

# Method for Isolating and Maintaining Cultures of *Geosmithia* from *Juglans nigra*.

Ned Tisserat

Bioagricultural Sciences and Pest Management

Colorado State University

*Geosmithia* is relatively easy to isolate from walnut cankers of all sizes. However, you need to make sure the submitter supplies you with the proper sample. Galleries and cankers are much more abundant in branches greater than 1 inch diameter and rarely occur in small diameter twigs at the ends of branches. Thus, the name walnut twig beetle is somewhat misleading in the case of black walnut. Samples should be collected from branches showing dieback or wilting. Although beetle galleries will be numerous in dead branches, the cankers will be difficult to delineate because the walnut bark oxidizes and turns brown at death. Cankers caused by *Geosmithia* usually are 3 – 6 inches in length and surround the beetle galleries. They rapidly coalesce to cause large irregular areas of phloem necrosis. The beetle galleries, and cankers often are more numerous on the bottom side of branches and the west side of the trunk. Young cankers may not extend all the way to the cambium, so be careful not to cut under the cankers and remove them. Eventually cankers will extend to the cambium. In all cases, the cankers will be covered by outer bark, even in advanced stages of the disease. Thus, you will not see the typical open-faced, target cankers we associate with diseases like butternut and *Nectria* canker.

After selecting a sample, remove the outer bark. The bark surface may be disinfested with ethanol but this isn't essential. Aseptically shave off the outer bark with a sterile scalpel to expose the brown to black diseased phloem surrounding the beetle galleries. Cut small bark chips approximately 5-10 mm long and 3-5 mm wide from canker margins and place directly on ¼ strength potato dextrose agar amended with 100 mg/L streptomycin sulfate and 100 mg/L chloramphenicol (¼ PDA++). It is not necessary to disinfest the bark chips in sodium hypochlorite prior to placing on the agar surface. The fungus initially grows very rapidly out of the wood chips and colonies commonly exceed 20-40 mm in diameter after 3-5 days at 25 °C. Conidia may be formed on the bark chips in as little as 2 days. The fungus is thermotolerant and will grow at 32 °C. Isolations from trunk cankers may be more difficult if the bark is macerated. *Fusarium solani* and other *Fusaria* may be isolated from these tissues.

Fungal colonies of *Geosmithia* on half-strength PDA are cream-colored to tan, and tan to yellow-tan on the reverse side of the plate. However, colonies may become attenuated (<20-30 mm diameter after several weeks) with appressed margins following successive transfers on ½ strength PDA. The fungus sporulates profusely in culture producing dry conidia on multi-branched, verticillate, verrucose conidiophores. Conidiophore morphology is similar to *Penicillium*, although this genus is not closely related. *Geosmithia* conidia are tan *en masse*,

cylindrical to ellipsoid, 2 to 6 x 6 to 14 (mean 2.7 x 6.5)  $\mu\text{m}$ , and form in chains. *Geosmithia* can be transferred and maintained on ½ strength PDA or malt agar.

The fungus will produce a yeast phase. This is more apparent if the conidia are streaked across a plate in a manner similar to streaking bacteria. This, in fact, is a good method for developing single spore isolates and for isolating the fungus from the beetles. Streaking beetle parts (thorax, elytra, entire beetle, etc.) across the agar will result in multiple yeast colonies. The yeast phase will revert back to mycelial growth within a few days.

Species- specific PCR primers have not been developed for this *Geosmithia*. One reason is that this fungus can easily be identified based on morphological characteristics and the ease by which it can be isolated from diseased tissue. Look for a buff-colored colony on PDA or MEA, penicilliate conidiophores, and barrel shaped conidia. The identity of the fungus can be confirmed by sequencing the rDNA ITS region using the primers ITS1 or ITS 5 and ITS4. There are at least 8 different ITS haplotypes associated with the *Geosmithia* from walnut.



