Thousand Cankers Disease Survey Guidelines for 2022

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

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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 standardized techniques for USDA-FS-, USDA-PPQ-, and State-supported surveys for thousand cankers disease (TCD). The focus of the surveys should be on symptomatic walnut or butternut trees; however, these guidelines should not be considered as the only possible survey process.

Background

In May 2008, walnut tree mortality, originally observed in Utah and Oregon (1990's) and New Mexico (2001) and Colorado (2003), was attributed to numerous cankers of the phloem developing in association with insect galleries. The new disease complex was named "thousand cankers disease" (TCD) and is considered to be native to the southwestern United States.

Although there is uncertainty regarding the roles of the organisms associated with TCD, the disease is thought to be caused primarily by the combined activity of a fungus, *Geosmithia morbida*, and the walnut twig beetle, *Pityophthorus juglandis*. While TCD was originally described from scattered locations throughout western states, it became clear by 2009 that the disease was more widespread in the West than previously thought. In many of these states, it appears that the walnut twig beetle (WTB) and, by association, TCD, is 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 *G. morbida*, walnut mortality has occurred in California, Oregon, Washington, Idaho, Utah, Arizona, New Mexico, Nevada, and Colorado. In the West, the WTB occurs from southern Arizona and New Mexico to northern Idaho and Washington, and from coastal California and Oregon to eastern Colorado.

In July 2010, TCD was reported in Knoxville, Tennessee, causing dieback on black walnut. The Tennessee infestation was believed to be at least 10 years old at the time of discovery and was previously attributed to drought stress. The find in Knoxville was 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. In July and August 2011, TCD was reported in Richmond, Virginia, and Doylestown, Pennsylvania. Surveys for the pathogen and beetle around Richmond revealed that the disease was present in five additional counties. In 2012, the WTB was trapped northwest of Cincinnati, Ohio, and the pathogen was isolated from branch material collected in North Carolina near its border with Tennessee. TCD was later confirmed in Ohio and North Carolina when both organisms were found in these states. In late 2013, WTB was collected in Cecil County, Maryland, and *G. morbida* was detected in this county in August 2014. In 2014 and 2015, the pathogen and WTB were discovered in Brown and Franklin Counties, respectively, in Indiana. In September 2013, TCD was confirmed in northeastern Italy on black walnuts of different ages: 80-year-old trees growing in a garden and 17-year-old trees in

a nearby walnut plantation for timber production. Later, TCD was also reported on English walnut in Italy. By 2015 the beetle had been detected across the complete tier of four northern Regions in Italy, whereas *G. morbida* had been detected in the Regions of Piemonte and Veneto. In 2018 both organisms were discovered in the more southerly Region of Tuscany.

G. morbida has been detected on other subcortical insects in Illinois, Minnesota and Missouri, but neither WTB nor TCD have been detected in these states. In addition, the detection of *G. morbida* on 17 insect species besides *P. juglandis* in the eastern U.S. has demonstrated there is not a unique relationship between this fungus and the WTB. These findings emphasize that we do not know the true distribution of this insect/disease association or of the pathogen across the USA and/or that new introductions may still be occurring in the East. While TCD has caused dieback and mortality across various climatic zones and among several walnut species, the level of risk and extent of impact to black walnut within its native range are 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 or the vector (WTB). In laboratory and greenhouse trials, it has consistently proved to be the host of highest susceptibility (*G. morbida*) and elicited the highest level of reproduction for WTB among other walnut and allied tree species. However, *G. morbida* is considered a weak pathogen and appears to be an annual canker pathogen on branches of pole timber-sized to mature *J. nigra* in Indiana and Ohio based on field inoculation experiments spanning two growing seasons. In 2011, WTB was collected and *G. morbida* was isolated from a butternut tree, *Juglans cinerea*, at a private residence in Lane County, Oregon. While no trees to date have been found with TCD within the native range of butternut, the apparent susceptibility of the species to TCD is troubling from a conservation perspective. The survival of butternut is already seriously threatened by butternut canker, among other issues. The only known non-*Juglans* host for WTB and *G. morbida* is wingnut (*Pterocarya* spp.) based on collection records and laboratory studies from California. Laboratory and field research with *G. morbida* have shown that all walnuts, butternut, and wingnut show significant amounts of dead phloem in response to controlled inoculations of the pathogen. Three species of hickories (including pecan) that were tested are not susceptible to *G. morbida* and provide no basis for reproduction by WTB.

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 to branch mortality (Figure 1). The fungus causes distinctive circular to oblong cankers in the phloem (i.e., just under the outer 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 emergence holes may be visible on dead and dying branches (Figure 3), and bark beetle galleries are often found in multiple layers within the

cankers in the phloem (Figure 4). In the final stages of disease, even the main stem may exhibit beetle attacks and cankers. (Taken from USFS Pest Alert: Thousand Cankers Disease, February 2013 – Appendix 1)

Figure 1. Wilting black walnut in the last stages.

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

Figure 3. Emergence holes made by adult WTB.

Figure 4. Cross section of egg galleries of the walnut twig beetle in black walnut phloem. Photo by Albert Mayfield, USDA Forest Service.

