*Botrytis cinerea* spore collection protocol

1. Grow *Botrytis* on Potato Dextrose Agar plates for 3 days.
2. Perform the following steps in laminar flow hood:
   1. Ethanol-sterilize your gloves each time your hands re-enter the hood.
   2. Do not place any items directly against the filter at the back. Do not cough, sneeze, or make rapid movements in the hood. As much as possible, do not block air flow from the back of the hood to your work space. Keep in mind, the front 6” of the hood have turbulent air flow as you work and are not sterile. Perform sterile work within the back 2/3 of the hood.
   3. Ethanol-sterilize all items brought into the hood in the front 1/3, before opening packages.
   4. Prepare small (5cm) petri dishes filled with 1-2 peach slices and a few ml of peach juice.
   5. Transfer 1-2 plugs (0.5cm2) of leading edge of *Botrytis* mycelia to peaches.
3. Grow *Botrytis* on peach plates at room temperature, ~12h light, for 1-2 weeks until mature spores are formed.
4. Prepare autoclave-sterilized materials:
   1. dI H2O (500 mL or more)
   2. beaker of glass wool
   3. beaker of 1.5 mL microcentrifuge tubes
   4. p1000 tips
   5. p200 tips
5. Perform the following steps in laminar flow hood, sterilizing between each isolate:
   1. Prepare 12ml syringes, one per isolate, by placing a small glass-wool filter into the tip of each syringe
   2. Flood peach plate with 3-5 ml dI H2O in 10 mL glass pipette
   3. If necessary to break surface tension, gently scrape surface of spores with flame-sterilized glass rod
   4. Using 10 mL glass pipette, pass the spore suspension through a sterile syringe with the glass wool filter, into sterile 15 mL centrifuge tube.
   5. Discard syringe and pipette.
   6. Immediately place centrifuge tubes on ice.
6. Centrifuge the suspensions in swinging bucket rotors at 1000 rpm for 10 minutes at 4°C.
7. Perform the following steps in the laminar flow hood, one isolate at a time:
   1. Gently decant and discard the supernatant
   2. Resuspend the spore pellet in 1 mL of 0.5x filter-sterilized grape juice.
   3. Place solution immediately on ice.
   4. Subsample 100 µL for quantification on hemacytometer.
8. Estimate spore concentration of 100μL subsamples using a compound microscope and a hemacytometer.
   1. Vortex spore solution on speed 5 for ~ 10 seconds.
   2. Pipette 10µL of spore subsample onto hemacytometer under coverslip.
   3. Count spores within all tiny squares (0.25 nL in 4 x 4 block) per larger (4 nL in 5 x 5 block) square.
   4. Count spores within only the 5 randomly selected larger (4 nL) squares:

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1. Calculate average concentration per larger (4nL) square using excel spreadsheet.
2. Rinse hemacytometer and coverslip with dI H2O between spore samples.
3. Calculate serial dilutions for each spore suspension using excel spreadsheet.
4. In the hood, pipette-mix and dilute spore suspension to produce 2, 1 mL suspensions of 10 spores/ μL using 0.5x grape juice.
5. Place all spore suspensions on ice until inoculation.
6. Infect leaves using 4 µL of solution.