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

7-004: Detection of *Phoma lingam* on *Brassica spp.*

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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: *Brassica* spp.
Pathogen: *Phoma lingam* (Tode ex Fr.) Desm. syn *Plenodomus lingam* (Tode ex Fr.) Hohn perfect state *Leptosphaeria maculans* (Tode ex Fr.) Ces. & de Not.

Prepared by: ISTA-PDC Method Validation Sub-committee

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Revised 19.11.2001 J. Sheppard, V. Cockerell
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Submitted by: ISTA-PDC Method Validation Sub-committee

Background

This method was originally published in the ISTA Handbook of Seed Health Testing in November 1964 as S.3. No. 31. The method appears in Annex 7.4.3.A.3 of the ISTA Rules (1999). It has been incorporated into the new Annexe to Chapter 7, Seed Health Testing Methods as method 7-004 and is subject to review before 2006.

Summary of Validation Study

Studied in International Comparative Testing 1959, 1978, 1981, 1983

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	-	Blotters
2,4 Dichlorophenoxyacetate (2,4D)	-	Sodium salt 0.2% 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 20ºC ± 2ºC. To stimulate sporulation, alternating 12-hour periods of darkness and near-ultraviolet light (NUV) during incubation are recommended. The recommended source is the *black light* fluorescent lamp (peak at 360 nm) but daylight fluorescent tubes are satisfactory.

Sample Preparation

The test is carried out on a working sample of 1000 pure seeds as described in Section 7.4.1 of the ISTA Rules.

Method

1. *Blotter*

   Place three pieces of filter paper (Whatman No. 1) in each petri dish and add 5 ml of a 0.2% solution of the sodium salt of 2,4 dichlorophenoxyacetic acid (2,4-D) to inhibit seed germination. Pour of the excess 2,4-D solution, rinse the seeds in sterile water and place 50 in each dish.

2. *Pretreatment*

   None.

3. *Incubation*

   Incubate for 11 days at 20ºC with alternating cycles of 12 hours light and 12 hours darkness.

4. *Examination*

   After 6 days examine at x25 magnification for loose growing silver white mycelium (Fig. 1) and pycnidial primordia of *Phoma lingam* on the seed and substrate. After 11 days make a second examination for pycnidia on infected seeds and on the filter paper near infected seeds. Seeds from which pycnidia of Phoma lingam have developed are recorded as infected.

   Pycnidia are relatively large, about 250µ, with papilla, sometimes developed as a neck (Figs. 2, 3). The ubiquitous saprophyte *Phoma herbarum* Westend. occurs also on brassica seed (Fig. 4), but has smaller pycnidia formed superficially on the seed coat, not papillate, with white yellow or pink but not purple (amethyst) exudate.

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 for most direct seed health tests. Where tolerance tables are not available in the ISTA Rules, the laboratory can develop these based on those given 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, 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.

Quality Assurance

Critical Control Points

None listed

References

The following references are extracted from the ISTA Handbook on Seed Health Testing, Working Sheet No. 31, 1964.

Boerema, G. H., (1964): *Phoma herbarum* Westend., the type species of the form genus *Phoma* Sacc. Persoonia 3, 9-16.

Boerema, G. H. & Van Kesteren, H. A., (1964): The nomenclature of two fungi parasitizing brassica. Persoonia, 3, 17-28.

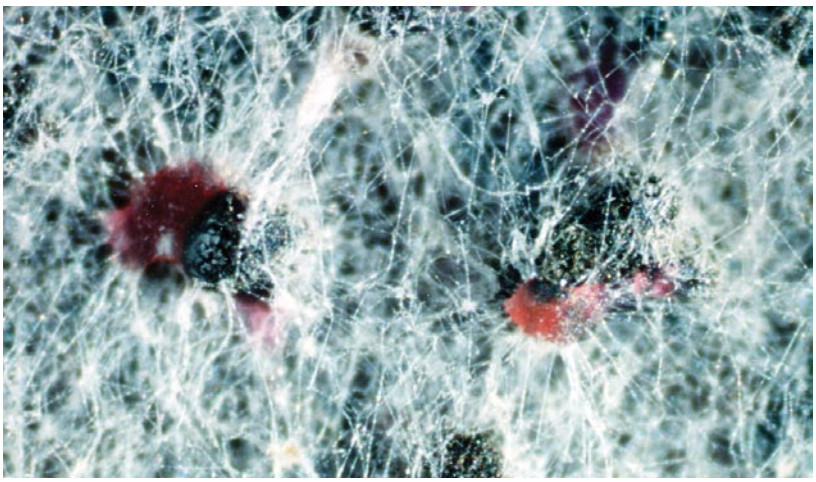

Fig. 1. Seed in blotter test showing white mycelium and pycnidia with amethyst coloured spore exudate.

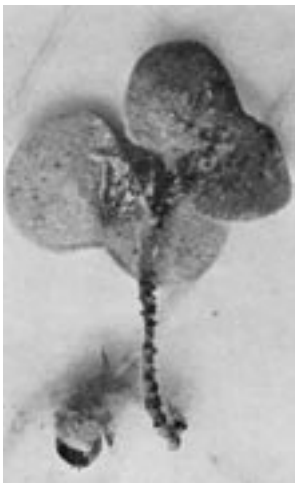

Fig. 2. Infected seedling in bloitter test showing pycnidia on the hypocotyl and cotyledons.

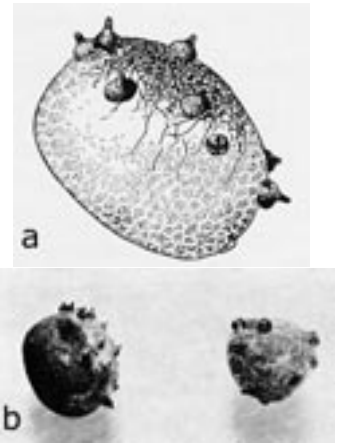

Fig. 3a and 3b. Pycnidia on seed

Fig. 4. *Phoma herbarum* on seed.