Thousand Cankers Disease Survey Guidelines for 2011

Photo credit: Tennessee Department of Agriculture

United States Department of Agriculture's Forest Service (FS) and Plant Protection and Quarantine (PPQ)

May 2011

Introduction

These guidelines were developed by the United States Department of Agriculture's Forest Service (FS) and Plant Protection and Quarantine (PPQ) with input from State agencies. The goal of this document is to provide guidance on how surveys can be conducted, in a standardized manner, for USFS and PPQ supported surveys. The focus of the surveys should be on symptomatic walnut trees; however, these guidelines should not be considered the only possible survey process. They can also be used for other TCD surveys carried out by the States.

Background

In May 2008, walnut mortality observed in Colorado as early as 2003 was attributed to numerous cankers developing in association with insect galleries. The new disease complex is referred to as "thousand cankers disease" (TCD) and is considered to be native to the United States.

TCD results from the combined activity of *Geosmithia morbida* fungus and the walnut twig beetle (WTB, *Pityophthorus juglandis*). While TCD is scattered throughout western states, the disease is thought to be more widespread than previously thought. In many of these states, it is believed that WTB and, by association, TCD, may be present wherever susceptible walnut species grow. The known geographical and host range of the WTB has expanded over the past two decades and, coupled with *Geosmithia morbida*, walnut mortality has occurred in California, Oregon, Washington, Idaho, Utah, Arizona, New Mexico, and Colorado. In July 2010, TCD was reported in Knoxville, Tennessee, causing dieback on black walnut. The infestation is believed to be at least 10 years old and was previously attributed to drought stress. This is the first report east of the 100th meridian, raising concerns that large native populations of black walnut in the eastern United States may suffer severe decline and mortality. This also emphasizes that we do not know the true distribution of this insect/disease association across the United States. While TCD has caused dieback and mortality across various climatic zones and among several walnut species, the extent of risk and impact to black walnut within its native range is still unknown.

Black walnut is a significant economic, social and environmental resource, and appears to be highly susceptible to TCD. Black walnuts exhibit little to no resistance to the pathogen and its vector.

Symptoms

The three major symptoms of this disease are branch mortality, numerous small cankers on branches and the bole, and evidence of tiny bark beetles. The earliest symptom is yellowing foliage that progresses rapidly to brown wilted foliage, then finally branch mortality (figure 1). The fungus causes distinctive circular to oblong cankers in the phloem under the bark, which eventually kill the cambium (figure 2). The bark surface may have no symptoms, or a dark amber stain or cracking of the bark may occur directly above a canker. Numerous tiny bark beetle entrance and exit holes are visible on dead and dying branches (figure 3), and bark beetle

galleries are often found within the cankers. In the final stages of disease, even the main stem has beetle attacks and cankers. (Taken from USFS Pest Alert: Thousand Cankers Disease, August 2010 – Appendix 1)

Figure 1. Wilting black walnut in the last stages of TCD.

Figure 2. Small branch cankers caused by *G. morbida*.

Figure 3. Exit holes made by adult WTB.

Survey

The first step in detection is to determine walnuts at risk by utilizing existing city tree inventories, locating walnut veneer and saw mills, and intensively managed stands of walnut trees. Once walnut trees are indentified, symptomatic trees with thinning crowns and leaf yellowing should be evaluated in early to mid-summer, with special attention given to the upper canopy of suspected diseased trees.

A decision tree to help illustrate the survey process is attached to this protocol (Appendix 2).

Roles

Resources provided by the Forest Service should focus on surveys of walnut trees in forested areas. Resources provided by PPQ should focus on surveys of walnut trees found in urban, residential, and industrial settings.

In addition, plantation and nursery owners, city foresters, and other tree care professionals should be encouraged to survey their walnut trees for TCD and report any suspicious trees to their State Forester, State Plant Health Director, or Cooperative Extension Office.

Data Collection

It is important that any surveys for TCD are documented to keep a record of both positive and negative TCD locations.

For FS surveys, survey data should be collected on the "Walnut Decline/Dieback Reconnaissance Survey" datasheet (Appendix 3) and is to be entered in the Forest Service TCD database.

For PPQ surveys, data should be collected on the spreadsheet available in the NAPIS database and should be entered into NAPIS. In addition, states may choose to also complete the "Walnut Decline/Dieback Reconnaissance Survey" datasheet (Appendix 3) to assist the Forest Service in collecting data. The purpose of this extra data is that the Forest Service is hoping to model walnut populations and TCD presence and absence, so this additional data will help that effort. Completed "Walnut Decline/Dieback Reconnaissance Survey" datasheets should be submitted to the PPQ TCD Program Manager at the end of the survey season for submission to the Forest Service.

Methods

To help determine if TCD is present, ask yourself or the property owner the following questions.

