International Rules for Seed Testing
2021

Validated Seed Health Testing Methods

7-002a: Detection of *Alternaria radicina* in *Daucus carota* (carrot) seed by blotter method

Including changes and editorial corrections adopted at the online Ordinary General Meeting 2020

Effective from 1 January 2021
Validation reports

See References. Copies are available by e-mail from the ISTA Secretariat at ista.office@ista.ch.

Please send comments, suggestions or reports of problems relating to this method to the ISTA Seed Health Committee, c/o ISTA Secretariat.

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 or damage, etc. resulting from the use of this method.

Safety precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during weighing out of ingredients. It is assumed that persons carrying out this test are in a laboratory suitable for carrying out microbiological procedures and familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic techniques. Dispose of all waste materials in an appropriate way (e.g. autoclaving, disinfection) and in accordance with local health, environmental and safety regulations.

Note on the use of the translations

The electronic version of the International Rules for Seed Testing includes the English, French, German and Spanish versions. If there are any questions on interpretation of the ISTA Rules, the English version is the definitive version.
7-002a: Detection of *Alternaria radicina* in *Daucus carota* (carrot) seed by blotter method

**Host:** *Daucus carota* L.

**Pathogen(s):** *Alternaria radicina* Meier, Drechsler & E.D.Eddy, syn. *Stemphylium radicinum* (Meier, Drechsler & E.D.Eddy) Neergaard

**Prepared by:** ISTA-PDC Method Validation Sub-committee

**Authors:** Sheppard, J.W., Cockerell, V & Roberts, S.J.; ISTA-PDC Method Validation Sub-committee

**Revision history**

Version 1.0, 2003-01-01
Version 1.1, 2013-01-01: Definition of sample size
Version 1.2, 2014-01-01: Addition of positive control; common name of host added
Version 1.3, 2017-01-01: Reporting results revised
Version 1.4, 2021-01-01: Sample size added and Methods revised

**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 *Anexe to Chapter 7: Seed Health Testing Methods* as method 7-002 (Sheppard & 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 for Vegetable Crops, ISF (ISHI-Veg) 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).

**Treated seed**

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

**Sample size**

The sample (total number of seeds tested) size to be tested depends on the desired tolerance standard (maximum acceptable percentage of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected). The minimum sample size should be 400 seeds.

**Materials**

**Reference material:** reference cultures or other appropriate material

**Substrate:** blotters or filter papers, 90 mm, circular (e.g. Whatman No 1 or equivalent), free from micro-organisms and inhibitors (3 per plate)

**Plates:** 90 mm 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**

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.

**Methods**

Critical control points are indicated by CCP.

1. **Pretreatment:** None.
2. Place three 90 mm filter papers in each plate and soak with sterile distilled/deionised water. Drain away excess water.
3. **Plating**
   3.1 Aseptically place 10 seeds, evenly spaced (CCP), on the surface of the filter paper in each plate.
   3.2 **Positive control (reference material):** Aseptically place seeds evenly spaced (CCP) onto the surface of the filter paper in an appropriate number of plates to obtain the reference culture, or plate a reference culture on media. The number of plates required will

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**Revision history**

Version 1.0, 2003-01-01: « Indications des résultats »
Version 1.1, 2013-01-01: Definition of sample size
Version 1.2, 2014-01-01: Positivkontrolle hinzugefügt; common name of host added
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depend on the level of contamination of the positive-control seed lot.
4. Incubate for 3 d at 20 ± 2 °C in the dark.
5. Transfer plates to freezer and maintain at −20 ± 2 °C for 24 h.
6. 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.
7. Examine seeds under a stereoscopic microscope at ×30 for fungal growth and up to ×80 for identification of conidia. Compare with positive control. 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).

**Critical control points (CCP)**

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 90 mm Petri dish (Step 3).

**References**


**Validation references**

Figure 1. a Conidiophores and conidia of *Alternaria radicina* and chains of conidia of the saprophyte *A. tenuis* on a rootlet initial. ×80. b Spreading hyphae and fructifications of the pathogen on the blotter. ×80. c Abundant growth and fructification of the pathogen on a rootlet initial. ×50. d Conidia of *Alternaria radicina*. ×350.