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

7-002a: Blotter method for the detection of *Alternaria radicina* on *Daucus carota*

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Crop: *Daucus carota* (carrot)

Pathogen: *Alternaria radicina* (syn. *Stemphylium radicinum*)

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ISTA-PDC Method Validation Sub-committee

Sponsored by: ISTA-PDC Method Validation sub-committee

Revision History: Version 1.0, 01 January 2003.

Background

This method was originally published in the *ISTA Handbook of Seed Health Testing* in November 1964 as S.3. No. 5 and was revised by Gambogi (1987). It was incorporated into the *Annexe to Chapter 7: Seed Health Testing Methods* as method 7-002 (Sheppard and Cockerell, 2002). It has been renumbered (7-002a) and slightly modified following studies conducted using six seed lots in 11 laboratories by the International Seed Health Initiative - Vegetables in 1999 and 2001 (Van Bilsen, 2003). The studies compared blotter and malt agar methods and concluded that the two were equivalent. Note that seeds can be simultaneously tested for the presence of *Alternaria dauci* using the same method (see method 7-001a).

Validation studies

Van Bilsen (2003)

Copies are available: by e-mail from ista.office@ista.ch; or by mail from the ISTA Secretariat.

Please send comments, suggestions or reports of problems relating to this method to the leader of the ISTA-PDC Mycology Working Group, c/o ISTA Secretariat

Safety Precautions

Ensure you are familiar with hazard data and take appropriate safety precautions. It is assumed that this procedure is being carried out in a 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 health, environmental and safety regulations.

Treated seed

Seed treatments may affect the performance of this test. It should only be performed on untreated seed.

Materials

Reference material	-	The use of reference cultures or other appropriate material is recommended.
Substrate	-	Blotters or filter papers, 9.0 cm, circular (e.g. Whatman No 1 or equivalent), free from micro-organisms and inhibitors (3 per plate).
Plates	-	9.0 cm sterile Petri dishes, one per ten seeds.
Incubator	-	Operating at 20 ± 2°C, equipped with timer-controlled near-ultraviolet lights (NUV, peak at 360 nm, e.g. colour number 08, Philips; BLB Sylvania).
Freezer	-	Operating at –20 ± 2°C.

Sample Preparation

1. It is vital to exclude any possibility of cross-contamination between seed samples. This can be achieved by swabbing/spraying equipment and gloved hands with 70% ethanol.
2. The test is carried out on a working sample as described in Section 7.4.1 of the International Rules for Seed Testing.

Method

[Critical control points are indicated by **CCP**]

1. Place three 9.0 cm filter papers in each plate and soak with sterile distilled/de-ionised water. Drain away excess water.
2. Aseptically place 10 seeds, evenly spaced (**CCP**), on the surface of the filter paper in each plate.
3. Incubate for 3 d at 20 ± 2°C in the dark.
4. Transfer plates to freezer and maintain at –20 ± 2°C for 24 h.
5. After freezing, incubate for 6 d at 20 ± 2°C with alternating 12 h periods of darkness and light, preferably NUV (ISTA,1984; Tempe, 1968). Plates should be approx. 25 cm below the lights and should not be stacked.
6. Examine seeds under a stereoscopic microscope at x30 for fungal growth and up to x80 for identification of conidia. Record the number of infected seeds in each plate. Conidiophores are simple or occasionally branched, arising usually singly from the surface of the seed, on the emerging radicle or on aerial mycelium (Fig. 1). Conidia are produced singly or in chains of 2 or rarely 3, ellipsoidal or barrel shaped, with

little evidence of beak, up to 75 µm long, olivaceous brown, (Ellis, 1971). Under the stereoscopic microscope, conidia appear blackish and glossy (Fig. 1).

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 Annexe 16 of the International Rules for Seed Testing or in Table G1 of the *Handbook of Tolerances and Measures of Precision for Seed Testing* (Miles, 1963).

2. *Reporting Results*

   The result of a seed health test should indicate the scientific name of the pathogen and the test method used. When reported on an ISTA Certificate, results are entered under *Other Determinations*.

   In the case of a negative result (pathogen not detected), the results should be reported in terms of the tolerance standard (e.g. infection level less than 1% with 95% probability). The tolerance standard depends on the total number of seeds tested, n, and is approximately 3/n (P=0.95) (see Roberts *et al.,* 1993).

   In the case of a positive result the report should indicate percentage of infected seeds.

