



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

**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 November 1964 as S.3. No. 31. 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.

## 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 has not been validated for the determination of *Phoma lingam* 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

- |  |   |
|--|---|
| <b>Reference Material</b>                | - The use of reference cultures or other appropriate material is recommended when ever possible.  |
| <b>Media</b>                             | - Blotters (filter paper), e.g. Whatman No 1 or equivalent  |
| <b>2,4 Dichlorophenoxyacetate (2,4D)</b> | - Sodium salt 0.2% solution   |
| <b>Petri dishes</b>                      | - When sowing density is given by a number of seeds per Petri dish, a diameter of 90 mm is assumed.   |
| <b>Incubator</b>                         | - Capable of operating in the range $20 \pm 2$ °C. To stimulate sporulation, alternating 12 h periods of darkness and near-ultraviolet light (NUV) during incubation are recommended. The recommended source is the <i>black light</i> 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 or equivalent) 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 20 °C with alternating cycles of 12 h light and 12 h darkness.

### 4. Examination

After 6 days examine at ×25 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 250 µm, 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. 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. 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.

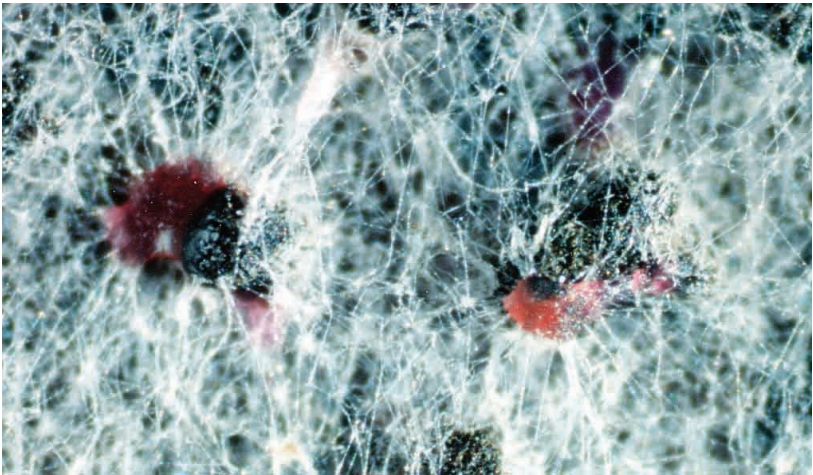


Fig. 1. Seed in blotter test showing white mycelium and pycnidia with amethyst coloured spore exudate.

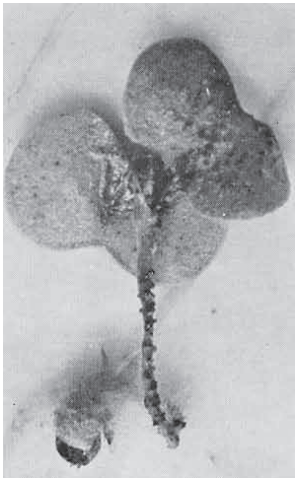
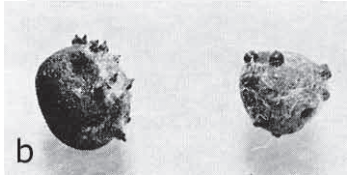


Fig. 2. Infected seedling in blotter test showing pycnidia on the hypocotyl and cotyledons.



a



b

Fig. 3a and 3b. Pycnidia on seed



Fig. 4. *Phoma herbarum* on seed.