1. Is this a walnut *(Juglans* sp.) tree? (Be aware that butternut trees may be susceptible to TCD in addition to butternut canker.) See Appendices 4 and 5 for guides to assist in identifying *Juglans* sp. and *Juglans nigra.*
2. Are there other possible causes?
    a. Any recent root disturbances?
    b. Any recent pesticide use?
    c. Any leaf spot diseases (anthracnose) or target-like (Nectria) cankers?
    d. Any toothpick-like sawdust projections from the bark (caused by ambrosia beetles)?
    e. Insect entrance or exit holes larger or differently shaped than those of WTB?
    f. Any other occurrences that may have affected tree health?
3. Do symptoms match TCD?
    a. Are there yellowing, wilting, or flagging leaves high in the crown?
    b. Did symptoms begin in late spring or early summer?
    c. Are the symptoms worst on the south and west sides of the trees?
    d. Do browning leaves remain attached to twigs?

    e. Are the limbs dying back starting at the top and moving downward?
    f. Are new sprouts growing from tree roots?
4. Are affected limbs easily accessible?
    a. Are numerous tiny brown beetles present (1.5-2 mm long)?
    b. Are pin-sized holes visible in the bark of affected limbs?
    c. If you remove the bark, are numerous brown cankers visible?
    d. If cankers are visible, are small beetle tunnels present in the center of them?

Note: Research into traps and lures to detect the walnut twig beetle is ongoing. A lure may be available in July; however, visual surveys of walnut trees should proceed in early to mid-summer to inspect for the symptoms described above. If a lure does become available, revised survey guidelines will be issued.

Sample Collection and Handling

If you determine that TCD is a possibility, prepare to take a sample of the tree.

Supplies

- Strong knife
- Tools to collect sample branches
- Tools to collect insect samples (e.g., aspirator, jewelers forceps or flexible insect forceps)
- Small glass or plastic vials
- Paper towels or newspaper
- Ziploc bags

Instructions for Collecting Tree Sample

1. Use whatever means necessary to collect samples from the affected limbs safely (e.g. pole pruner, bucket truck, etc.).
2. If possible, collect samples from the south or west exposure.
3. Look for pin-sized, round holes. Peel away the bark with a sturdy knife on affected branch. Avoid cutting into the cambium and wood.
4. If holes or dark cankers and beetle galleries are present, prepare to collect a sample.
    a. Find the transition zone between healthy and damaged or dead wood.
    b. Ideally, cut 2-4 different branches 2-4 inches in diameter into 6 -12 inch long sections each that include healthy and damaged wood.
    c. Trim off excess twigs and branches.
    d. If any tiny (1.5-2 mm long), reddish-brown adult beetles (figures 4 and 5) are found during inspection or sampling, collect them in a leakproof vial of 75% ethyl alcohol. Larvae are not able to be identified at this time.
    e. Wrap each branch in paper towels or newspaper.

f. Put sample in two Ziploc bags while on site and seal. Multiple segments (maximum of 3) from the same branch can be packaged together as a single sample.
g. Record sample information on "TCD Sample Collection Datasheet" (Appendix 6).
h. Sterilize tools before collecting additional samples.

Figure 4. Walnut twig beetle-top view.

Figure 5. Walnut twig beetle-side view.

Determining Presence of WTB or Geosmithia morbida

Once samples are collected from a symptomatic tree, it is encouraged that the state screens the insects or fungus if the state has the capability to identify the WTB or *Geosmithia morbida*. This screening is sufficient for confirmation. Screening aids to help identify the WTB and *Geosmithia morbida* are included in Appendices 7 and 8, respectively.

WTB:

If, after using the screening aid, the state is unsure if the specimen is a WTB, the state may submit the sample to James LaBonte (Oregon Department of Agriculture, Plant Division, 635 Capitol Street N.E., Salem, OR 97301-2532, (503) 986-4749, jlabonte@oda.state.or.us) to find out if the specimen is WTB. The preferred shipping method is unmounted beetles in a leakproof vial of 75% ethyl alcohol. Place the vial in a whirlpack or zip lock bag. Mounted specimens are less desirable, but if they are all that is available, securely package them to

avoid damage during shipment. Contact James LaBonte if there are any questions about how to ship beetle samples.

Geosmithia morbida:
For those who do not have the capacity to identify *G. morbida* or if screening is inconclusive, samples may be sent to the National Plant Diagnostic Network. Locations can be found at www.npdn.org.

Instructions for Shipping Tree or Fungus Samples to NPDN

1. Suspect plant material, in double Ziploc bags, should be stored in a refrigerator awaiting shipment to a diagnostic facility. It is recommended that samples be frozen for 24 hours prior to shipment to kill any potential WTB in the sample. The preferred method for shipment is triple packaging, two Ziploc bags and an outer container. Tubes and plates should be sealed with tape. Shatter-proof containers should be used for the cultures. The outer shipping container should be an approved cardboard shipping box, and the seams of the box should be closed with approved shipping tape.
2. If submitted by regulatory personnel, the inspector will label and complete the appropriate forms. The inspector should record the State, identifier, the grower's license number (if applicable), the host(s), the inspector's initials as well as the location and date of inspection. If submitted by the State's Department of Agriculture, please include the Department of Agriculture designation: XXX-state-XXX. Upon receipt of the sample, this number will be placed in the notes section of the laboratory's database program so that it can be cross referenced with NAPIS.
3. It is suggested that samples be accompanied by a supplementary data sheet indicating the number of hosts present at each site. Save this data sheet in accordance with the NPDN format.
4. Samples should be shipped via overnight delivery or hand delivered to the diagnostic facility.
5. Many of the NPDN regions have established FedEx accounts that can be used to ship samples to expert labs. Please check with your regional center before forwarding samples.
6. Call the NPDN lab ahead of time or send an email so laboratory staff will be expecting sample. Mail packages early in the week to avoid having samples at unrefrigerated temperatures over the weekend.

