Diagnosing Thousand Cankers Disease of Walnut

Thousand cankers disease (TCD) is a newly recognized disease of various species of walnut (*Juglans*). Black walnut (*Juglans nigra*) is particularly susceptible to TCD and during the past decade it has devastated plantings of black walnut in most western states. In July 2010 thousand cankers disease was found over an extensive area around Knoxville, Tennessee, the first finding of TCD within the native range of *J. nigra*. Subsequently, in 2011, it was detected in the vicinity of Richmond, Virginia and in Bucks County, Pennsylvania. It is very possible that other unrecognized infestations of thousand cankers disease may be present in other areas in the eastern and Midwestern United States. It is very important in the management of this disease that the existing range of TCD is thoroughly documented.

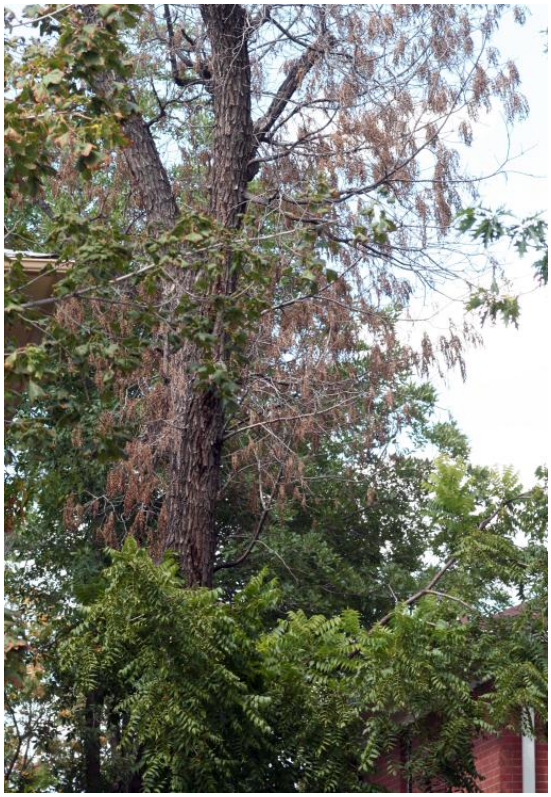

Figure 1. Rapid foliage wilting in end stage of thousand cankers disease.

Thousand cankers disease is produced by the combined activity of canker-producing fungus (*Geosmithia morbida*) that is introduced into trees by an insect vector, the walnut twig beetle (*Pityophthorus juglandis*). The disease, which is lethal in susceptible hosts, results from repeated infestations of *Geosmithia*-bearing twig beetles. This produces cumulative wounding that result in a progressive decline. It is likely that both organisms (fungus, twig beetle) are consistently found together so that identification of either organism can be used in diagnosing thousand cankers disease. A second fungus (*Fusarium solani*) is also associated with trunk cankers in advanced stages of TCD.

Figure 2. Oval shaped cankers in the cambium of black walnut produced by growth of *Geosmithia morbida*.

Visual Symptoms of TCD-Affected Trees

Crown thinning commonly develops following infestation with

thousand cankers disease and can be used to identify suspect trees. Further symptoms that are useful in TCD detection are the presence of isolated branches that show leaf yellowing (flagging) or sudden leaf wilting. Often such symptoms will rapidly progress over the subsequent years to produce progressive dieback and, often, ultimate death of

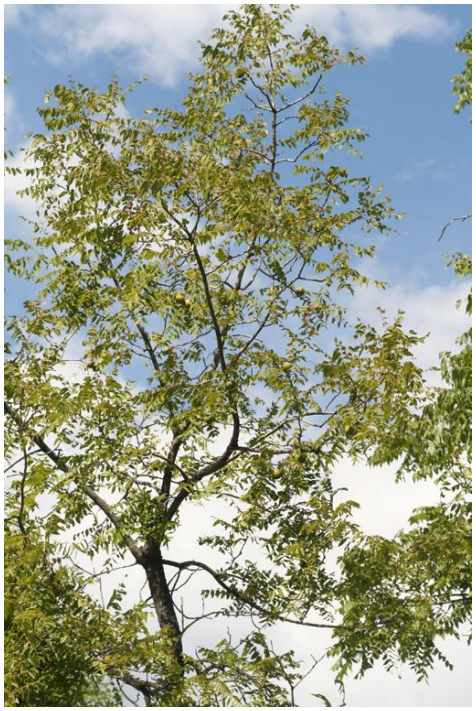

Figure 3. Crown thinning is a symptom useful in identifying trees that may be TCD-affected.

