Entire procedure for detached leaf assay experiments

1. 3 weeks before big experiment, grow isolates on new PDA plates
2. 2 weeks before big experiment, grow isolates on peaches (option for fast growth)
3. Prepare the following items:
4. Autoclave glass wool, make a plug and put into 12 ml syringes, then seal it with parafilm (1 per each isolate, plus 2 spares)
5. Autoclave ddH2O (500 ml per each 50 isolates)
6. Autoclave tooth picks (in two small beakers) OR can use glass rods
7. Autoclave boxes of yellows tips (200 ul) (1 box per 10 isolates)
8. Autoclave 1.5 ml tubes (1 large beaker per 15 isolates)
9. Autoclave 3 sets of scissors and forceps in foil packet
10. Prepare 70% ethanol (1 L per 8 isolates)
11. Count 1 L bottles (2 per tray), weigh 7.5 phytoagar into each bottle and cap the bottle
12. Wash trays and dome lids with soap, rinse with deionized water, let them dry on counter
13. Design your experiment (randomize plant genotype and isolate genotype in three replicates), Print the tray labels and cut them in strips, print inoculation maps and stick onto the cabin.
14. Make sure we have in stock the following items:
15. bottles of 95% ethanol (4L each, 1 per each 50 isolates)
16. 10 ml pipettes (1 per isolate)
17. boxes of 1ml sterile tips with filters (1 per 15 isolates)
18. 15 ml- tubes (1 per isolate)
19. boxes of kimwipes (1 per 30 isolates)
20. 1 box of cover plates for hemacytometer
21. Items to check
22. 50% filter-sterilized Grape juice (about 100 ml per 25 isolates)
23. Vortex (2), gloves, paper towels
24. Laptop
25. Timer
26. Sharpies
27. At least two sets of 1 ml pipette, 200 ul pipette and 20 ul pipette
28. Racks for 1.5 ml tubes (the more the easier)
29. large garbage can
30. One cart from CEF-A with good wheels to move plants and trays
31. One day before big experiment, prepare the agar flats and it will take at least 2 hours
32. Fill the bottles with 750 ml deionized water, cap the bottle and autoclave all the agar using autoclave machine in Asmudson Hall
33. After putting agar in autoclave machine, surface sterilize all the trays and domes with 70% ethanol, let them dry on counter.
34. When autoclave is done, briefly shake the bottle and make the medium even, then pour to the tray, after a while, get rid of any bubbles in the tray agar with a sterile pipette tip
35. When the medium is solidified, put tray labels on and cover with the lid
36. Cart plants into lab during daylight (for experiments in winter) – DO NOT allow them to get rain on them
37. Immediately (less than 12h) before the big experiment, collect spores from each isolate
38. Collecting spores from all isolates. DON’T scratch too much of the spores. Once you notice the solution becomes a little bit cloudy, you have enough spores already. If you scratch too much, it will take forever to count spores of one isolate. After centrifuge step, suspend spores in 1-3 ml of grape juice depending on the size of the pellet. It will greatly shorten the following counting step.
39. Counting spores (takes about 1 hour)
40. Serial dilution to prepare the final inoculum (takes about 1 hour)
41. Prepare the detached leaf and put on the agar (takes about 2 hours)
42. Inoculate on detached leaf (takes about 1 hour)