



# **International Rules for Seed Testing 2022**

**Validated Seed Health Testing Methods**

**7-001a: Detection of *Alternaria dauci* in *Daucus carota* (carrot) seed by blotter method**

**Including changes and editorial corrections adopted  
at the online Ordinary General Meeting 2021**

**Effective from 1 January 2022**

## Validation reports

See References. Copies are available by e-mail from the ISTA Secretariat at [ista.office@ista.ch](mailto: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)  
Zürichstr. 50, CH-8303 Bassersdorf, Switzerland

© 2022 International Seed Testing Association (ISTA)

Online ISSN 2310-3655

All rights reserved. No part of this publication may be reproduced, stored in any retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without prior permission in writing from ISTA.

## 7-001a: Detection of *Alternaria dauci* in *Daucus carota* (carrot) seed by blotter method

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

**Pathogen(s):** *Alternaria dauci* (J.G.Kühn) J.J.Groves & Skolko, syn. *A. porri* f.sp. *dauci* (J.G.Kühn) Neerg., syn. *A. carotae* (Ellis & Langlois) Stevenson & Wellman

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

**Authors:** Sheppard, J.W., Cockerell, V. & Roberts, S.J.

### 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, 2016-01-01: New Figures 1a, 1b

Version 1.4, 2017-01-01: Reporting results revised

Version 1.5, 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. 4 and revised in 1987 (Gambogi, 1987). It was incorporated into the *Annexe to Chapter 7: Seed Health Testing Methods* as method 7-001 (Sheppard & Cockerell, 2002). It has been renumbered (7-001a) and slightly modified following studies conducted 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 radicina* using the same method (see method 7-002a).

### 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  $20 \pm 2$  °C, equipped with timer-controlled near-ultraviolet lights (NUV, peak at 360 nm, e.g. colour number 08, Philips; BLB, Sylvania)

**Freezer:** operating at  $-20 \pm 2$  °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

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 NUV light (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 magnification for identification of conidia. Conidiophores are simple or slightly branched (Fig. 1), arising singly or in small groups from the surface of the seed or on aerial mycelium. Conidia are usually solitary, obclavate, up to 450 µm long (including beak), pale olivaceous brown at first, becoming brown with age, with a long pale beak up to 3 times the length of the body (Ellis, 1971). Groups of sunken conidia are sometimes visible by the emerging clusters of their bright long beaks (Fig. 1c). 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

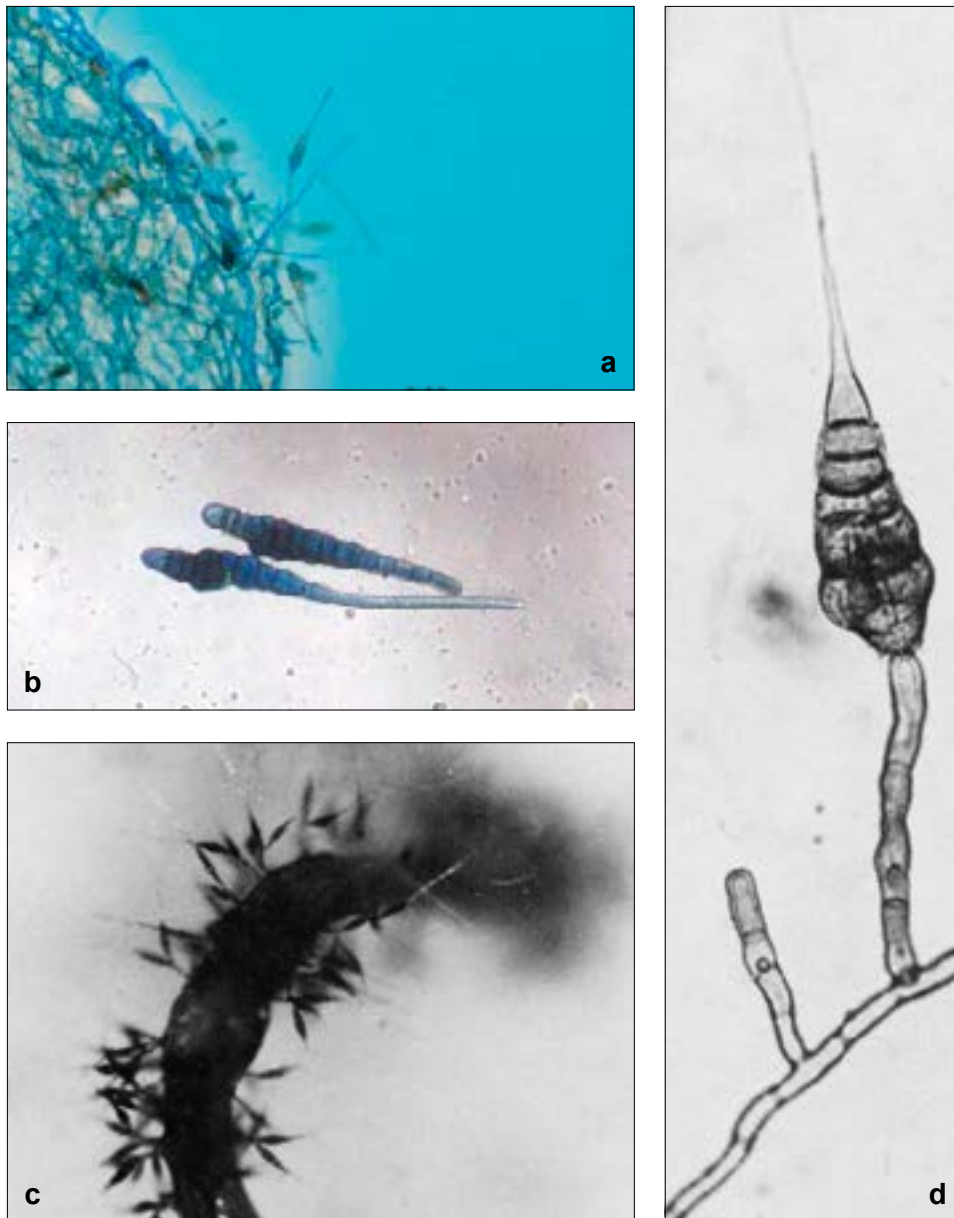
This test should only be performed by persons who have been trained in fungal identification or under the direct supervision of someone who has.

