

### Conidial Inoculum Prep: Liquid Culture Method

1. Begin fungal source plates 1-2 weeks prior to starting liquid culture (AT LEAST 3-4 weeks prior to making GH or field inoculations)
  - a. Put a few silica gel particles on APDA plates.
  - b. Grow in the incubator.
    - i. Black-light for 24hrs/day
    - ii. Day Temp=24.7C, 12 hours light
    - iii. Night Temp=22C, 12 hours dark
2. One day prior to starting liquid culture, make Mung Bean Broth.
3. Add APDA plugs to the broth in good proportion (1 plate per 1 L broth).
4. Cover the flask opening with aluminum foil.
5. Shake flasks at 200 rpm, 23.4°C in the dark for 1 week.
6. Use cheesecloth and a funnel to filter out the mycelium.
7. Measure spore concentration with an hemacytometer.
8. Spore suspension can be stored at 4°C for up to 4 months and at -20°C for up to 6 months.

### Conidial Inoculum Prep: Culture Plate Method

*Protocol provided by Dr. Dill-Macky*

1. Begin fungal source plates 1-2 weeks prior to starting liquid culture (AT LEAST 3-4 weeks prior to making GH or field inoculations)
  - a. Put a few silica gel particles on APDA plates.
  - b. Grow in the incubator.
    - i. Black-light for 24hrs/day
    - ii. Day Temp=24.7C, 12 hours light
    - iii. Night Temp=22C, 12 hours dark
2. Inoculate several Mung Bean Agar plates with a mycelial plug or with 1 mL from stock, sterile conidia.
3. Allow to grow 10-14 days in the same conditions as 1b.
4. Wash plates with 20 mL sterile, distilled H<sub>2</sub>O.
5. Fix a Buchner funnel to a large Erlenmeyer (2-6 L) flask and cover the holes with cheesecloth. Filter the mycelium from the wash solution.
6. Measure spore concentration with an hemacytometer.
7. Inoculum can be stored in 1 L plastic bottles for future use. It is preferred to store at a standard concentration (ie. 800,000 spores/mL). It can be stored at 4°C for up to 4 months and at -20°C for up to 6 months.