Isolate Collection
Modified with input from Drs. Ruth Dill-Macky and Jeannie Gilbert.

1. Annually collect scabby heads and thresh seed for isolation. Try to get a mixture of 20-30 samples (heads or kernels) from all locations where we grow wheat.
2. One day prior to isolation, make APDA plates.
3. Put seeds in 1.5 mL eppendorf tubes to surface disinfest:
   a. Add 12.5% bleach solution to each tube and let sit 30 seconds.
   b. Pipette off the bleach and add sterile, distilled water to wash the seeds. Let sit 30 seconds, repeat for a total of 3 washes.
   c. Blot seeds on sterile filter paper to dry
4. Plate 2-3 seeds per APDA plate
5. Allow mycelium to grow for about 1 week in the growth chamber:
   a. Black-light for 24hrs/day
   b. Day Temp=24.7C, 12 hours light
   c. Night Temp=22C, 12 hours dark
6. After 1 week, use a wire loop to scrape off mycelia from the best seed for each sample and streak onto a new APDA plate. Allow to grow 1 week.
   a. Identify isolates to the species level.
   b. Once there is sufficient growth, transfer a plug to Mung Bean Agar.
7. Single spore each isolate and get spores into silica gel with minimal subculturing to maintain isolates in an inactive state.
8. Test virulence by GH inoculations.