## 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 with not more than 10 seeds per 90 mm Petri dish (Step 3).
- Samples may be difficult to examine due to the growth of contaminants, especially *Alternaria tenuis*, and/or *A. radicina*. Experience and great care is required for the detection of all occurrences (ISTA 1984) (Step 7).
- Supplementary examination may be required 13–14 d after plating of seeds to allow hampered or deeper inoculum to exceed contaminants by its fructification (Hewett, 1964) (Step 7).

## References

- Ellis, M. B. (1971). *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England. 609 pp.
- Gambogi, P. (1987). *International Seed Testing Association, Handbook of Seed Health Testing*, Working Sheet No. 4 (2nd Ed.): *Alternaria dauci* on *Daucus carota*. International Seed Testing Association, Zurich, Switzerland.
- Hewett, P. D. (1964). Testing carrot seed infected with *Alternaria porri* f. sp. *dauci*. *Proceedings of the International Seed Testing Association*, **29**, 463–471.
- International Seed Testing Association (1984). *Report of the 18th International Seminar on Seed Pathology, 9–16 July, 1984*, Washington State University, Puyallup, U.S.A.
- Miles, S. R. (1963). Handbook of tolerances and of measures of precision for seed testing. *Proceedings of the International Seed Testing Association*, **28** (3), 525–686.
- Roberts, S. J., Phelps, K., Taylor, J. D. & Ridout, M. S. (1993). Design and interpretation of seed health assays. In *Proceedings of the First ISTA Plant Disease Committee Symposium on Seed Health Testing, Ottawa, Canada* (ed. J. W. Sheppard), pp. 115–125, Agriculture Canada, Ottawa, Canada.
- Sheppard, J. W. & Cockerell, V. (2002). Detection of *Alternaria dauci* on *Daucus carota* (Carrot). *International Rules for Seed Testing. Annexe to Chapter 7: Seed Health Testing Methods*. 7-001.
- Tempe, J. de (1968). The quantitative effect of light and moisture on carrot seed infections in blotter medium. *Proceedings of the International Seed Testing Association*, **33**, 547–553.



**Figure 1.** **a** Conidia of *Alternaria dauci* on simple or slightly branched conidiophores. **b** Single conidia. **c** Conidia of *A. dauci* on simple or slightly branched conidiophores borne on a single rootlet initial.  $\times 80$ . **d** Conidium and simple conidiophores.  $\times 350$ .

### Validation references

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