Figure 4. Branch dieback and bushy foliage growth is a symptom associated with thousand cankers disease. At some sites tree may remain in this condition for a considerable period.

the tree within a few years. Wilting of large areas of branches is often rapid during end stages of thousand cankers. In Colorado, black walnut usually is killed within 3-4 years after initial leaf yellowing symptoms. However, progress of the disease may vary depending on such conditions as site characteristics, initial plant vigor, host species, and other factors including natural controls of the walnut twig beetle. For example, in some sites disease progression can be considerably slower. Where dieback occurs but disease progression slows bushy growth often develops at areas below affected areas of limbs.

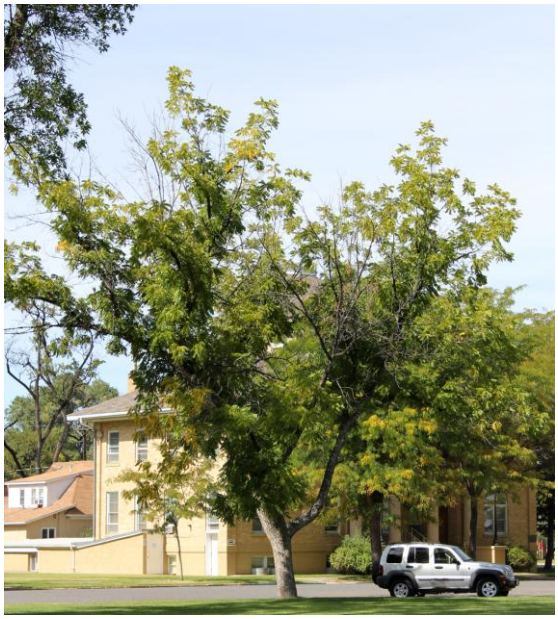

Figure 5. Yellow flagging of branches is a symptom associated with thousand cankers disease..

Development of leaf yellowing and

flagging symptoms useful in TCD detection often do not develop until early summer, and early to midsummer is usually the best time for TCD surveys based on symptoms expression. Conditions of stress from heat and drought may also increase development of symptoms.

Of course, leaf yellowing/flagging of black walnut can have many other - and far less serious – causes. Limb injuries by squirrels, hail, or storm damage may cause scattered branches to wilt. Certain insects (e.g., walnut aphid, leafhoppers) and spider mites may produce foliage yellowing that can mimic TCD-associated leaf yellowing. However, in areas where the disease is suspected to occur there should be additional examination to determine if thousand cankers disease is the cause.

Surveying for Presence of Walnut Twig Beetle

Figure 6. Exit holes and walnut twig beetle tunnels exposed on small walnut branch.

Walnut twig beetles can be most easily found by examination of branches symptomatic of TCD infection. The most easily observed symptom of twig beetle presence is the occurrence of minute exit holes in the bark made by walnut twig beetles. In areas where these are present the galleries of the bark beetles can usually be exposed easily when the bark is peeled away with a knife. These exit holes are most easily found in limbs with relatively smooth bark that are at least ¾-inch diameter. Despite the name "twig beetle", walnut twig beetles are not found in the smallest diameter twigs. Branches of 1-2 inch diameter with smooth bark are easiest to exam for the presence of the beetle.

(*Note:* Ambrosia beetles also are commonly associated with walnut limbs. Exit holes produced by ambrosia beetles are a bit larger than those produced by walnut twig beetle, are more scattered on limbs and ambrosia beetle tunnels extend deeply into the wood. Walnut twig beetle larval tunneling is almost entirely limited to the bark (phloem) and exit holes are often clumped due to the multiple insects developing in close proximity.)

Figure 7. Walnut twig beetles on head of a penny for size comparison.

The walnut twig beetle *Pityophthorus juglandis* is a minute (1.5-1.9 mm) yellowish-brown bark beetle, about 3X as long as it is wide. It is the only *Pityophthorus* species associated with

Juglans but can be readily distinguished from other members of the genus by several physical features. (Most *Pityophthorus* species in North America are associated with conifers; *Pityophthorus lautus* is a hardwood-infesting species present in eastern North America.) Among these are 4 to 6 concentric rows of asperities on the prothorax, usually broken and overlapping at the median line. The declivity at the end of the wing covers is steep, very shallowly bisulcate, and at the apex it is generally flattened with small granules.

Figure 8. Walnut twig beetle, *Pityophthorus juglandis*. Photograph courtesy of Jim LaBonte, Oregon Department of Agriculture.

