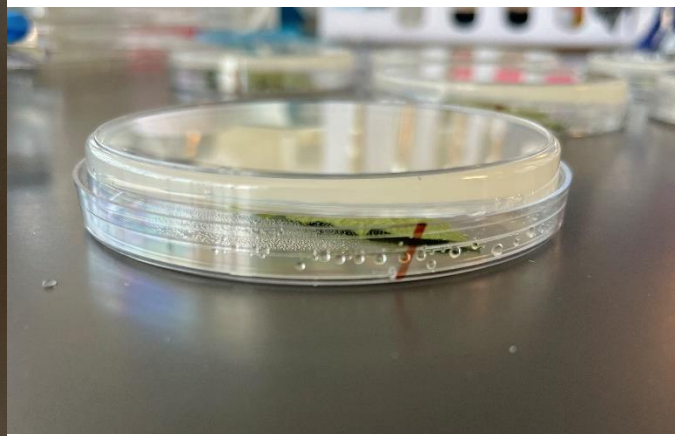
P. infestans **Live Leaf Transfers (Updated April 2023)**

For General Maintenance

Method 1: Leaf Smash (Best for inoculating detached leaves)

1.) Gather the following materials: Infected leaf, healthy leaf to be inoculated, a spray bottle of dH_2O, parafilm, and a 1.5% water agar plate.
2.) Invert the 1.5% water agar plate. The leaf will be sitting on the non-agar side of the plate. The water agar is present to maintain humidity. The images below show examples of a leaf under water agar in preparation for inoculation.



3.) Spray the abaxial (underside) side of the healthy leaf lightly with dH_2O
4.) Pick up the healthy leaf and press the abaxial side of the healthy leaf to the abaxial side of the infected leaf. Typically with an actively sporulating leaf, you only need to press a small portion of the healthy leaf to a small portion the infected leaf to get infection. In this way you can distribute the sporangia on an infected leaf across multiple leaves.
5.) Place the newly infected leaf in the water agar plate, abaxial side up.
6.) Seal the plate with Parafilm, and incubate at room temperature.
7.) If maintaining a culture on leaves, the culture should be transferred to fresh leaves once a week.

Method 2: Leaf Spray (Best for inoculating whole plants)

1.) Gather the following materials: Infected leaf, healthy plant to be inoculated, Parafilm, a 1.5% water agar plate, two 15mL Falcon tubes, and a spray cap (Order spray caps from Container & Packaging Supply, item number S009). Fill one of the Falcon tubes with about 10mL of dH_2O.
2.) Place the infected leaf in the Falcon tube with water and cap. Vortex or shake vigorously for about 10 seconds.
3.) Dilute the sporangia rinse in the second Falcon tube with dH_2O. You can do this as a 1:10 or 1:100 solution. Note that increased dosage will increase disease severity and speed. Cap this Falcon tube with the spray cap.
4.) Spray the plant all over with approx. 1-2mL of sporangia spray. Mix spray frequently to keep sporangia in suspension.
5.) Seal the plants either in an inoculation box or place a clear plastic bag over the plant to maintain humidity and incubate at room temperature.

For Quantified Application (Detached leaves or whole plants)

1.) Follow the method for leaf spray above through step 2.
2.) Swirl the sporangia solution and extract 1 mL of solution. If needed, add 1-2 drops of dye (e.g. cotton blue) for visualization during quantification (NOTE: If dye is added, do not use this aliquot in inoculations!). If you do not need to use dye, you can sample directly from the rinse tube.
3.) Use the aliquot to estimate the number of sporangia per mL using a hemocytometer. In our lab we load 10µl of solution into each well with the coverslip in place (solution will wick into the counting chamber), but follow the instructions your hemocytometer comes with for loading. There are also multiple tutorials online for performing the calculations. Note that if you add dye, you will need to account for how much the dye dilutes your solution when performing the calculation.
4.) Use the estimate to dilute the remaining sporangia solution to your desired concentration in the empty Falcon tube and attach the spray cap. Gently invert the tube to mix the spray.
5.) Spray the healthy leaf/plant with the sporangia spray. If using detached leaves, spray the abaxial side. If using a whole plant, spray the entire plant. Use the number of mLs needed in order to achieve your desired concentration of sporangia. Mix spray frequently to keep sporangia in suspension.
6.) If using detached leaves, seal the plates with Parafilm and incubate at room temperature. If using whole plants, seal the plants either in an inoculation box or place a clear plastic bag over the plant to maintain humidity and incubate at room temperature.

Transfer from Pure Culture to Leaf

1.) Gather the following materials: Pure culture for transfer, healthy leaves to be inoculated (~5-10), parafilm, and 1.5% water agar plates, enough for the number of leaves you plan to inoculate. Note: To prevent contamination, this should be performed in a sterile hood with appropriate sterile tools for culture transfer.
2.) Invert the 1.5% water agar plates. The leaves will be sitting on the non-agar side of the plate (see images above). The water agar is present to maintain humidity. Place the leaves abaxial side up in the plates.
3.) Aseptically cut agar plugs from the culture you wish to transfer to leaves.
4.) Using aseptic technique, place a plug onto a leaf, with the mycelial side of the plug face down on the leaf. If you are transferring to multiple leaves, sterilize your transfer tool between plugs to avoid contaminating your culture plate.
5.) Seal the plate and incubate at the appropriate temperature for your pathogen. The image below shows a prepared plate with plug.

6.) Once growth occurs onto the leaf, remove the plug. The leaf can then be used to inoculate fresh leaf tissue as outlined in the methods above. The images below show an example of a leaf infected with *P. infestans*. Note that this method may require several plates to successfully achieve transfer. Some cultures, especially those that have been on media for extended periods, may have lost the ability to infect leaves and will no longer be suitable for in vivo experiments. Maintain cultures for in vivo

experiments on fresh leaves to preserve virulence.