Diagnostic Updates

Tips for Isolating *Phialophora gregata*, the Brown Stem Rot (BSR) Pathogen, from Plant Material

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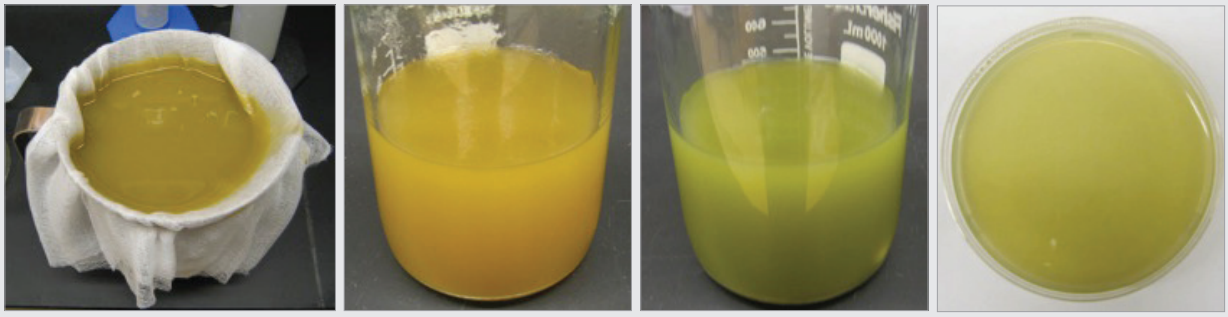
Although PCR and real-time PCR protocols are available for detecting the presence of *Phialophora gregata* in soybean tissues, there are advantages to confirming a diagnosis of BSR by isolation of the pathogen. The following describes a standard protocol for isolating *P. gregata* from plant material that includes several modifications and tips, which have proven to be very successful.

The medium. The semi-selective medium for *P. gregata* (PGM media) was first reported back in 1991. Although it has been tweaked a number of times by different labs, the recipe presented here is still relatively simple as well as effective.

160 g	Gerber® 2nd Foods® green bean baby food (not 1st or 3rd)
0.8 g	$CuSO_4 \bullet 5H_2O$ (Copper (II) sulfate pentahydrate a.k.a. Cupric sulfate pentahydrate)
0.0148 g	Terrachlor 75% Wettable Powder *
20 g	Bacto Agar
1 L	de-ionized water

*The active ingredient of Terrachlor is pentacloronitrobenzene or PCNB, at 75%. Technical grade PCNB has limited solubility in water so it is best to get it as a wettable powder fungicide formulation. It may be difficult to find Terrachlor but I have also seen it sold under the name Quintozene (Chemtura Co.)

Mix green bean baby food with water and filter through three layers of cheesecloth. Add the strained liquid to agar, add a stir bar, and sterilize for 40 minutes. After sterilization, place medium in a 60° C water bath to cool. While the medium is cooling, add the copper and Terrachlor to 9 ml of sterile, de-ionized water and vortex till mixed. Once medium is cooled, add the copper/Terrachlor solution and mix well (stir bar comes in handy here). The medium will turn from a dull pea-green color to a brighter blue-green. Store plates in the dark until they are ready to use (good for about 2 months).

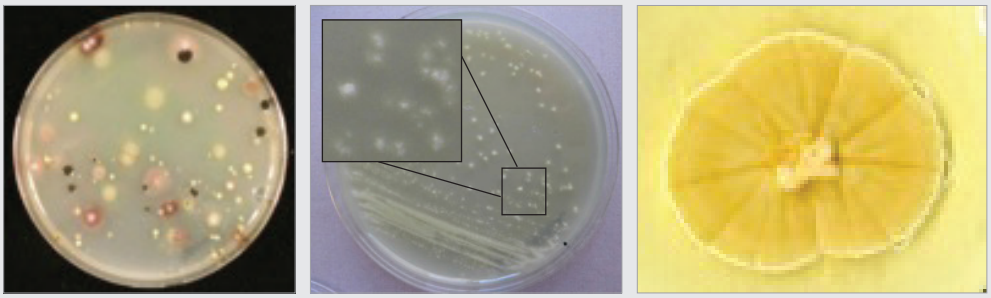


Media preparation. *Images from left-right:* straining of green bean baby food solution through cheesecloth. Color of medium after sterilization and before addition of copper and terrachlor. Color of medium after addition of copper and terrachlor. Appearance of PGM plate after solidification. Photos courtesy of Teresa Hughes, Purdue University.

The sample. How the sample is treated can have a significant influence on isolation of *P. gregata*. Plants can be collected at anytime during the season but chances of isolating *P. gregata* improve once soybean has started to flower and increases as the plant matures. Regardless of the growth stage at which the plants are collected, the sample should be processed within two weeks.

Remove leaves, petioles, flowers, pods, as well as secondary roots and nodules if your including root tissue. The best material for isolation is the 3–4 inches of the main lower stem and the first 2–3 inches of the taproot. Plants should be washed to remove dirt and allowed to air dry. Once plants are dried (they snap when bended), grind material through a Wiley Mill followed by a second grinding through a Udy cyclone sample mill (or equivalent). The powder sample (particle size is extremely important for isolating *P. gregata*) can then be dilution plated onto PGM.

Images from left to right: example of sample (clean; no petioles, leaves, etc); appearance of plant sample after grinding through Udy mill; appearance of the 10–2 dilution. Photos courtesy of Teresa Hughes, Purdue University.



Images from left to right: example of *P. gregata* colonies (circled in red) on PGM from stem material; example of *P. gregata* colonies on PGM, pure culture; image of an isolate of *P. gregata* on PDA. Photos courtesy of Teresa Hughes, Purdue University.

Weigh out 100 mg of ground tissue and add to a test tube containing 9 ml of sterile, de-ionized water (10–2 dilution). Vortex and remove 1 ml and add to a second test tube (10–3 dilution). Plate 100 µl of each dilution onto PGM and spread with a hockey stick. (Note, large orifice tips are very useful for dilution plating the ground material). Incubate plates upside down (no need to parafilm) in the dark at 12–16° C for 2–3 weeks (the cooler temperature aids the selectivity of the medium). Colonies of *P. gregata* on PGM will be small and whitish-yellow with a slightly raised center. Selected colonies can then be plated directly onto PDA. On this medium, *P. gregata* has a distinct appearance. It will grow very slowly, produce little or no spores, and will appear dense with ridges.