Results of the identification of samples sent to Jim LaBonte or NPDN will be reported back directly to the submitting agency and will not be shared with other states without the permission of the submitting agency.

Outreach

Educating plantation and nursery owners, city foresters, and other tree care professionals about TCD should be considered an essential part of any survey plan. By collaborating with stakeholders and sharing these survey guidelines, more trees can be surveyed with the limited resources available.

Appendices

#1 - USFS Pest Alert: Thousand Cankers Disease, August 2010 (NA-PR-02-10). Available at: http://na.fs.fed.us/pubs/palerts/cankers_disease/thousand_cankers_disease_screen_res.pdf

#2 - TCD Survey Decision Tree

#3 - Walnut Decline/Dieback Reconnaissance Survey Datasheet (for FS surveys)

#4 - Key Identification Features of Juglans species

#5 - ID Guide for *Juglans nigra* (black walnut)

#6 - TCD Sample Collection Datasheet

#7 - A Screening Aid for the Identification of the Walnut Twig Beetle, *Pityophthorus juglandis* Blackman

#8 - Isolation and Morphological Identification of *Geosmithia morbida*

Appendix 1

Pest Alert

United States
Department of Agriculture
Forest Service
Northeastern Area
State and Private Forestry
NA–PR–02–10
Revised August 2010

Thousand Cankers Disease

Dieback and mortality of eastern black walnut (*Juglans nigra*) in several Western States have become more common and severe during the last decade. A tiny bark beetle is creating numerous galleries beneath the bark of affected branches, resulting in fungal infection and canker formation. The large numbers of cankers associated with dead branches suggest the disease's name—*thousand cankers disease*.

The principal agents involved in this disease are a newly identified fungus (*Geosmithia* sp. with a proposed name of *Geosmithia morbida*) and the walnut twig beetle (*Pityophthorus juglandis*). Both the fungus and the beetle only occur on walnut species. An infested tree usually dies within 3 years of initial symptoms.

Thousand cankers disease has been found in many Western States (figure 1). The first confirmation of the beetle and fungus within the native range of black walnut was in Tennessee (July 2010). The potential damage of this disease to eastern forests could be great because of the widespread distribution of eastern black walnut, the susceptibility of this tree species to the disease, and the capacity of the fungus and beetle to invade new areas and survive under a wide range of climatic conditions in the west.

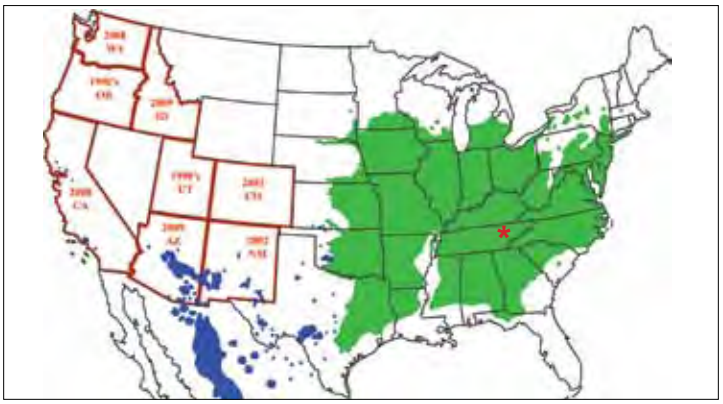

Figure 1. Thousand cankers disease occurs in eight western states (outlined in red) and in the east was first confirmed in Knoxville, TN in July 2010 (see *). In the west the year when symptoms were first noted is given. Native distributions of four species of western walnuts (blue) and eastern black walnut (green) are also shown. Eastern black walnut is widely planted in the West, but not depicted on this map.

Disease Symptoms

The three major symptoms of this disease are branch mortality, numerous small cankers on branches and the bole, and evidence of tiny bark beetles. The earliest symptom is yellowing foliage that progresses rapidly to brown wilted foliage, then finally branch mortality (figure 2). The fungus causes distinctive circular to oblong cankers in the phloem under the bark, which eventually kill the cambium (figure 3). The bark surface may have no symptoms, or a dark amber stain or cracking of the bark may occur directly above a canker. Numerous tiny bark beetle entrance and exit holes are visible on dead and dying branches (figure 4), and bark beetle galleries are often found within the cankers. In the final stages of disease, even the main stem has beetle attacks and cankers.

Figure 2. Wilting black walnut in the last stages of thousand cankers disease.

Figure 3. Small branch cankers caused by *Geosmithia morbida*.

Geosmithia sp.

Members of the genus *Geosmithia* have not been considered to be important plant pathogens, but

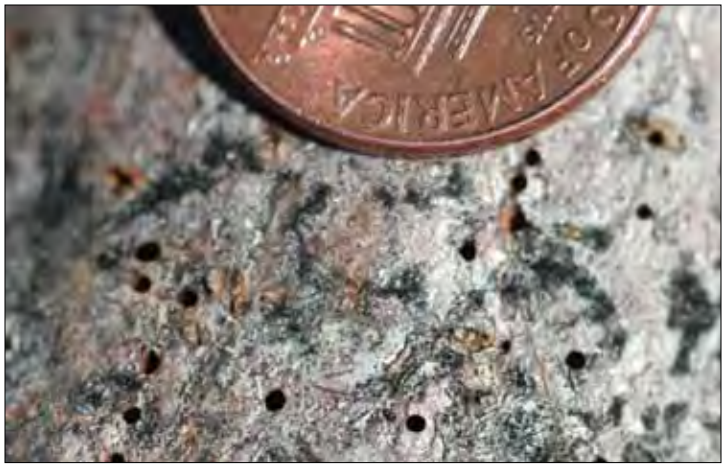

Figure 4. Exit holes made by adult walnut twig beetles.

