Isolating *F. graminearum* from scabby seeds

Potato Dextrose Agar – 1 L

One liter of PDA is enough for approximately thirty 100 x 15mm Petri dishes. Pour 1 liter of water into a large Erlenmeyer flask and add 39 g of PDA. Stir until most of PDA is dissolved. Plug flask with a cotton stopper, cover with aluminum foil, and autoclave at 121°C for 45 minutes, liquid cycle. Let the flask cool slowly to 51°C in an incubator. Pour agar into plates under laminar flow hood. You should wait two days before using the PDA to see if any contaminants have grown on the plates.

Culturing *F. graminearum* isolates

Surface sterilize scabby wheat kernels in 10% bleach solution for 5 minutes. Then, rinse with sterilized water. Place three seeds on each PDA plate. Store plates at room temperature for 2 or 3 days until you see mycelia. Prompt culturing at this point is key to securing clean isolates. Transfer mycelia to a PDA + rifamycin plate (acidified PDA may also be used). Take only one plug from the mycelia tips surrounding each wheat kernel. Each plug is an isolate and should be labeled accordingly. A record should be kept of the source of each isolate. Try to culture 20 – 30 isolates with about 3 isolates from each source. Keep numbers of subcultures to a bare minimum (Dr. Don White, U of I plant pathologist) or isolates may lose their pathogenicity. Seal cultures with parafilm and store in the refrigerator.

Potato Dextrose Agar + Rifamycin – 1 L

As the sterilized PDA is cooling in the incubator, measure out 0.025 g of Rifamycin (antibiotic) and dissolve in 4 ml of EtOH. Aspirate rifamycin solution with a 10 ml syringe add a sterile 0.2 micron syringe filter and dispense solution directly into PDA. Gently swirl agar in large circles as not to create air bubbles. *Note: Rifamycin is light sensitive and must be stored in the dark.*

Acidified PDA

Reduce the pH of pre-mixed PDA to around 4.5 using 10% tartaric acid, then autoclave.

Transferring *F. graminearum* onto new PDA (sub-culturing)

1) Prepare 2 liters of PDA
2) Sterilize hood with 95% EtOH
3) Sterilize knife with 95% EtOH and flame
4) Cut a 5 mm plug from the hyphal tips of the isolate
5) Place plug upside down in the center of the new plate
6) Store FHB isolate at room temperature until it has colonized the whole plate
7) Check plate for contamination, use for making inoculum or wrap with parafilm, to prevent desiccation, and store in the refrigerator.
8) Isolates can remain viable for over a year when stored on PDA in the refrigerator.