Research into the chemical ecology of the walnut twig beetle by USFDA Forest Service has identified compounds that are attractive to this insect. One of these (Compound X) has become commercially available in 2012, distributed by Contech Enterprises. The lure is recommended to be used with a Lindgren funnel style trap, which has the advantage of collecting clean, intact insects that can be more easily examined. Lures can also be used in combination with sticky traps.

The presently available lure has a fairly limited range of attraction of a few dozen feet. Thus it is recommended that traps be placed on or in close proximity to walnut trees suspected of harboring walnut twig beetles. A strong advantage of the traps is that they may more easily detect beetles than a physical survey of branches, particularly when beetle populations are low, few branches are affected and/or they are inaccessible being high in the canopy.

Figure 9. Lindgren funnel trap used to sample for walnut twig beetle. A lure (upper left) is attached that will draw nearby beetles. The cylindrical form of the trap mimics a tree limb and the beetles drop into a collecting cup at the bottom when impacting the trap.

Symptoms of Cankers

Thousand cankers disease kills trees by the production of numerous small, dark, dead areas (cankers) under the bark largely due to the growth of *Geosmithia morbida*. Each canker is associated with tunneling by the walnut twig beetle, although beetle inoculations of *Geosmithia morbida* may sometimes result from small wounds that are not easily observed. Cankers can be detected by carefully removing the bark from symptomatic limbs. When peeling the bark to visualize cankers, be sure to not cut too deeply; the beetle galleries and fungus initially are found in the bark (phloem) and not in the cambium or sapwood.

Figures 10, 11. Bark completely removed to show discoloration of sapwood during advanced stages of the disease. Note the white, dusty appearance of *Geosmithia morbida* at the canker margin in the upper picture.

Individual cankers may originally be only a few millimeters in diameter, but ultimately can be 3 cm or greater and often assume an elongate oval form. Typically a shallow tunnel produced by the walnut twig beetle will be present near the center of the canker. Eventually the cankers coalesce to damage larger, irregular areas of the bark. They also extend down to the vascular cambium, resulting in a brown to black discoloration of the sapwood.

The combination of a dark canker with the beetle tunneling is almost certain confirmation of TCD. However, dark cankers occur under the bark following wounding and other injuries. Culturing the *Geosmithia* fungus from the canker will allow positive confirmation of thousand cankers disease.

Method for Isolating and Maintaining Cultures of *Geosmithi morbida* from *Juglans nigra*

Geosmithia morbida is relatively easy to isolate from walnut cankers of all sizes. However, properly collected samples are critical for accurate detection. 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. Samples should be collected from living 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. 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.

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. As early stage cankers may not extend all the way to the cambium, care needs to be taken to avoid cutting under and removing affected tissue during dissection. Eventually cankers will extend to the cambium.

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 *Fusarium* species may be isolated from these tissues. The colony morphology of *Fusarium solani* is similar to Geosmithia morbida, but tends to have a more whitish, pinkish appearance. Also cultures appear more moist because of the presence of spores in gelatinous beads in the colony.

Figure 12. Typical colony morphology of *Geosmithi morbida* growing out of bark chips on ¼ strength PDA.

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 long chains of dry conidia on multi-branched, verticillate, verrucose conidiophores.

Condiophore 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) µm, and form in chains. *Geosmithia* can be transferred and maintained on ½ strength PDA or malt agar

