Schmale Lab Protocol for Isolating *Gibberella zeae* from Wheat Heads

Dr. David G. Schmale III, Assistant Professor of Mycotoxicology and Fungal Plant Pathology
413 Latham Hall, Department of Plant Pathology, Physiology, and Weed Science
Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0390
Lab Phone: (540) 231-0733; Email: dschmale@vt.edu

Pathogen Isolation and Purification

Collect FHB samples around 2 to 4 weeks after flowering. Surface-disinfest individual wheat heads in 10% sodium hypochlorite for 1 min and rinse in sterile DI water. Cut heads into three equal portions (bottom, middle, and top) and plate on *Fusarium*-selective medium (**Figure 1**, see recipe for FSM). Incubate plates at room temperature for 3-5 days. Use a sterile wooden toothpick and transfer fungal colonies to ¼-strength potato dextrose agar (PDA). Let the cultures grow for 7-10 days at room temperature. Streak macroconidia onto plates of 2% water agar and single-spore onto ¼-strength PDA. Let the single-spored culture grow out for 7-10 days. For a simple single-sporing method, use a pair of forceps and stick a minuten pin (www.bioquip.com, 0.15mm stainless steel pins, #1207SA) into the end of a matchstick. Use a stereomicroscope and cut out a small square agar block that contains an individual macroconidium and transfer to ¼-strength PDA. For refrigerated storage, transfer a small agar plug from the single-spored culture onto sterile filter paper placed on ¼-strength PDA (the fungus grows over the top of the filter paper and all of the spores and mycelium are attached to the paper). Let the culture grow for 7-10 days. Remove paper and dry overnight in a new Petri dish in the laminar flow hood. Store the dry paper in a sterile coin envelope (inside plastic bags) in a standard fridge (NOT THE FREEZER!). For cryogenic storage, create a spore suspension by adding 20% glycerol to your single-spored culture. Scrape the mycelium and spores up into a spore suspension/mycelium goop with a sterile wooden coffee stirrer and transfer to cryogenic vials for storage at minus 80.

Figure 1. Isolation of *G. zeae* from wheat heads on *Fusarium*-selective medium.

Recipe for Modified Nash-Snyder *Fusarium*-Selective Medium (FSM)

1. Add **500 ml of DI H2O and a magnetic stir bar** to a 2L flask.
2. Add the following ingredients:

Agar	**15.0g**
Peptone	**15.0g**
KH2PO4	**1.0g**
MgSO4.7H20	**0.5g**
Terrachlor	**1.0g** (contains PCNB 75% w/w)

3. Add an additional **500 ml of DI H2O** (for a total of 1 liter). Place two pieces of foil over the top of the flask and seal well. Tape the foil to the flask. Autoclave the basal medium for 30 minutes. Remove from autoclave and cool to approximately 55C. Once cool add the following antibiotics in a **10ml sterile DI H2O** solution:

Streptomycin sulfate	**1.0g**
Neomycin sulfate	**0.35g**

Reference for FSM

Schmale, D.G., Leslie, J. F., Saleh, A. A., Shields, E. J., and Bergstrom, G. C. 2006. Genetic structure of atmospheric populations of *Gibberella zeae*. Phytopathology 96: 1021-1026.