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Raffaelea plant inoculation

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Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

The purpose of this protocol is to test pathogenicity of the ambrosia fungi Raffaelea.

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

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Note: cultures of pathogenic fungi may need to be inoculated in plant material, and then subcultured on media prior to use in experiments. This increases the likelihood that the fungus will express pathogenic traits.

Spore suspension preparation

1. Pick a culture with substantial and active growth (3-5 weeks for most Raffaelea).
2. Add 2 mL of sterile DI water to plate
3. Using a plate spreader, apply modest pressure and homogenize water with fungal culture.

4. Tilt the plate and gather the water and fungi together at the bottom end of the plate. Then transfer the sample to a 2 mL microcentrifuge tube using a pipette.
5. Dilute about 200-500 µL (depending on fungus) of the original spore suspension to 2 mL of sterile DI water.
6. Use this dilution to determine spore concentration using hemocytometer (see separate protocol) and create desired spore concentration.
7. Although often an extraneous detail, you may want to aim for 100,000 spores/100 µL.

Inoculation procedure

1. Use an electric drill/screwdriver with 3/32" or 7/64" bit for mimicking a beetle gallery.
2. First score a point of inoculation about a half inch below an active branch node.
3. Point the drill downwards at about a 45 degree drilling angle at the inoculation point. Hold the drill bit steady with your thumb and forefinger, cradling the tree trunk using the same hand.
4. While still guiding the drill bit with your fingers, quickly and steadily drill about a half inch deep into the tree, enough to hold 50 uL of the spore suspension.
5. Pipette spore suspension into the tree.
6. Parafilm the trunk of the tree, surrounding and sealing the point of inoculation.
7. Later, plate some of your spore suspension to ensure that the inoculum contained viable fungus material.

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