Figure 5. Walnut twig beetle: top view (A) and side view (B).

Geosmithia morbida appears to be more virulent than related species. Aside from causing cankers, the fungus is inconspicuous. Culturing on agar media is required to confirm its identity. Adult bark beetles carry fungal spores that are then introduced into the phloem when they construct galleries. Small cankers develop around the galleries; these cankers may enlarge and coalesce to completely girdle the branch. Trees die as a result of these canker infections at each of the thousands of beetle attack sites.

Walnut Twig Beetle

The walnut twig beetle is native to Arizona, California, and New Mexico. It has invaded Colorado, Idaho, Oregon, Utah, and Washington where walnuts have been widely planted. The beetle has not caused significant branch mortality by itself. Through its association with this newly identified fungus, it appears to have greatly increased in abundance. Adult beetles are very small (1.5 to 2.0 mm long or about 1/16 in) and are reddish brown in color (figure 5). This species is a typical-looking bark beetle that is characterized by its very small size and four to six concentric ridges on the upper surface of the pronotum (the shield-like cover behind and over the head) (figure 5A). Like most bark beetles, the larvae are white, C shaped, and found in the phloem. For this species, the egg galleries created by the adults are horizontal (across the grain) and the larval galleries tend to be vertical (along the grain) (figure 6).

Survey and Samples

Visually inspecting walnut trees for dieback is currently the best survey tool for the Eastern United States. Look for declining trees with the symptoms described above. If you suspect that your walnut trees have thousand cankers disease, collect a branch 2 to 4 inches

Figure 6. Walnut twig beetle galleries under the bark of a large branch.

in diameter and 6 to 12 inches long that has visible symptoms. Please submit branch samples to your State's plant diagnostic clinic. Each State has a clinic that is part of the National Plant Diagnostic Network (NPDN). They can be found at the NPDN Web site (www.npdn.org). You may also contact your State Department of Agriculture, State Forester, or Cooperative Extension Office for assistance.

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Photographs:
Figure 1: Andrew Graves
Figure 2: Manfred Mielke, U.S. Forest Service
Figures 3, 4, 6: Whitney Cranshaw, Colorado State University, www.forestryimages.org
Figure 5: Steve Valley, Oregon Department of Agriculture

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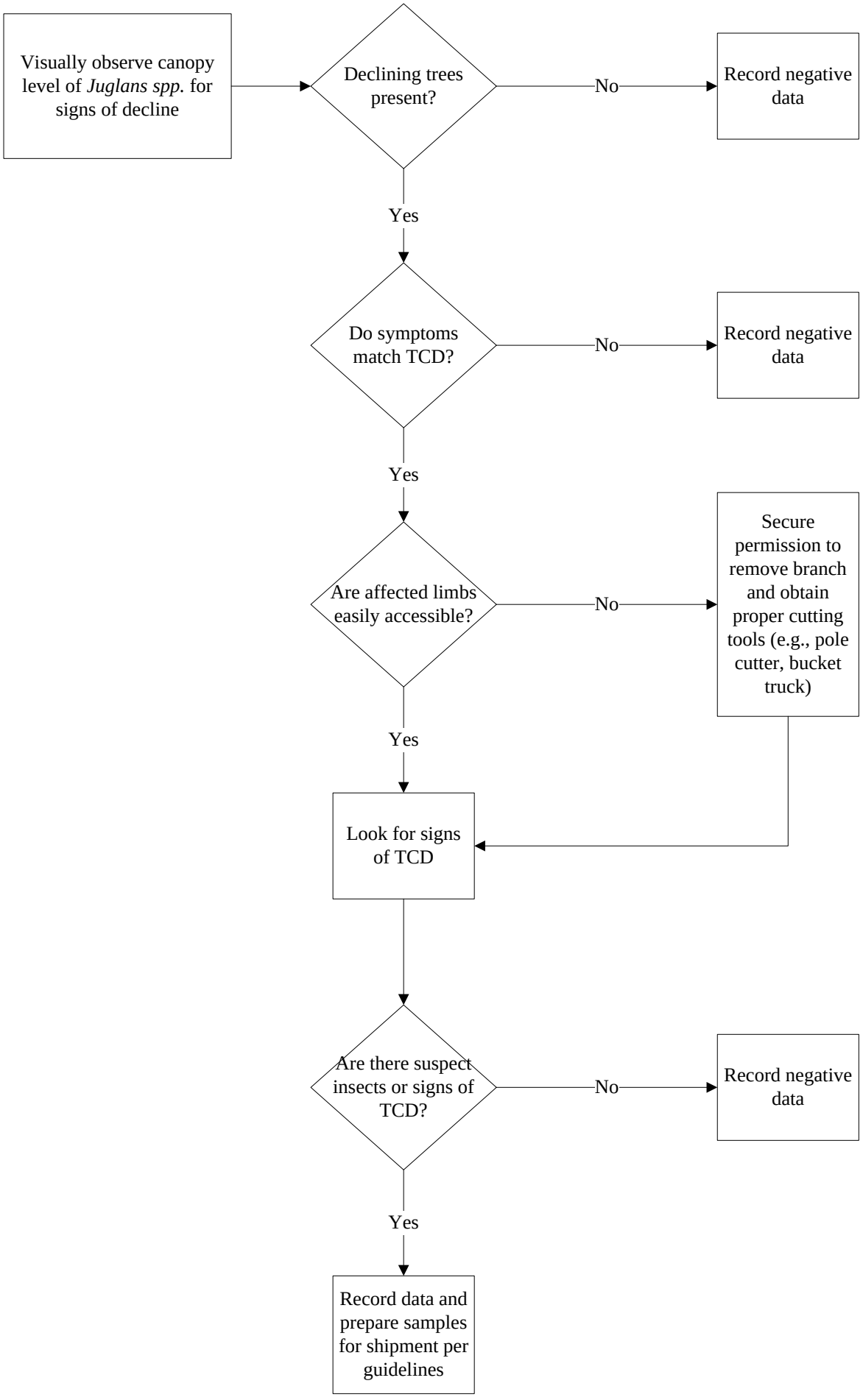
Appendix 2 - Thousand Cankers Disease Survey Decision Tree



