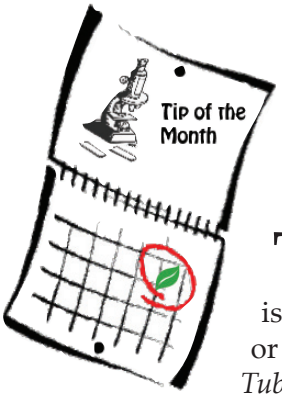

Isolation Method for Obtaining Endophytic *Tubakia* spp. from Oak Twigs

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The following is an abbreviated methods procedure for isolation from living twigs, petioles or leaves of the bur oak blight (BOB) *Tubakia* sp. and other endophytic *Tubakia* spp.

Cut twigs into small discs 1/8-1/4" thick;

Place into straight bleach (5.25% sodium hypochlorite);

Stir 3 minutes (use stir bar);

Rinse in 95% ethanol;

Rinse in sterile water;

Plate (4 discs/plate) onto any of these media: PDA, ¼ strength PDA, 2% malt, or 2% malt with 0.2% yeast;

Incubate plates at room temp. (21-23 C) and normal lab lighting;

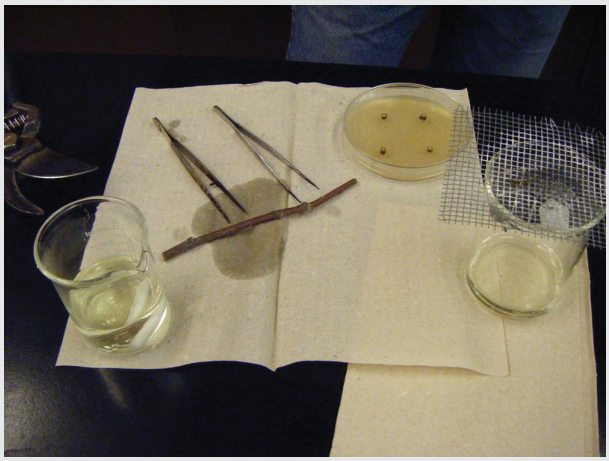
Check for growth:

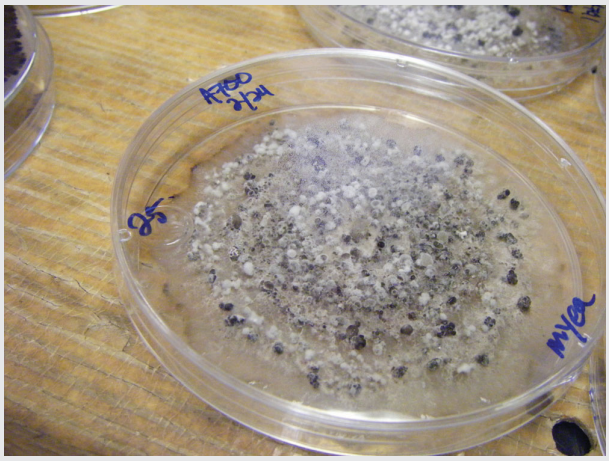
> 4 -14 days for *Tubakia* spp., ID by mycelium (the BOB *Tubakia* sp. sporulates rarely in culture) or transfer to obtain pure culture.

The *Tubakia dryina* species complex includes:

Tubakia dryina sensu stricto
T. sp. BOB
T. sp. A – BH type
T. sp. B – post oak type
Actinopelte americana - spot type
Actinopelte americana - veinal type

Tubakia is in the Diaporthales, related to *Phomopsis* spp. and the fungi that cause anthracnose. The BOB *Tubakia* infects expanding shoots, petioles and leaves in the spring and leaves in the summer. Primary inoculum in the spring comes from black petiole pustules on dead, overwintering leaves still hanging on the twigs.





From top to bottom: *Tubakia* sp. on red oak, endophyte isolation, and *Tubakia* sp. in culture. Photos courtesy of Gail Ruhl, Purdue University.