International Rules for Seed Testing 2027

Validated Seed Health Testing Methods

7-002b: Detection of *Alternaria radicina* in *Daucus carota* (carrot) seed by malt agar method

Including changes and editorial corrections adopted at the Ordinary General Meeting 2026 in Calgary, Canada

Effective from 1 January 2027

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.

Published by
The International Seed Testing Association (ISTA)
Richtiarkade 18, CH-8304 Wallisellen, Switzerland

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Online ISSN 2310-3655

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7-002b: Detection of *Alternaria radicina* in *Daucus carota* (carrot) seed by malt agar 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: International Seed Health Initiative for Vegetable Crops, ISF (ISHI-Veg)

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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; addition of streptomycin sulphate; 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
Version 1.5, 2024-01-01: Sample size and Figures revised
Version 1.6, 2025-01-01: Methods and figure legends revised
Version 1.7, 2027-01-01: Flowchart added in Methods

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 has been 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. The major modification is evaluation after 10 d incubation rather than 7 d. Note that seeds can be simultaneously tested for the presence of *Alternaria dauci* using the same method (see method 7-001b).

Treated seed

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

Sample size

The sample size (total number of seeds to be tested) depends on intended use, the maximum acceptable infection level and the analytical sensitivity of the method. The minimum sample size should be 400 seeds.

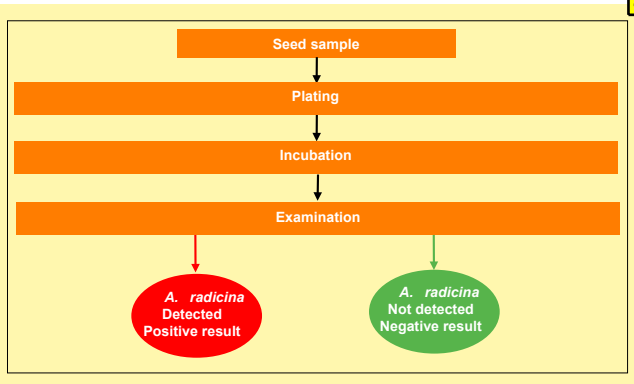
Materials

Reference material: reference cultures or other appropriate material
Malt agar plates with streptomycin sulphate: 90 mm Petri dishes, one plate 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).

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. Plating

2.1 Aseptically place a maximum of 10 seeds, evenly spaced, on the agar surface of each malt agar plate.

2.2 Positive control (reference material): Aseptically place seeds evenly spaced (CCP), onto the agar surface of an appropriate number of malt agar plates to obtain the reference culture, or plate a reference culture on one malt agar plate. The number of plates required will depend on the level of contamination of the positive control seed lot.

3. Incubate plates for 10 d at 20 ±2 °C, with alternating 12 h periods of darkness and NUV light. Plates should be approx. 25 cm below the lights and should not be stacked.
4. Subculture a reference culture to a malt agar plate at the same time the seeds are plated and incubate with the test plates.
5. Examine plates visually, and under a stereomicroscope at ×30 magnification, for fungal growth. Use a magnification of ×50 to ×80 for identification of conidia. Colonies of *Alternaria radicina* are irregular to circular with luxurious aerial mycelium, dark olive grey to greyish-black from above, bluish-black from below (Meier, *et al.,* 1922). 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 groups 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 stereomicroscope, conidia appear blackish and glossy (Fig. 1). Compare with positive control. Record the number of infected seeds in each plate (CCP).

General methods

Checking tolerances: Tolerances provide a means of assessing whether or not the variation in results within or between tests are 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 Table 5B Part 1 of Chapter 5 of the ISTA Rules, or Table G1 in Miles (1963).

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 Certificate, results are entered under 'Other Determinations'.

The report must indicate the number of seeds tested.
In the case of a negative result (pathogen not detected), the results must be reported as 'not detected'.
In the case of a positive result, the report must indicate the 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 (CCP)

Contaminants may compete strongly with the pathogen on the malt agar medium, so that detection may be laborious and difficult (Step 5).

The malt agar source can influence the results. Whenever a new batch of malt agar is used a check on the quality should be made using a reference lot with a known infection level (Preparation of malt agar).

Media and solutions

Malt agar + streptomycin

Malt agar (CCP): as specified by manufacturer
Streptomycin sulphate: 50 mg
Distilled/deionised water: 1000 ml

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 (or 500) ml of distilled/deionised water.
3. Steam to dissolve.
4. Autoclave at 121 °C and 15 psi for 15 min.
5. Allow agar to cool to approx. 50 °C and add streptomycin sulphate dissolved in water.
6. Pour 15–22 ml of molten agar into 90 mm plates (Petri dishes) and allow to solidify at room temperature (20–25 °C) for 24 h before use.

