Method for the Detection of *Alternaria radicina* on Carrot seed using the ARSA Method

Crop: Carrot (*Daucus carota*)
Pathogen: *Alternaria radicina (A. radicina*) Meier, Drechsler and Eddy

Revision history: Version 4, July 2017

Sample and sub-sample size

The test is done on a minimum sample size of 200 seeds and a maximum sub-sample size of 100 seeds.

Principle

- This is an agar plating method using the Alternaria Radicina Selective Agar, ARSA (5), which allows for the detection of viable colonies of *A. radicina*.
- The blotter and malt agar methods for simultaneously detecting *Alternaria radicina* and *Alternaria dauci* are described in the Annex to Chapter 7 (Table 7.4.3.A.1 ISTA Official Seed Health Testing Methods) of the ISTA Rules (3).

Restrictions on Use

- This test method is suitable for untreated seed.
- This test method is suitable for seed that has been treated using physical (hot water) or chemical (chlorine) processes with the aim of disinfestation/disinfection, provided that any residue, if present, does not influence the assay. It is the responsibility of the user to check for such antagonism and/or inhibition by analysis, sample spiking, or experimental comparisons.
- The ability to detect *A. radicina* on plates can be influenced by the presence of other fungi. This can influence the reliability of the test.
- This test method has not been validated for seed treated with protective chemicals or biological substances. If a user chooses to test treated seed using this method, it is the responsibility of the user to determine empirically (through analysis, sample spiking, or experimental comparisons) whether the protective chemicals or biological substances have an effect on the method results.

Validation

In a comparative test organized by ISHI-Veg, the ARSA method was compared with the blotter and the malt agar methods (1). As there were no significant differences in the results, it was concluded that the methods are equivalent.

Method description

1. Incubation

1.1. Plate 50 seeds per 9 cm petri plate with ARSA medium using a vacuum head or sprinkle the seeds on the agar surface. Separate the seeds that are clustered with a sterile probe.

Note: The method was reviewed recently and found to be fit for purpose.

1.2. Prepare a spore suspension of low concentration from a known *A. radicina* reference culture in sterile de-ionized water and spread across the surface of an ARSA plate.

- The spore concentration should be low enough to allow spore germination and individual colony formation. To achieve this low spore concentration a dilution series of the original spore suspension should be made and plated.

1.3. Incubate the petri plates at 28 °C for 14 days in the dark.

2. Identification on ARSA medium

2.1. Check the morphology of the *A. radicina* reference culture (2) on the ARSA medium. Examine the seeds visually for fungal growth of *A. radicina* (Fig. 1).

- This is best accomplished by viewing the undersurface of the plates. The pathogen produces black irregularly branching hyphae radiating into the medium. Conidia are not generally produced.

Medium

- Use de-ionized water.
- Autoclave medium at 121°C, 115 psi for 15 min.
- The formulation of the fungicides Mertect 340-F and Bayleton 50 WP is critical. Use the original fungicides. If other formulations of the active ingredient are used, a check on the quality should be made using a reference seed lot of a known infection level.
- Use ARSA within 3 weeks to ensure activity of antibiotics.

ARSA (Alternaria Radicina Selective Agar) per liter (2)

Prepare solutions A and B separately in 500 ml water and mix them after they have cooled down to 50°C. Add solution C just prior to pouring.

Solution A

K2HPO4	1.0 g
KNO3	1.0 g
KCl	0.5 g
MgSO4.7H2O	0.5 g
Bacto agar	15.0 g

Solution B

Sodium Polypectate[4]	0.5 g

Solution C

Streptomycine sulphate stock solution 10 mg/ml[1]	5 ml
Chlortetracycline HCL stock solution 10 mg/ml[1]	5 ml
Mertect 340-F stock solution 0.25 ml/ml[2]	1 ml
Bayleton 50 WP stock solution 40 mg/ml	5 ml
2,4-Dichlorophenoxy acetic acid (2,4-D) stock solution 2 mg/ml[3]	5 ml

[1] The streptomycine sulphate and chlortetracycline HCl stock solutions can be combined. Dissolve 1 gram each of streptomycine sulphate and chlortetracycline HCl in 100 ml sterile water and use 5 ml/L

[2] Make the Mertect stock solution by suspending 25 ml Mertect in 75 ml sterile water and use 1 ml/L. Make sure the viscous Mertect is completely suspended

[3] Make the 2,4-D stock solution by dissolving 200 mg of 2,4-D in 5 ml of hot ethanol and add this slowly to 100 ml sterile water

[4] The source of the sodium polypectate (polygalacturonic acid) can influence the results of ARSA. Good results were achieved with Sigma P-3850. Whenever another brand of sodium polypectate is used, a check using a reference seed lot of a known infection level should be made on the quality.

References

1. Van Bilsen, J.G.P.M. (2003) Report of a compararive test on *Alternaria dauci* and *Alternaria radicina* on carrot seed. ISHI-Veg Research Report 1-2003-Ad+Ar. Nyon, Switzerland: International Seed Federation.
2. Grogan, R.G. and W.C. Snyder (1952) *Alternaria radicina*. CMI Descriptions of Pathogenic Fungi and Bacteria No 346. The Commonwealth Mycologist Institute, Ferry lane, Kew, England: The Eastern Press Ltd. London.
3. See the ISTA website: http://www.seedtest.org/en/content---1--1132.html
4. Jukema, N.J. and P.L. de Wolf (2005) Optimalisering zaadproductie van peen: Een perspectievenstudie. Wageningen, The Netherlands: Praktijkonderzoek Plant & Omgeving B.V.
5. Pryor, B.M., Davis R.M. and R.L. Gilbertson. Detection and eradication of *Alternaria radicina* on Carrot seeds. *Plant Disease* **78**: 452-456.

Fig. 1. *Alternaria radicina* fungal growth