Isolation of *Phytophthora* species from infected leaves

(non-*infestans* species only)

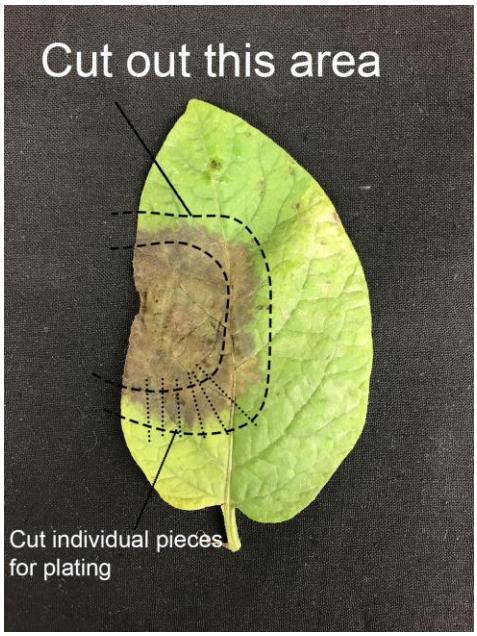
Before isolating, prepare four petri plates in the hood:

1.) Bleach - Make a 10% bleach solution (e.g. 90mL dH2O and 10mL bleach) and add to the first dish. Pour enough to cover ~¾ of the surface area of the plate, then swirl to cover the last ¼.
2.) Sterile water 1 - Pour sterile water into the second plate in the same manner.
3.) Sterile water 2 - Pour sterile water into the third plate in the same manner.
4.) Dry - Aseptically rip up a sterile paper towel and lay inside the petri dish.

Ensure that you have a scalpel with a fresh blade, forceps, and antibiotic (e.g. PARP) plates.

<u>Isolation Procedure:</u>

1.) Select a leaf with a large, distinct lesion. Ideally you want a lesion where there is a distinct border between infected and healthy leaf tissue. Place this leaf in an empty petri dish
2.) Using the scalpel, carve a strip of tissue out of the leaf that encompasses the margin of the lesion. Then, cut this strip into several small slices. Each piece should include both green and brown tissue. Exact size isn't important, but smaller leaves will be easier to sterilize and plate. No more than 1cm x 0.5cm, roughly.



3.) Using a pair of forceps, transfer the leaf pieces to the bleach plate. Dry leaf pieces are frequently hydrophobic, so use the forceps to push the leaf pieces into the bleach solution

to make sure they are completely submerged. Wash the leaf pieces in the bleach solution for approximately 30 seconds, gently swirling the bleach plate.

4.) Immediately transfer the leaf pieces to the first water plate. Avoid keeping the leaf pieces in the bleach solution for too long, as this can start to degrade them. Gently swirl in the water for approximately 1 minute.
5.) Transfer the leaf pieces to the second water plate. Gently swirl in the water for approximately 1 minute.
6.) Place the leaf pieces on the paper towels to blot dry.
7.) Resterilize the forceps. Use the forceps to bury leaf pieces under PARP agar. Since bacteria can't grow through agar, it's important to push the leaf pieces a bit away from the agar entry point to help filter out bacterial contamination - do not push leaf pieces into the agar and then leave them exposed.
8.) Plate several leaf pieces on a plate. Typically a plate will fit approximately 8-10 pieces. Do not crowd pieces close together so any isolations have room to grow out.
9.) Seal and label the plate. A standard labeling structure could be (suspected species)-(state)(year)-(field number)-(leaf number), such as Pnic-NC24-1-1. Store at the appropriate temperature (e.g. room temp) and monitor for hyphal growth.
10.) Repeat procedure for remaining leaves. If all leaves came from the same field/bag there is no need to replace the rinse/drying plates. However, if doing leaves from multiple fields, especially if they are not close together, changing out rinse/drying plates between leaves is recommended.