

<b>VERSION:</b> 1.0	<b>DATE:</b> 2002
<b>PATHOGEN:</b> <i>Alternaria dauci</i> (syns: <i>A. brassicae</i> var. <i>dauci</i> ; <i>A. carotae</i> )	
<b>HOST:</b> carrot ( <i>Daucus carotae</i> )	
<b>COMMON NAME:</b> leaf blight of carrot	
<b>METHOD:</b> Rb 2.2 Direct plating/Blotter Method (Sunseeds)	
<b>METHOD CLASS:</b> STANDARD (A)	
<b>SAMPLE:</b> 3,000 seeds	

**PROCEDURE:**

**Direct Plating**

1. Six 9" x 12" plastic boxes with covers are surface sterilized with 95% denatured alcohol.
2. Germination blotters are cut to fit and placed in the bottom of each box.
3. The blotters are moistened with sterile deionized water.
4. 500 seeds are sprinkled over the blotters in each box.
5. The boxes are incubated at 22°C for 2 days under UV lights with a 12 hr dark period.
6. The boxes are then placed in a -20°C freezer overnight.
7. The boxes are then returned to 22°C for 10-14 days under UV lights with a 12 hr dark period.

**Pathogen Identification**

1. The boxes are read by scanning them with a binocular stereoscope at 7x power.
2. Infested seed may show a soft brown rot and are covered with a fine mass of mycelium with large sword shaped conidia protruding from the mass.

3. Transfer suspect spores to a microscope slide and confirm spore morphology at 100x power.
4. Remove spores with a sterile needle to plates of Carrot Extract Media for a further pathogenicity test.
5. No pathogenicity test is normally conducted, but if desired, the following inoculation can be conducted.

### **Pathogenicity Test**

1. Grow seedlings until the 4-5 true leaf stage.
2. Spray seedlings with conidial suspension ( $1.0 \times 10^4$ ) until runoff.
3. Incubate in plastic bags for 48 hr at 25-30°C. Place in greenhouse at 22-28°C, spray with water and cover seedlings each night with plastic.
4. Symptoms can take up to 14 days to appear and consist of small dark brown to black lesions with a yellow border that first appears on leaf margins. Lesions increase in size and may encompass the entire leaflets giving the seedlings a scorched appearance.
5. Complete Koch's postulates.