Quality Assurance

Specific Training

This test should only be performed by persons who have been trained in fungal identification or under their direct supervision.

Critical Control Points

[Identified by **CCP** in the methods]

Spreading hyphae may lead to contamination of other seeds. Seeds must therefore be spaced at least 20 mm from each other, i.e. no more than 10 seeds per 9.0 cm Petri dish (Step 2).

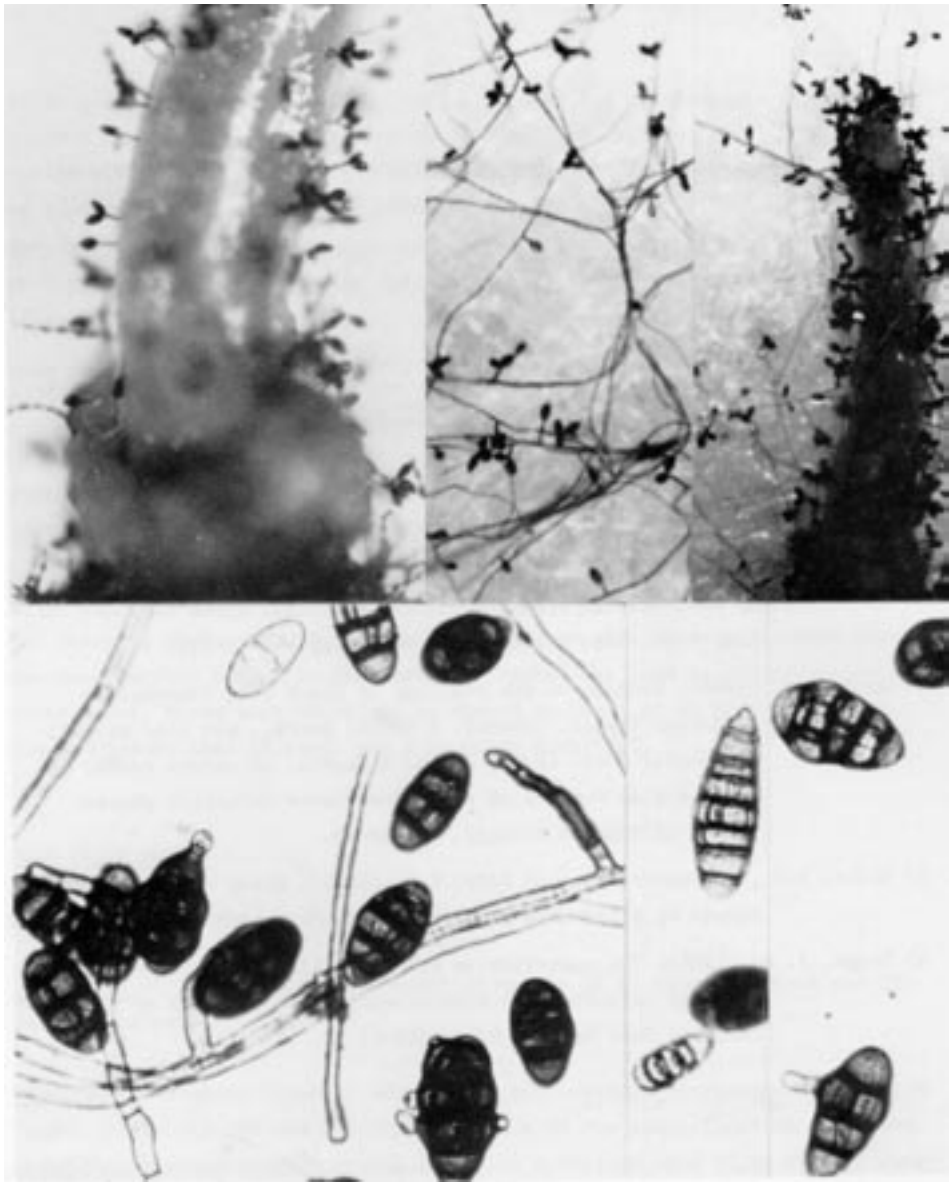

Fig. 1. Top: conidiophores and conidia of *Alternaria radicina* and chains of conidia of the saprophyte *A. tenuis* on a rootlet initial x80 (left); spreading hyphae and fructifications of the pathogen on the blotter, x80 (centre); abundant growth and fructification of the pathogen on a rootlet initial, x50 (right). Bottom: conidia of *Alternaria radicina,* x350.

References

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