

## **Producing Apothecia and Collecting Ascospores of *Sclerotinia sclerotiorum***

(Based on procedures published by Russo et al. 1982. Experimental Mycology. 6:259-267, and by Steadman and Cook. 1974. Plant Disease Rep. 58:190)

### **Producing Sclerotia**

1. Peel and dice potatoes. Place in an autoclavable container, like 2000 mL Erlenmeyer, beaker or glass jar. Cover bottom, about two layers of cubes. Do not put too many potatoes in one vessel, 2-3 potatoes per container.
2. Autoclave for three consecutive days, 40 min ea.
3. Inoculate with actively growing *Sclerotinia* culture: several pieces of agar in each.
4. Incubate at room temperature for 1-2 months. No light requirement.
5. After sclerotia are mature (=dry) and the substrate starts liquefying, wash sclerotia off with repeated changes in running water. Be gentle, avoid breaking sclerotia.
6. Dry clean sclerotia in hood and store desiccated at +4°C.

### **Inducing Apothecia**

1. Cover bottom of glass petri dishes (10 cm) thinly with vermiculite and saturate that with water. Autoclave. Fifty ml sclerotia fill about nine petri plates. Also autoclave 2 L deionized water, paper towels and cheesecloth.
2. Surface sterilize sclerotia: Add 46 ml ultrableach to final volume of 500ml water; treat sclerotia for 5 min. Rinse 3x with sterile water, 5 min each. Dry sclerotia in the hood on sterile paper towels for a few hours.
3. Check moisture in the vermiculite dishes and place sclerotia on surface, not buried in, close together. Parafilm plates.
4. Place plates into -20°C for 24 hours and then room temperature for 24 h. Repeat two more times. After third time in -20°C, place in a +15°C incubator with constant lights, fluorescent, cool white.
5. Mature apothecia usually develop in 4-5 weeks.

### **Collecting Ascospores**

Device: Plastic Powder funnel (Nalgene PF 100), bottom part of plastic Büchner funnel (55 mmØ supporting filter grid from a sterilizing unit to fit a 47 mm filter (0.45 µm).

Funnel assembly is attached to a water aspirator to produce vacuum to collect spores.

Plates are kept in the 15°C incubator under lights. Before harvesting bring them close to the vacuum source and spread them on the counter. Let them stay several hours without disturbing. Lift top and quickly cover plate with the funnel, vacuum on. Spore release should be easily visible.