Site ID: _____

Appendix 3 – For Forest Service Surveys
WALNUT DECLINE/DIEBACK RECONNAISANCE SURVEY DATASHEET

Name of Inspector: _____ **Date(s) of Site Visit:** _____

State: __ **County:** _____ **Site Description:** _____
Nearby Towns/Nearest Intersection

GPS Location: Lat: _____ **Waypoint Recorded? Yes** ☐ **No** ☐

Long: _____ **Waypoint Name / #:** _____
Preferred: Decimal Degrees (DD.DDDDD); WGS 84

Landowner / Manager Name and Contact Info: _____

_____

◄ *CHECK THE RESPONSE THAT BEST DESCRIBES THE SITUATION* ►

Species Present: ☐ *J. nigra* ☐ *J. cinerea* ☐ *Unknown* ☐ *Other Juglans sp.:* _____

Site Category: ☐ *B.L. Hardwood* ☐ *Plantation* ☐ *Urban/Community/Roadway* ☐ *Rural* ☐ *Other*

Stand Size: ☐ *Isolated Tree* ☐ *Small cluster* ☐ *< 1 Acre* ☐ *1-5 acres* ☐ *> 5 Acres*

% Juglans in Stand: ☐ *< 10%* ☐ *10-25%* ☐ *25-50%* ☐ *50-75%* ☐ > 75%

% Juglans Affected: ☐ *Widely Scattered Individuals* ☐ *< 50%* ☐ *>50%*

Size of Trees Affected: (Check all that Apply) ☐ *Sapling* (< 5 inches) ☐ *Pole* (5-12 inches) ☐ *Sawtimber* (> 12 inches)

Dist. of Affected Trees: ☐ *Scattered* ☐ *Grouped* ☐ *Follows Geographic Feature:* _____

% Crown Affected: ☐ < 10% ☐ 10-25% ☐ *25-50%* ☐ *50-75%* ☐ > 75%

Symptoms/Signs: (Check all Present)

☐ *Entrance/Exit Holes (pin-size)* ☐ *Beetle Galleries* ☐ *Beetles*

☐ *Wilting Leaves* ☐ *Brown Leaves Still Attached* ☐ *Dieback*

☐ *Recently Dead Branches* ☐ *Cankers (Vascular Tissue)* ☐ *Yellowing Leaves*

☐ *Cankers (Outer Bark)* ☐ *Other:*

(*Please Complete TCD Sample Collection Datasheet for EACH Sample Collected from this Site*)

Samples Taken: ☐ *Yes* ☐ *No* ***Sample IDs:*** _____

_____

_____

Pictures taken: ☐ *Yes* ☐ *No*

Please include any additional notes about the site, trees affected, symptoms/signs, samples taken, pictures taken, etc. on reverse

Key Identification Features of Juglans species

Leaves are alternate, with 9-23 leaflets that are pinnately compound and each leaflet is sessile or nearly sessile. The leaflets are serrated with a stout rachis which is usually hairy. The base of leaflet is nonsymmetrical and the tips gradually taper to a sharp tip. The leaflets are rounded at the base but are elongated out to the tip.

Black Walnut leaves. Source: Steven Baskauf, Vanderbilt University, 2005

Butternut leaves. Source: Steven Baskauf, Vanderbilt University, 2002

Persian Walnut leaves. Source: Cristina Comanici, 2008

Bark is somewhat diamond shaped though more so in *J.nigra* and *J. regia* and varies from dark brown to grayish black to ashy gray.

Black Walnut bark. Source: Ji-Hyun Park, University of Minnesota, 2011

Butternut bark. Source: Dr. Andrew Nelson, SUNY, 2009

Persian Walnut bark. Source: Gerhard Elsner, 2007

Twigs are stout with an acrid taste, hairy or naked; pith is chambered after the first season except between season's growth, stellate in transverse section; terminal buds with few scales, often appearing naked; lateral buds regularly occur one on top of the other, leaf scars with groups of three equidistant U-shaped bundle scars.

