

## Greenhouse Inoculations-Feb to March

1. Start conidial inoculum prep at least 2 weeks prior to making inoculations, either by liquid culture or using culture plates. See the file Conidial Inoculum Prep.doc
2. Transport spore suspension in a small cooler with ice packs - Heat and UV light will deteriorate the suspension
3. Be sure that the mist tent is operating when inoculations are initiated (just plug it in). The mist tents increase the humidity of the greenhouse and help ensure good infection for the fungus.
  - a. The timers are already programmed
    - i. On 20 seconds every 6 minutes-John made this
  - b. Also, the mist is only on 9am-3pm, so an appliance timer is used to accomplish this
4. Once a wheat head is elongated out of the boot and reaches anthesis it is ready to be inoculated.
  - a. Do NOT wait until anthers are extruded from floret, this may be too late
  - b. Just want receptive, fluffy flowers
5. Gather all plants to be inoculated onto one table
6. Place the appropriate colored tape onto the stem; each day has a different color, and write on the tape the month and date, 3-25. This facilitates easy identification of plants when taking severity.
  - a. Monday = Red
  - b. Tuesday = Blue
  - c. Wednesday = Orange
  - d. Thursday = Green
  - e. Friday = Pink
  - f. Saturday = Yellow
  - g. Sunday = White
7. Record the pot numbers to be inoculated into the Allegro
  - a. See the file Allegro.doc

8. Gently pipette 10  $\mu$ l of 50,000 spores/ml spore solution into the floret (do not damage the ovary).
  - a. REMEMBER, spores will settle to the bottom so be sure to shake the suspension frequently, about every 10<sup>th</sup> to 20<sup>th</sup> inoculation
9. Staple a glassine bag onto the inoculated head
10. Remove the glassine bag after 72 hours. Don't worry about leaving bags longer if they are supposed to come off during a weekend, but get them off ASAP
  - a. Use the tape color to decide what bags come off:
    - i. Monday = Pink bags are removed
    - ii. Tuesday = Yellow
    - iii. Wednesday = White
    - iv. Thursday = Red
    - v. Friday = Blue
    - vi. Saturday = Orange
    - vii. Sunday = Green
11. Place all the inoculated plants for each day in one place so that it will be very easy to find the plants when severity ratings are taken
12. Severity ratings are recorded 21 days after inoculation
  - a. Severity ratings are taken simply by counting the number of infected florets (bleached florets) and the total number of florets
  - b. Severity = # infected / total number
13. Discard Plants after they have been read. Just pile them all together and let the students know so they can discard them and stop watering them.
14. Analyze data.