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

7-014: Detection of *Septoria nodorum* on *Triticum aestivum* (Wheat)

Published by: International Seed Testing Association (ISTA), Bassersdorf, Switzerland
2002

Crop: *Triticum* spp (Wheat)
Pathogen: *Stagonospora nodorum* Berk= syn *Septoria nodorum* Berk., Perfect state: *Leptosphaeria nodorum* Mailer

Prepared by: ISTA-PDC Method Validation Sub-committee

Revision History: Version 1.0 November 20, 2001
Revised 20.11.2001 J. Sheppard
Reprinted with editorial changes 2003

Submitted by: ISTA-PDC Method Validation Sub-committee

Method Abstract

This method was originally published in the ISTA Handbook of Seed Health Testing in November 1964 as S.3. No. 19 and revised in 1984 by M. Kietreiber, Bundesanstalt für Pflanzenbau, Wien, Austria. The method appears in Annexe 7.4.3.A.7 of the ISTA Rules (1999). It has been incorporated into the new Annexe to Chapter 7, Seed Health Testing Methods as method 7-014 and is subject to review before 2006.

Summary of Validation Study:

Studied in International Comparative Testing: 1959, 1961, 1962, 1964 and 1979-81

Using potato dextrose in darkness, Hewett (1975) found a correlation coefficient of 0.95 between counts in the laboratory and the number of diseased seedlings in the field. Comparative tests organized by the ISTA Plant Disease Committee gave reasonable agreement between stations (Rennie, 1982).

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	-	Malt Agar or Potato Dextrose Agar containing 100 ppm streptomycin sulphate.
Sodium hypochlorite solution		(1% available chorine) for seed disinfection.
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 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 International Rules for Seed Testing.

Method

1. *Pretreatment*
   10 minutes in 1% (avilable chlorine) sodium hypochlorite.
2. *Agar Method*
   Malt agar or Potato Dextrose Agar containing 100 ppm streptomycin sulphate.
3. *Incubation*
   7 days at 20º C in darkness.
4. *Examination*.
   After seven days examine each seed by naked eye for slow growing circular colonies of dense white or cream mycelium that often covers infected seeds. The reverse of the colony is yellow/brown becoming darker with age.

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.

2. Malt Agar

Compound	g/l	g/500 ml
Malt Agar[1]	45	22.5
Deionized/Distilled Water	1l	500 ml
Streptomycin sulfate	1 mg	0.5 mg

CCP[1] *Malt agar constituents should be equivalent to those of the following manufacturers Difco, USA or Oxoid, UK.*

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 (or 500) ml of deionized/distilled water.
3. Dissolve powdered Malt Agar in deionized/distilled water by stirring.
4. Autoclave at 15 PSI and 121°C for 15 min.
5. Allow agar to cool to approx. 50°C.
6. Pour 15-22 ml of molten agar into 90 mm Petri plates and allow to solidify before use.

Plates may be stored at room temperature in a dry cabinet or at 4ºC.

Potato Dextrose Agar

Compound	g/l	g/500 ml
Potato Dextrose Agar[1]	39	19.5
Deionized/Distilled Water	1l	500 ml
Streptomycin sulfate	1 mg	0.5 mg

CCP [1] *PDA constituents should be equivalent to those of the following manufacturers Difco, USA or Oxoid, UK.*

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 (or 500) ml of deionized/distilled water.
3. Dissolve powdered PDA in deionized/distilled water by stirring.
4. Autoclave at 15 PSI and 121°C for 15 min.
5. Allow agar to cool to approx. 50°C.
6. Pour 15-22 ml of molten agar into 90 cm Petri plates and allow to solidify before use.

Plates may be stored at room temperature in a dry cabinet or at 4ºC.

Quality Assurance

Critical Control Points

Where the wording of the original Working Sheet suggests that an action is critical this has been marked with ***CCP***.

References

The following references are extracted from the ISTA Handbook on Seed Health Testing, Working Sheet No. 19, M. Kietreiber, 1984.

Hewett, P.D. (1975). *Septoria nodorum*. Seedlings and stubble of winter wheat. Transactions of British Mycological Society, 65, 7-18.

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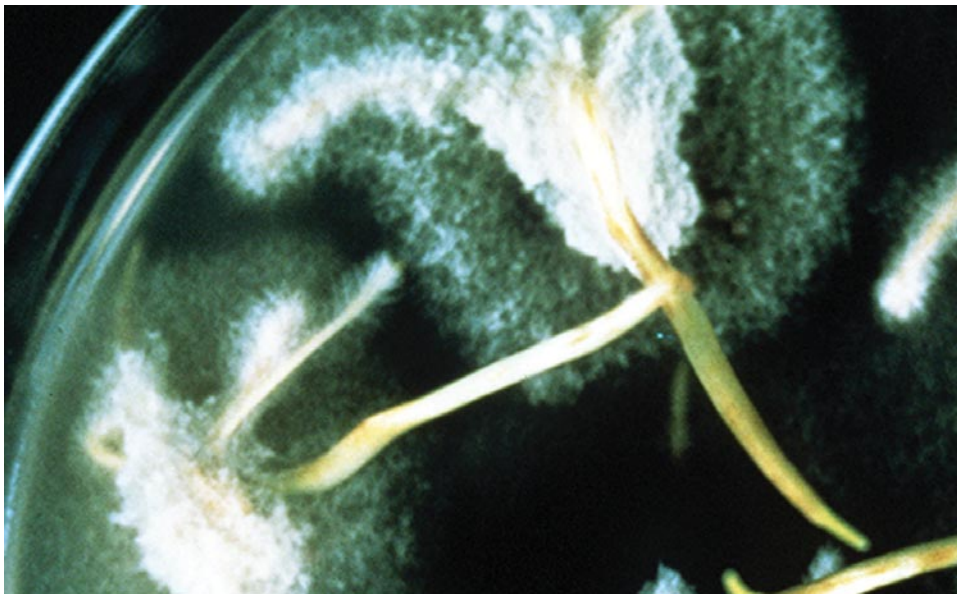

Fig. 1. Slow growing, finely tufted, white aerial mycelium of *Septoria nodorum* covering grain in an agar plate test.