Greenhouse Inoculation – Macroconidia Production

Plant Preparation

Plan ahead by allowing 9 weeks for vernalization and 4-6 weeks until flowering. You don't want to start inoculating during vacation or a meeting out of town. Plant three reps of two seedlings per 5 inch pot.

Inoculum Preparation

Choose 10 of the most aggressive isolates for inoculum production. Culture each isolate in two flasks of CMC broth. Begin a batch of inoculum one month before expected anthesis in the greenhouse. This way if you don't produce enough spores in the first batch, you have time to make a second batch. Quantify CMC cultures using a hemacytometer and combine equal macroconidial concentrations from each isolate into a large flask. This will be your bulk inoculum. Make sure you have enough conidia to inoculate all the plants. The inoculum can be stored for 6-8 weeks in the refrigerator.

CarboxyMethyl-Cellulose (CMC) Medium for Production of Macroconidia of *Fusarium graminearum*

CarboxyMethyl-Cellulose (Sigma C-4888)	15.0 grams
NH_4NO_3	1.0 gram
KH_2PO_4 monobasic	1.0 gram
$MgSO_4 \cdot 7H_2O$	0.5 gram
Yeast extract	1.0 gram
Distilled or de-ionized water	fill to 1.0 liter

From: Plant Pathological Methods: Fungi and Bacteria, John Tuite
Burgess Publishing Company, 1969, pg 25.

Add all ingredients to gently boiling water, mixing vigorously with a magnetic stirrer. Cover to control evaporation and continue heating and stirring until all lumps of CarboxyMethyl-Cellulose (CMC) dissolve. CMC is barely soluble in water and will take up to 3 hours to dissolve without a hard boil. The Sigma-Aldrich formulation of CMC (C-4888) is the only known CMC product, which will dissolve in water alone.

Pour about 70 ml of liquid CMC into 250 ml flasks, plug with cotton, cover with tinfoil, and autoclave at 250° F and 18 psi for 25 minutes. It is important to only fill the flasks about 25 – 30% full, in order to allow aeration on the shaker table.

Inoculate each flask in a laminar flow hood with several plugs (approximately 10mm x 10mm) cut from a purified *F. graminearum* isolate grown on agar (PDA works well, even when dried out). Place the flasks on a shaker table, and swirl gently at 150 rpm for 3 to 4 days at room temperature under fluorescent lights. Count conidia with a hemacytometer to prepare the inoculum. Expect most conidia counts to be in the range of $0.5 – 4.0 \times 10^6$ spores per ml, depending on the isolate. Expect an occasional isolate to mostly produce mycelia, not conidia. CMC cultures can be stored 1½ months in a 40 F / 4°C refrigerator.

Inoculation

Prepare inoculum (50,000 spores/ml) by diluting a sample of the stock inoculum with sterilized water. *Note: we prepare a separate working concentration of inoculum to keep our stock inoculum from being contaminated.* Use a hemacytometer to calculate spore concentration. Select two good heads close in maturity in each pot for inoculation. Heads at late boot to just headed are best for inoculation. Keep in mind, it is better to inoculate a day after anthesis than a day before. Tag heads with tape. Different colored tape can be used for each day of the week. Inject inoculum (20 µl =1000 spores) into one primary floret in the center of the spike with the repeat syringe. Avoid an inoculation technique that causes inoculum to drip out of the floret or down the spike, or that damages any floral structures. Move plants into the mist chamber for three days with optimal growing conditions. Maintain the temperature between 75 and 80°F with 100% relative humidity. Lower temperatures delay symptoms and may require plants to spend a fourth day in the chamber. DO NOT let the temperature in the chamber reach above mid-90's°F. This will kill the fungus. If the mist chambers are too hot, raise the sides during the day. Program the timer to mist for 5 seconds every 10 minutes, continuously. Too much moisture will cause mycelia to grow outside the spike which does not imitate field conditions. Infection expression (incidence) should be 99%.

Rating

Rating for scab is done by counting the number of infected spikelets. Label one of the heads so you differentiate it for the second rating. Rating is done exactly 15 and 21 days after inoculation. One day will make a difference in scab growth, and consequently how you rate the plants. On day 21, count the total number of spikelets in the infected spike to calculate FHB severity.