Black Walnut Twig. Source: Steven J. Baskauf, Vanderbilt University. 2002

Butternut Twig. Source: Dr. Andrew Nelson, SUNY, 2009

Persian Walnut Twig. Source: Sten Porse, 2008

Fruit are drupaceous; outer shell is semifleshy, and when ripe will remain unopened, the core is nutlike, with thick walls, wrinkled or deeply grooved; seed is sweet and usually oily.

Black Walnut fruit. Source: Steven J. Baskauf, Vanderbilt University, 2002

Butternut fruit. Source: Karren Wcisel, 2005

Persian Walnut fruit. VegTalk.org, 2007

Descriptive text taken from:

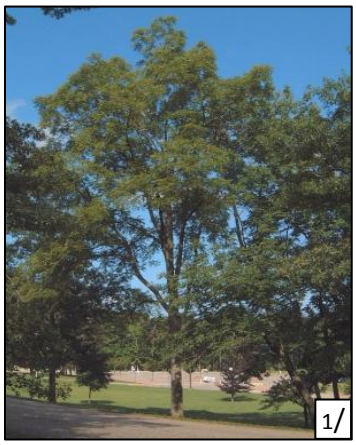
Harlow, William M. and Harrar, Elwood S. "Textbook of Dendrology" 5th ed. McGraw – Hill, Inc., New York. 1968.

Document created by: P. Castillo, NRS, U.S. Forest Service, April 2011

Common name: Black Walnut

Scientific name: Juglans nigra

Species Identification Features:


1/

Juglans nigra (Black Walnut) form

Leaves are alternate, pinnately compound, 12-24 inches long. **Leaflets** total 9-21, and are broadly lanceolate, uneven at base, finely serrate, 2.4-5.1 inches long, dark green, glabrous above and soft-hairy below.


1/

Twigs are thick, gray, finely hairy when young, with raised 3-lobed leaf scars, and chambered brown pith. End bud single, narrow, with paired gray hairy scales.


1/


2/

Bark gray to dark brown, becoming deeply furrowed into scaly ridges.


3/

Fruits are often in pairs, 1.6 - 2.4 inches in diameter, with a thick and pulpy husk.


1/

1/ Photos courtesy of Virginia Tech Department of Forest Resources and Envir. Conserv.

2/ Photo by Steven J. Baskauf, Dept. of Bio. Sci., Vanderbilt University

3/ Photo by Ji-Hyun Park, Ph.D. Student, University of Minnesota Department of Plant Pathology

Online guide to black walnut: http://www.tn.gov/agriculture/regulatory/tcdchecklist.html

Document created by: P. Castillo, NRS, U.S. Forest Service, April 2011

Site ID: _____

Appendix 6 - TCD Sample Collection Datasheet

(Please complete Walnut Decline/Dieback Reconnaissance Survey Datasheet (FS) or NAPIS TCD Datasheet (PPQ) for each site from which samples are taken)

Sample ID: _____
Site ID – Sample #

Name of Sampler: _____ **Date of Sample:** _____

GPS Location: Lat: _____ **Waypoint Recorded? Yes** ☐ **No** ☐

Long: _____ **Waypoint Name / #:**_____
Preferred: Decimal Degrees (DD.DDDDD); WGS 84

Species: ☐ *J. nigra* ☐ *J. cinerea* ☐ *Unknown* ☐ *Other Juglans sp.* _____

Crown Class: ☐ *Dominant* ☐ *Co-dominant* ☐ *Intermediate* ☐ *Suppressed* ☐ *Open grown*

% Crown Affected: ☐ < 10% ☐ 10-25% ☐ *25-50%* ☐ *50-75%* ☐ > 75%

Tree DBH: _____ *inches* **Tree Height:** _____*ft*

Type of Sample: ☐ *Stem* ☐ *Branch* ☐ *Adult Beetle(s)*

Aspect of Sampled Branch: _____ **Diameter of Branch:** _____*inches* **Height of Branch** _____*ft*

Symptoms/Signs: (Check all Present on Tree)
☐ *Entrance/Exit Holes (pin-size)* ☐ *Beetle Galleries* ☐ *Yellowing Leaves*
☐ *Wilting Leaves* ☐ *Brown Leaves Still Attached* ☐ *Dieback*
☐ *Recently Dead Branches* ☐ *Cankers (Vascular Tissue)* ☐ *Cankers (Outer Bark)*
☐ *Other:*

Number of Branch Segments in Bag / Package: _____

Sample Description: _____

_____

Appendix 7

A Screening Aid for the Identification of the Walnut Twig Beetle, *Pityophthorus juglandis* Blackman

James R. LaBonte[1],
Robert J. Rabaglia[2]
[1] Plant Division, Oregon Dept. of Agriculture, Salem, OR;
[2] USDA Forest Service, Forest Health Protection, Arlington, VA .