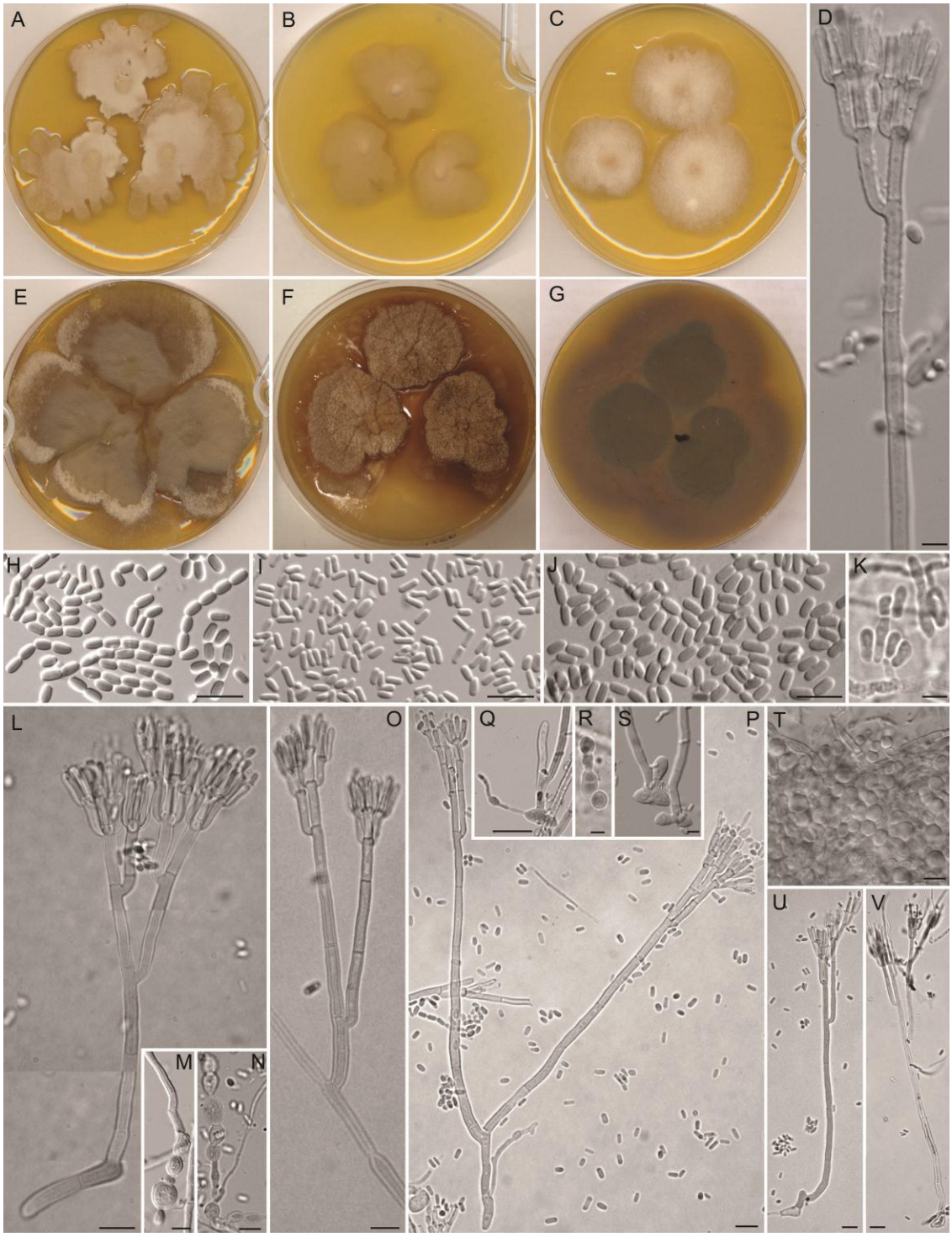

Figure 13. *Geosmithia* from *Juglans nigra*. Two-week old colonies grown on malt extract agar (A–C) and Czapek yeast agar (E, G) (at 25°C unless otherwise noted. Conidiophores (D,L,O, P, U, V) Conidia (H, I,J). Substrate conidia (K). Conidophore bases (M, Q,S). Monillioid mycelium and budding and inflated cells forming the basis of the colony (N). Yeast stage (T). Bars: D, K, R, S = 5 µm; H–J, L–Q, T–V = 10 µm. Photo courtesy of Miroslav Kolařík *Institute of Microbiology; Czech Republic*.

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 11 different ITS haplotypes associated with the *Geosmithia* from walnut.

Verifying the Disease

It is particularly important to verify TCD in locations where the walnut twig beetle and *Geosmithia morbida* have not previously been reported. If a new state record of this disease is suspected, often the most appropriate agency to first contact is the State Department of Agriculture or state forestry offices. Walnut branch samples from trees suspected of having TCD should be sent to a plant diagnostic lab that is a member of the National Plant Diagnostic Network (NPDN).

When collecting samples, select branches that are still alive but have evidence of either beetle galleries/exit holes, suspect cankers under the bark or recent branch wilting that may be associated with TCD-related injuries. *Branches provided as samples should be at least ¾-in diameter* as twig beetle tunneling and canker production rarely are found in the smallest diameter twigs.

When mailing samples, place 6-inch branch sections in a Ziploc bag *without* water or wet paper towels. Place this bag in a second Ziploc bag, seal tightly and mail to your nearest plant diagnostic lab. For a listing of a NPDN lab nearest you go to NPDN.org. Phoning ahead of sample shipments is strongly encouraged so that samples can be properly handled.

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