

## **Field Inoculation**

We rely on ascospores to infect our FHB nursery. In the fall we spread corn stover, as a source of *Gibberella zeae*. In the spring we prepare at least 200 lbs/acre of corn inoculum. We spray the corn stover with macroconidia and scatter corn spawn at four weeks and two weeks before anthesis. We then irrigate just enough to keep the ground wet for good ascospore production. We start the misting schedule for one hour at 2am, 8am, 2pm, and 8pm one week before anthesis.

## **Scabby Seed Production**

We use large, clear polypropylene (autoclave) bags to produce infected corn spawn. We decided that the use of milk jugs or flasks for inoculum production was too cumbersome for producing large amounts of inoculum. We also switched from wheat spawn to corn spawn, because the wheat seeds tend to get buried in the mud.

1. Soak the corn seed in a large tub overnight.
2. Drain as much excess water as possible through cheese cloth.
3. Add 4 liters of wet grain to each bag. (more than this, and it might not get sterilized)
4. Secure a very large foam plug in the opening of the bag with rubber bands.
5. Autoclave bags for 45 minutes.
6. Let the seed cool overnight
7. Autoclave again, 45 min. (This will kill off basidiospores which germinate under high heat and humidity)
8. Let the seed cool overnight
9. Inoculate each bag with CMC culture (explained below), each bag gets its own isolate. *An alternative to CMC culture is to use chopped up PDA cultures.*
10. Do not shake the bags for two days, then shake every day thereafter.
11. Incubate cultures on the benchtop for 2-3 weeks, until white mycelia cover most of the kernels.
12. Dry the infected kernels and store at 4°C.

## **CarboxyMethyl-Cellulose Medium for Production of Macroconidia of *F. graminearum***

CarboxyMethyl-Cellulose (Sigma C-4888)	15.0 grams
NH <sub>4</sub> NO <sub>3</sub>	1.0 gram
KH <sub>2</sub> PO <sub>4</sub> monobasic	1.0 gram
MgSO <sub>4</sub> ·7H <sub>2</sub> O	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 for 20 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 like shoe leather). Place the flasks on a shaker table, and swirl gently at 150 rpm for 3 to 4 days at room temperature under fluorescent lights. Pour macroconidia onto sterilized corn, or stored for up to 8 weeks in a 40 F / 4°C refrigerator.