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

7-001b: Malt agar method for the detection of *Alternaria dauci* on *Daucus carota*

Published by: International Seed Testing Association (ISTA), Bassersdorf, Switzerland
2003

Crop: *Daucus carota* (carrot)

Pathogen: *Alternaria dauci*

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Sponsored by: International Seed Health Initiative - Vegetables

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. 4 and revised in 1987 (Gambogi, 1987). It has been slightly modified following studies conducted 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. 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 radicina* using the same method (see method 7-002b).

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, especially during preparation of media, autoclaving, and weighing out of ingredients. 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.
Malt Agar plates	- See page 6. 9.0 cm plates (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

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

2. 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.

3. Sub-culture a reference culture to a malt agar plate at the same time seeds are plated and incubate with the test plates.

4. Examine plates visually, and under a stereoscopic microscope at x30 magnification, for fungal growth. Use a magnification of x50 – x80 for identification of conidia. Colonies of *Alternaria dauci* are brown or dark brown with olive-grey aerial mycelium and produce a brown diffusible pigment in the medium. 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. 1, bottom left). Record the number of infected seeds in each plate (**CCP**).

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]

Contaminants may greatly compete with the pathogen on the non-selective medium making detection laborious and difficult (Step 4).

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).

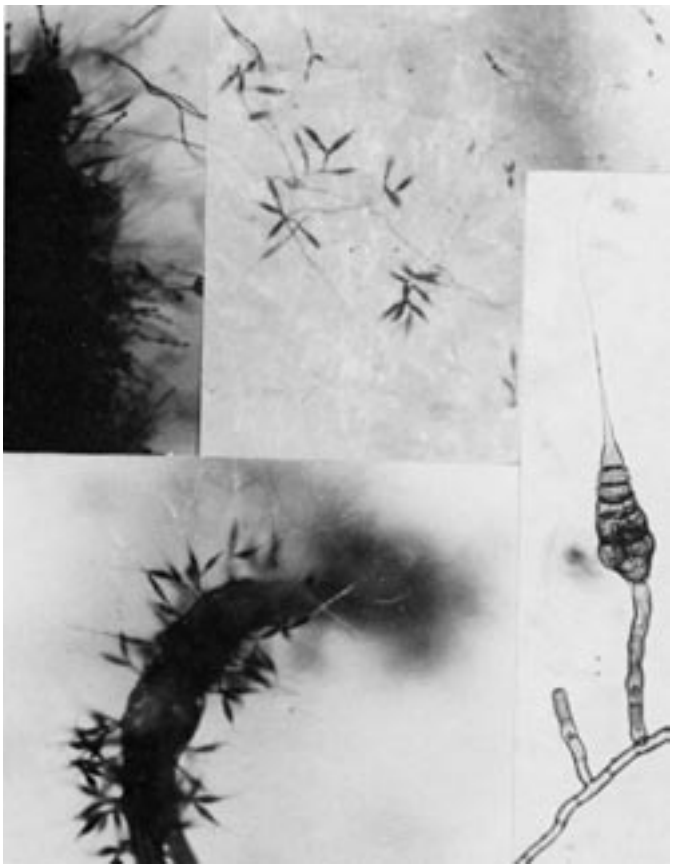

Fig. 1. Top: single conidia of *Alternaria dauci* and chains of conidia of the saprophyte *A. tenuis* on seed surface (left) and simple or slightly branched conidiophores with conidia of *A. dauci* developing from creeping hyphae (right), x80 magnification. Bottom: conidia of *A. dauci* on simple or slightly branched conidiophores borne on a single rootlet initial at x80 magnification (left); conidium and simple conidiophores at x350 magnification (right).

Preparation of Malt Agar

Compound	g/l	g/500ml
Malt Agar (**CCP**)	as specified by manufacturer	
De-ionised/distilled Water	1000 ml	500 ml

Preparation

1. Weigh out ingredients into a suitable autoclavable container.
2. Add 1000 (or 500) ml of distilled water.
3. Steam to dissolve.
4. Autoclave at 15 PSI and 121°C for 15 min.
5. Allow agar to cool to approx. 50°C.
6. Pour 15-22 ml of molten agar into 9.0 cm Petri plates 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.

References

Ellis, M.B. (1971) *Dematiaceous Hyphomycetes*. Commonwealth Mycological Institute, Kew, Surrey, England. 609 pp.

Gambogi, P. (1987) *International Seed Testing Association, Handbook on Seed Health Testing, Working Sheet No 4 (2nd Ed.)*: Alternaria dauci *on* Daucus carota. International Seed Testing Association, Zurich, Switzerland.

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Roberts, S.J., Phelps, K., Taylor, J.D. and Ridout, M.S. (1993) Design and interpretation of seed health assays. In: Sheppard, J.W., (Ed.) *Proceedings of the First ISTA Plant Disease Committee Symposium on Seed Health Testing, Ottawa, Canada.* pp. 115-125. Agriculture Canada, Ottawa, Canada.

Van Bilsen, J.G.P.M. (2003) Report of a comparative test on *Alternaria dauci* and *Alternaria radicina* on carrot seed. *ISTA Method Validation Reports* (submitted).