Spore extraction: The sucrose method

* Allow yourself four to five hours to complete this procedure.

* This procedure allows for small soil sample sizes

1. In a 15mL centrifuge tube, place 5 g of soil (mark sure not to exceed the 7 ml mark of the centrifuge tube). The soil can either be dried soil ran through a 2mm sieve or you can wet sieve the soil (look at protocol) prior to using this method. The latter method works best for desert and Mediterranean type soils.

2. Fill tube with distilled water to the 14mL mark. Shake tube vigorously and place on its side for 15 minutes. Check that the water is infiltrating the soil-if it is not moistening all the way through, there are likely to be air bubbles preventing water infiltration. Use a spatula (if needs be) to gently break the air pockets. The water should infiltrate to the soil at the bottom of the tube.

3. Centrifuge for 10 minutes at 2500 rpm.

4. Poor off the water with floating organic debris.

5. Fill the tubes with 2M sucrose-calgon solution to the 14 mL mark. Once again shake it vigorously and lay them on their sides for 10 minutes. (the sucrose creates a density gradient while the calgon gives the spores a charge) CAN WAIT IN THE REFRIGERATOR IF NEEDS BE…..

6. Centrifuge tubes for 20 minutes at 2500 rpm.

7. Place a filter paper into the filter funnel. Put a membrane (grid membrane) on top. Moisten with water, and turn on vacuum line.

8. Gently pour the sugar-calgon solution containing the spores over the membrane.

9. Once the solution has filtered, gently rinse the membrane with water to remove excess sugar.

10. When filtered, remove filter paper/membrane and place in a small Petri dish.

11. Store samples in the refrigerator. (For longer term storage put a little ethanol in the Petri dish with the spores. When you want to observe the spores simply filter away the ethanol).

For Highly organic Soils (or otherwise problematic soil samples)

1. Follow steps 1-6 as describe above.

2. This time pour the sucrose-calgon solution into a separatory funnel and let sit for 10 minutes.

3. Open stopcock to allow sucrose out slowly. It should take at least 30 minutes to empty each separatory funnel. At this time, mycorrhizal spores will be attached to the glass walls of the separatory funnel.

4. Run water down the sides of the separatory funnels. Place your hand over the top of the separatory funnel and shake (this will suspend the spores in the aqueous solution for vacuum filtration).

Literature

References: Allen et al. 1979. Mycologia 71:666-669 Ianson and Allen. 1986. Mycologia 78(2):164-168

Preparation of Sucrose-calgon Solution (2M sucrose, 10% calgon)

1. For 1 L solution, weigh out 685.0 g sucrose

2. Measure 450 mL water into a large flask and heat

3. Add a stir bar and stir rapidly (but not top speed), then slowly add sucrose.
   NOTE: *watch solution carefully, it will tend to boil over and burn on the hot-plate!*

4. Continue heating and stirring until solution in clear. Turn of heat.

5. Add 100 g Calgon bath salts to sucrose solution and continue stirring until Calgon is just dissolved.

6. Add 550 mL water to the solution and continue stirring until thoroughly mixed.

7. Cool mixture and refrigerate in a tightly-capped plastic bottle. Label the bottle.