathogen: *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi et Cav.

Prepared by: ISTA-PDC Method Validation Sub-committee

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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 appears in annex 7.4.3.A.4 of the ISTA Rules (1999). It has been incorporated into the new Annexe to Chapter 7, Seed Health Testing Methods as method 7-006 and is subject to review before 2006.

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 may be influenced by treatment applied to the seed lot. Seed health tests on chemically treated seeds will generally deliver unreliable test results caused by masking or inhibition of the growth of the target organism.

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 chorine) for seed disinfection.
Incubator	-	Capable of operating in the range 20ºC ± 2ºC.

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 minutes 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 20ºC 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 x25 magnification (Fig. 3). The septate setae measure approx. 6µ x 100µ. The pale orange acervuli contain cylindric, hyaline conidia with rounded ends containing one or two guttulaes. Conidia measure 2.5 5.5µ x 11 20µ (Mordue, 1971; Kulshrestha, Mathur, and Neergaard, 1976). The use of a high power microscope (magnification x200) 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. A tolerance table, which can be applied to most direct seed health tests, can be found in Table 5.1 of Annex 15 of the ISTA Rules or in Table G1 of 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, pp 644).

2. Reporting Results

   The result of a seed health test should indicate the scientific name of the pathogen detected and the percentage of infected seeds. When reported on an ISTA Certificate results are entered under Other Determinations. The results should be accompanied by information on the test method used, including any pretreatment.

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 $V_{stock} = V_{final} \times C_{final} / C_{stock}$ (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 liter solution of sodium hypochlorite containing 1% chlorine from a stock of commercial bleach containing 12% available chlorine:

$V_{stock} = V_{final} \times C_{final} / C_{stock}$ $V_{stock} = 1\mathbf{L} \times 1/12 = 0.083$

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 fur Pflanzenbau and 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.

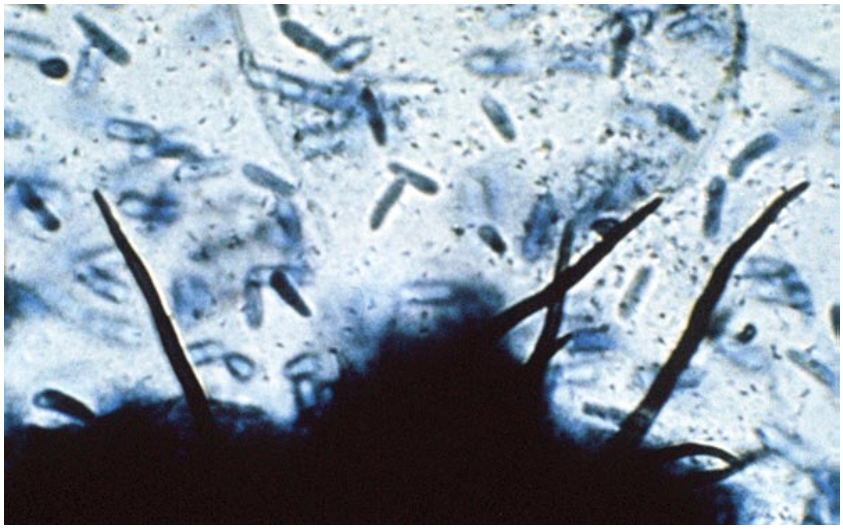

Fig. 3. Acervuli with cylindric, hyaline conidia and dark brown septate setae. x 150.