Introduction: The walnut twig beetle (WTB), *Pityophthorus juglandis* Blackman (Scolytidae), is a small (~2 mm long) bark beetle native to the southwestern United States (AZ, CA and NM) and northern Mexico (Chihuahua). Recently, WTB has been linked to decline and death of walnut trees (*Juglans*) in several western states, including states outside its native range, e.g., CO and OR. Twig, branch, and eventual tree death (known as thousand cankers disease, TCD) is the result of WTB attack and canker development around beetle galleries caused by a fungal associate (*Geosmithia morbida*). WTB and TCD have recently been detected in TN, raising concerns about the impacts on eastern black walnut and butternut in their native ranges. The early detection and identification of WTB is important to the successful prevention and management of TCD in the east and other areas of North America where these pests are unknown. This screening aid will help differentiate WTB from other bark beetles in trap samples or specimens collected from suspect walnut trees.

Reality check: *Pityophthorus* is a large genus (>100 species in North America) and identification to species can be difficult since these are very small beetles and the distinguishing characters are often hard to discern without high magnification and good optics. Suspect specimens should be submitted to an identification specialist for verification.

Actual size

Actual size

Key:

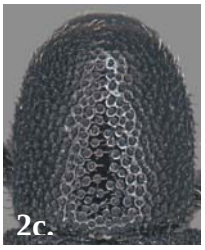
1. Total body length less than 3 mm.....2
   Total body length greater than 3 mm.....NOT *P. juglandis*

2. The pronotal asperities from the middle to the anterior margin form two or more well-defined concentric rows, adjacent asperities in each row normally in contact basally (2a).....3

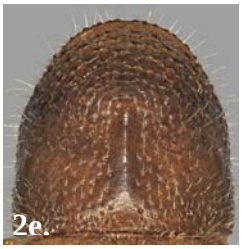
Pronotum lacking asperities (2b-c) or, if asperities present, those from the middle to the anterior margin not in well-defined concentric rows (2d-f), if rows more or less evident, adjacent asperities in each row not in contact (2g-h).....NOT *P. juglandis*


2a.


2b.


2c.


2d.


2e.

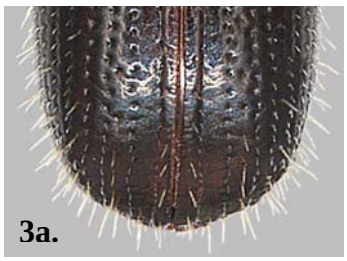

2f.


2g.


2h.

3. Apex of elytra evenly rounded (3a).....4

Apex of elytra not evenly rounded (3b-c).....NOT *P. juglandis*


3a.


3b.


3c.

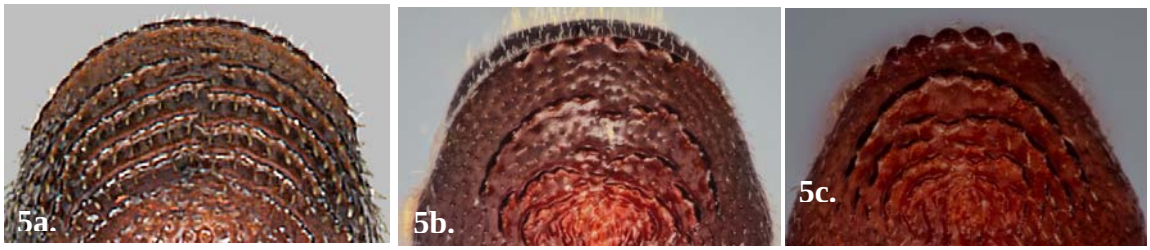
4. Apical elytral declivity flattened on either side of suture laterad of impressed striae 1 and striae 2 not impressed (4a), declivity finely roughened and dull (3a).....5

Apical elytral declivity distinctly depressed on either side of suture laterad of striae 1 and striae 2 deeply impressed, declivity shiny (4b-c).....NOT *P. juglandis*


4a.


4b.

5. Anterior margin of pronotum with more than 12 asperities (5a).....YES – *P. juglandis*
*Two rarely collected species from the desert Southwest shrub *Franseria* might key here but these are so unlikely to be encountered that it is not worth trying to differentiate between them and *P. juglandis*.
Anterior margin of pronotum with no more than 12 asperities (5b-c).....NOT *P. juglandis*



Appendix 8 - Isolation and Morphological Identification of *Geosmithia morbida*

Taken from National Plant Diagnostic Network Standard Operating Procedure for Plant Diagnostic Laboratories Thousand Cankers Disease of Black Walnut *Geosmithia morbida* and its vector, *Pityophthorus juglandis*. Modified from: Tisserat N. 2009. Diagnosing Thousand Cankers Disease of Walnuts. Colorado State University

Isolations:

