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.**