FHB Type I Resistance in Wheat
Greenhouse Screening Protocol

By Jennifer Patton-Ozkurt, Rene Navarro, and Christina Cowger, USDA-ARS, Raleigh, NC.
Email: Jennifer.Patton-Ozkurt@ARS.USDA.GOV

In wheat, FHB Type I resistance is resistance to initial infection. FHB Type II resistance is resistance to spread within the head. The Type II phenotype could confound the assessment of Type I resistance – how many separate infections occurred in a set of four adjacent infected spikelets? We make some assumptions; see below.

Planting

1) Plant in greenhouse by mid-October for heading by late January. Plan vernalization accordingly.
2) Plan to inoculate only the primary head in each pot. Secondary heads will flower at different times, so it's impossible to incubate 1º and 2º heads consistently. So plant lots of reps (5-8 if possible), with each plant a rep.
3) We plant in Deepots, http://www.stuewe.com/products/deepots.html. One plant per pot.
4) Medium: 10 shovelfuls sterilized soil to 1 bag Metromix; add Osmocote at recommended rate and mix very well (no clumps).
5) Place piece of paper towel in bottom of each pot to retain medium. Fill about two-thirds of pot with soil/Metromix/Osmocote, then fill to top with soil/Metromix/no Osmocote to keep root growth in lower pot. Then transplant sprouted wheat seed. After planting, there should be one inch of head-space in pot to allow watering.
6) Plant only 3 rows of pots in each frame, to facilitate watering with a shower head watering attachment.
7) Temperature = 70 degrees F, or slightly higher for strong symptom development.
8) Starting about one week after planting, fertilize once a week with Miracle-Gro (1 teapoon/gallon water, or use Miracle-Gro hose attachment) until flowering, then discontinue fertilization.

1 to 2 weeks before heading

Mung Bean Broth for production of *Fusarium graminearum* macroconidia (courtesy of George Buechley, Purdue University)

1) Bring distilled water to boil, turn off heat and let sit for several minutes. Throw in 2-3 beans to be sure their addition does not trigger boiling, which leads to mycelium production.
2) Add green Mung beans, 40g of beans per liter of water. You can purchase a few lbs of Mung beans at an Asian grocery store. It's a small green-colored bean, sometimes spelled "moung" or "moong" bean.

3) Steep beans for tea 10-15 minutes.
4) Filter the tea through cheesecloth to remove beans, autoclave, and cool to 65º C.
5) Add *Fusarium* inoculum from PDA agar plate. We use a mixture of three isolates with good proven aggressiveness. To a 500-ml Mung bean shake in a 1 L flask, we usually add about a 0.5 X 0.5-inch chunk of PDA.
6) For 4 days at room temperature, shake at 100 to 200 strokes per minute or bubble solution vigorously with sterilized forced air.
7) Isolate spores via centrifugation at 4,000 rpm for 3 minutes – no faster, or spores will have reduced viability.
8) Count spores with hemocytometer to determine concentration.
9) Store at 4 º C.

At Flowering

Inoculation of Primary Head

1) Inoculate when anthers on primary head are bright yellow-green.
2) On the day of inoculation, prepare an inoculum solution with a concentration of 1 X 10^5 spores/mL in an atomizer. Place on ice.
3) Tag primary head with date of inoculation.
4) Physically isolate primary head and, with a sweeping motion, spray the head 4 times with atomizer.
5) Allow head to air-dry 30 minutes.
6) Place plant in misting chamber for 72 hrs incubation. Mist so that plants remain moist, which depends on the amount of sun. We usually mist for about 2 seconds every 5 minutes, but this will depend on your set-up.
7) Assess disease at 7 days after inoculation.[a] On each head, record the number of infected spikelets and total number of spikelets. We suggest that where there are adjacent infected spikelets, each spikelet be counted separately.[b]
8) Type I resistance level is number of infected spikelets in the head over total number of spikelets in the head.[c]

[a]We want to assess as soon as possible with Type I, to minimize the spread of infection from one spikelet to another. The first lesions appear at 4 days. A few well-isolated infections don't appear until 14 days. Seven days is a happy medium.

[b]At seven days, there ARE some adjacent infected spikelets. Are these separate infections or the result of spread from a single infection? Of course, a single infected spikelet might also have been infected twice. One has to make arbitrary assumptions. At seven days, we suggest assuming each infected spikelet is the result of a separate infection, even if it is adjacent to another infected spikelet, because there has been little time for spread through the head.

[c]Schroeder and Christensen (1963, Phytopathology, 53:831-838) report Type I resistance as number of infected spikelets per head. This saves the counting of total spikelets, but introduces an additional source of error, as cultivars vary considerably in average spikelet number.