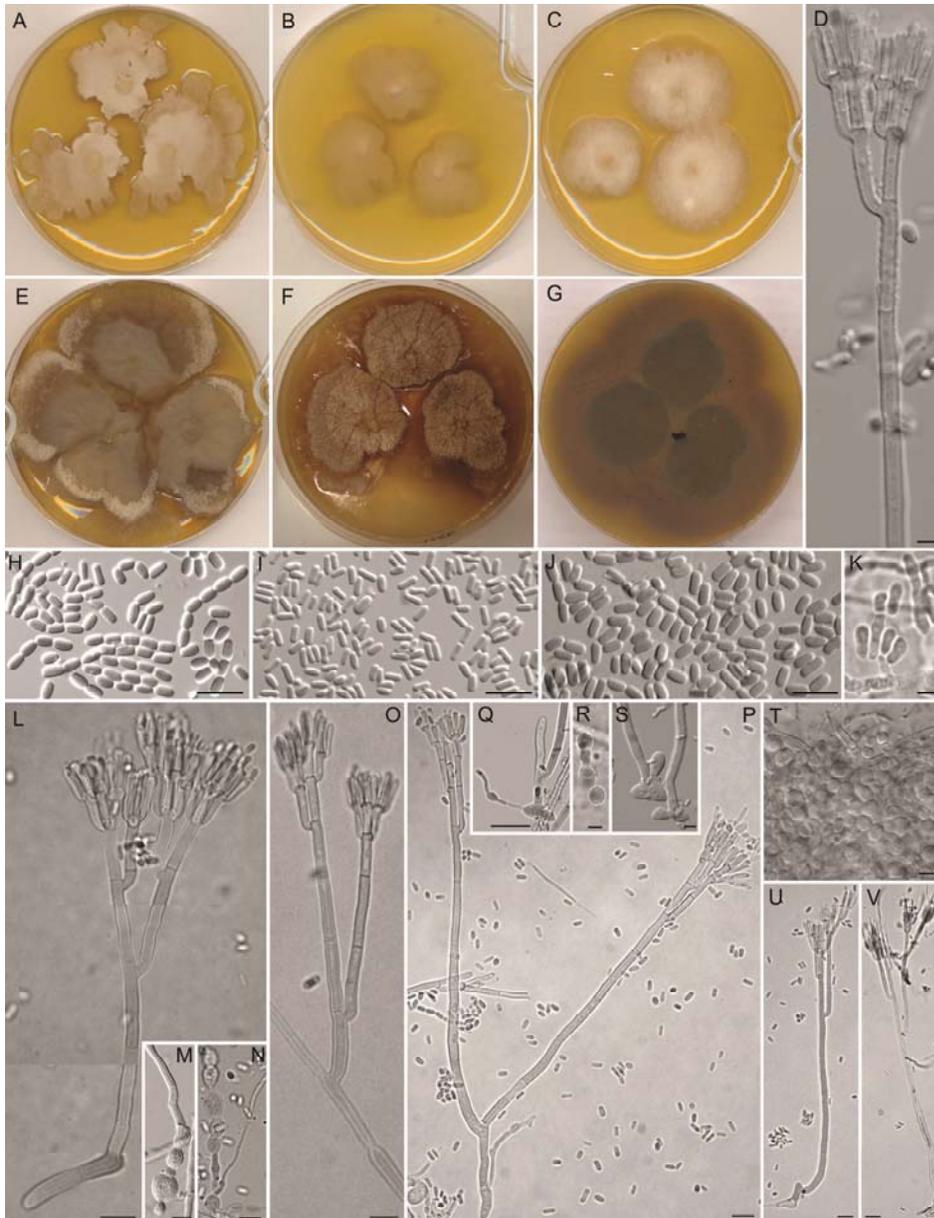
Cankers can be seen by carefully shaving off the outer bark. Note how they extend beyond the beetle galleries and coalesce to blight large, irregular areas of the phloem. *Geosmithia* can be isolated from anywhere in the necrotic phloem.



The bark has been completely removed from this branch to show the extent of cankering. However, most of the damage is in the phloem, so I have removed most of the necrotic tissue by shaving this deep. Note the white sporulation of *Geosmithia* in the coalescing cankers (right side of picture).



Typical colony morphology of *Geosmithia* growing out of bark chips on  $\frac{1}{4}$  strength PDA.



*Geosmithia* from *Juglans nigra*. Photo courtesy of Miroslav Kolařík *Institute of Microbiology; Czech Republic*