Storage

Prepared plates may be stored at room temperature or at 4 °C for up to one month before use.

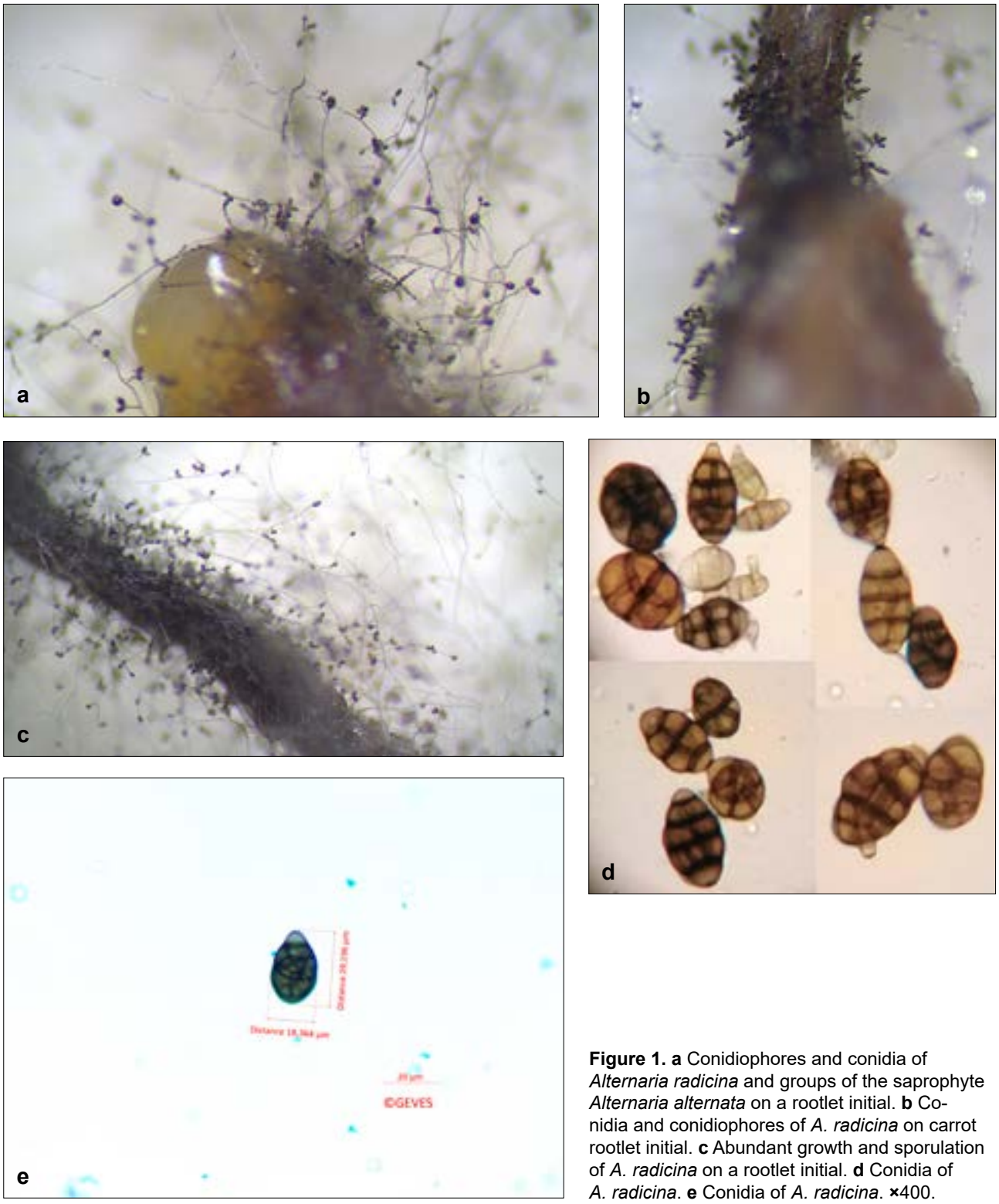


Figure 1. a Conidiophores and conidia of *Alternaria radicina* and groups of the saprophyte *Alternaria alternata* on a rootlet initial. **b** Conidia and conidiophores of *A. radicina* on carrot rootlet initial. **c** Abundant growth and sporulation of *A. radicina* on a rootlet initial. **d** Conidia of *A. radicina*. **e** Conidia of *A. radicina*. ×400.

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Validation references

ISTA (2003). Report of the comparative test on *Alternaria dauci* and *Alternaria radicina* on carrot seed. *Method Validation Reports*. International Seed Testing Association, Bassersdorf, Switzerland.