Corn Inoculum Preparation

This procedure is modified from a protocol used by Dr. Jeannie Gilbert's lab in Winnepeg, Manitoba, Canada.

1. **Calculate corn inoculum need based on a rate of 20-40 g/m^2 (we use 40 g/m^2 in the non-irrigated nurseries and 30 g/m^2 in the irrigated nursery).**

2. **Pour 8-9 kg corn into 7 gallon Rubbermaid tubs. Add tap water to ~1 in. above the corn level and allow imbibing for 16 hours. (I do this around 4:30 PM.)**

3. **Pour off water and cover the tub with aluminum foil. Put the lid over the foil and autoclave for 90 minutes at 10 to 15 atmospheres. (I usually do this around 8:30 AM.)**

4. **Remove the corn from the autoclave and let cool.**

5. **Using aseptic techniques, inoculate the corn with 3-5 APDA plates colonized with *Fusarium graminearum*, using plates no more than 2 weeks old.**
   a. **Cut the APDA into small pieces with a scalpel.**
   b. **Empty the plates onto the autoclaved corn**
   c. **Pour 150 mL sterile water with 0.2 g streptomycin sulfate over the corn.**
   d. **Mix the inoculum throughout the corn using a sterile implement (I use a sterile, disposable serological pipet to stir it.)**
   e. **Re-cover the corn with the aluminum and lid.**

6. **After inoculation, let the corn incubate for no less than 2 and no more than 3 weeks. Don't seal tightly seal the tubs or stack them because *F. graminearum* needs an aerobic environment to grow. It grows great at room temperature and can tolerate low (10°C) overnight temperatures if the daytime temperature gets above 20°C.**

7. **Following incubation, the corn should have a dense, thick layer of white/pink/yellow mycelium. It should be purely *F. graminearum*, but if there is limited secondary growth, you can scoop it out at this point.**

8. **Cover an area with plastic and surface disinfest with 95% EtOH. Spread the corn over top to a thin layer (1-3 kernels deep), breaking up clumps.**

9. **Allow the corn to dry for 3-8 days, breaking up any clumps that form. Using fans and a dehumidifier makes drying faster. After it is dry, put it in mesh "grapefruit" bags and store in a cold room or freezer until needed.**

10. **Spread in the field three weeks prior to heading with hands, <u>wearing a mask and gloves</u>.**