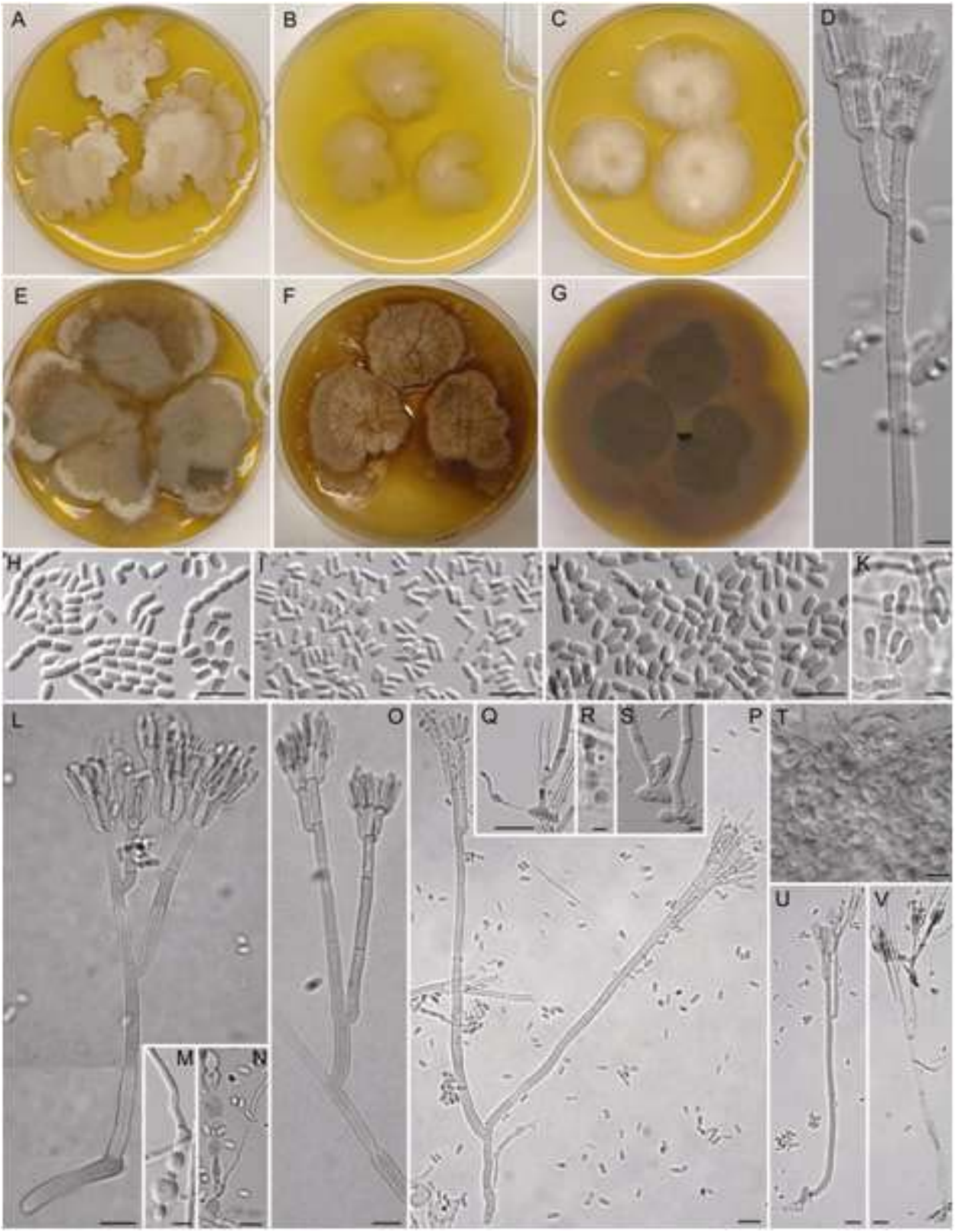
- Gently peel away outer bark to expose discolored phloem.
- Identify samples most symptomatic for *Geosmithia morbida.* These samples should have brown to black cankers ranging from a few millimeters up to 3 cm or greater. *Geosmithia morbida* can be isolated from cankers of any size. The cankers are often elongate to oval with a shallow tunnel near the center of the canker produced by the WTB.
- Place small bark chips on ¼ PDA++.
- (Seal plates with parafilm and incubate at 25°C. Colonies will grow rapidly.

Identifying *Geosmithia morbida:*

- 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.
- Fungal colonies on half strength PDA are cream-colored to tan, and tan to yellow-tan on the reverse side of the plate.
- The fungus sporulates profusely in culture producing dry conidia on multi-branched, verticillate, verrucose conidiophores. Condiophore morphology is similar in appearance to *Penicillium* although these genera are not closely related.
- *Geosmithia sp.* 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

Culture Maintenance:

Geosmithia morbida can be transferred and maintained on ½ strength PDA or malt agar.



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*. Reprinted with permission from *Mycologia*.

Media Recipes

1. ¼ strength Potato Dextrose Agar with streptomycin sulfate and chloramphenicol (¼ PDA++.)

a. Dissolve 7.6g Potato dextrose agar and 5g granulated agar into 1000ml distilled water.
b. Autoclave mixture for 30 minutes (121°/15psi).
c. Cool to around 50°C.
d. Add 100mg streptomycin sulfate and chloramphenicol to sterilized agar mix.
e. Pour into sterilized plates.

2. ½ strength Potato Dextrose Agar (½ PDA)

a. Dissolve 19.5g Potato dextrose agar and 7.9g granulated agar into 1000ml distilled water.
b. Autoclave mixture for 30 minutes (121°/15psi)
c. Cool to around 50°C
d. Pour into sterilized plates.

3. Malt Agar (MA)

a. Dissolve 20g granulated agar and 25g Malt extract* into 1000ml distilled water.
b. Autoclave mixture for 30 minutes (121°/15psi)
c. Cool to around 50°C
d. Add 1ml Lactic Acid (25%)
e. Pour into sterilized plates.

*34g Malt agar can be used in place of granulated agar and malt extract.