Survey

The first step in detection is to locate walnut trees at risk by utilizing existing city tree inventories to delineate the urban walnut resource, and by identifying the locations of intensively managed stands of walnut trees in peri-urban or rural areas. Because the WTB may be transported beneath the bark on logs, burls, or large branches, walnut trees may also be at risk if they are growing near sites where enterprises such as walnut veneer or sawmills import and stockpile this material. Once walnut trees are identified, symptomatic trees with thinning crowns and fading leaves (i.e., yellow or bronze in color) should be evaluated in early to mid-summer, with special attention given to the upper portion of the crown of suspected diseased trees. A field identification guide for WTB and TCD is available (Appendix 2).

If TCD is suspected, the surveying agency may take a sample (see below) and elect to install a pheromone-baited, multiple funnel trap to target the WTB near walnut trees on the site (see below and refer to Appendices 8 and 9 or videos available at http://ipm.ucdavis.edu/PMG/menu.thousandcankers.html). A short, pheromone-baited walnut

branch section may be installed below the trap or on a nearby pole to lure live beetles to aid in the detection of the pathogen (*G. morbida*) (Appendix 11).
A decision tree is attached to this protocol to illustrate the visual survey process (Appendix 3).

Roles

Resources provided by the Forest Service should focus on surveys of walnut trees and WTB trapping in forested areas. Resources provided by PPQ should focus on surveys of walnut trees and WTB trapping 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. All surveyors (FS, PPQ, and State) should collect data on the "Walnut Twig Beetle Trap Result Reporting" spreadsheet (Appendix 4). FS will post the state specific datasheets to use at <http://www.fs.fed.us/foresthealth/technology/survey_tcd.shtml>. Please follow the instructions on the first tab and submit your data annually to Bruce Moltzan <bmoltzan@fs.fed.us> prior to November 1. Though latitude and longitude are requested, individual data points will not be made available outside the agencies sponsoring the collection or recording of that particular data (Federal and State).

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; wingnut trees may also be susceptible). See Appendices 5 and 6 for guides to assist in identifying *Juglans nigra* and other *Juglans* sp.
2. Are there other possible causes?
    a. Any recent root disturbances?
    b. Any recent pesticide use?
    c. Any leaf diseases (anthracnose) or target-like (Nectria) cankers?
    d. Any toothpick-like sawdust projections from the bark (caused by ambrosia beetles)?
    e. Insect entrance or emergence holes larger or differently shaped than those of WTB?
    f. Any other nearby activity or unusual weather patterns 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 worse 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 or the lower stem?

4. Are affected limbs easily accessible?
    a. Are numerous tiny reddish-brown beetles (1.5-2 mm long) and/or tunneling present beneath the bark?
    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?

Sample Collection and Handling

If you determine that TCD is a possibility, prepare to take a sample from the tree and consider the location for installation of a WTB trap (see below).

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 containing 70% ethanol
- Paper towels
- Gallon 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. Carefully peel thin layers of the bark away 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 (figure 5) or larvae are found during inspection or sampling, collect them in a leak-proof vial of 70% ethanol.
    e. Wrap each branch in paper towels. Double bag the sample in two Ziploc bags while on site and seal both bags. Multiple segments (maximum of 3) from the same branch can be packaged together as a single sample.
    f. Record sample information on “TCD Sample Collection Datasheet” (Appendix 7).
    g. Sterilize tools before collecting additional samples.

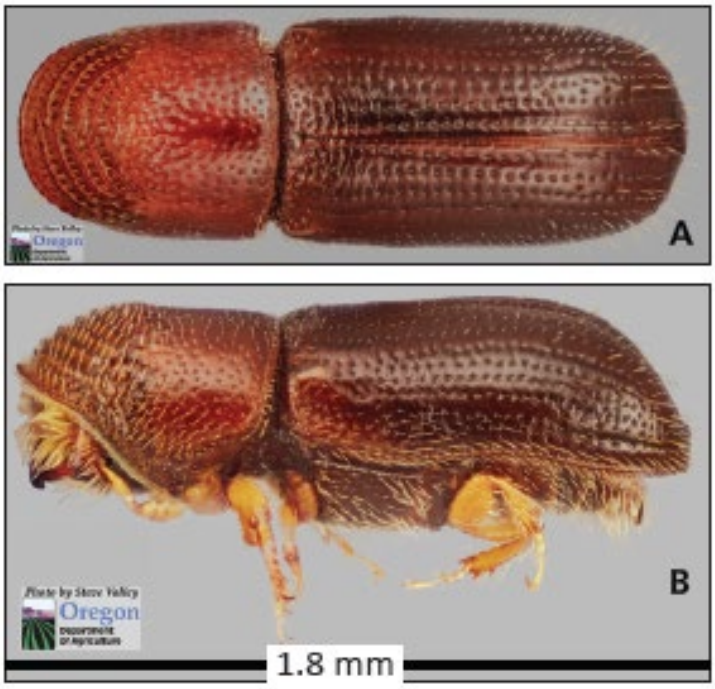

Figure 5. Walnut twig beetle: top view (A) and side view (B).
Credit: Steve Valley, Oregon Department of Agriculture
USFS Pest Alert: Thousand Cankers Disease, February 2013 (Appendix 1)

Trap Placement and Monitoring

The following information is excerpted from detailed trapping guidelines (Appendix 8) entitled: *Detecting and Identifying the Walnut Twig Beetle: Monitoring Guidelines for the Invasive Vector of Thousand Cankers Disease of Walnut*. A short version of these guidelines for field use is also available (Appendix 9). Users are encouraged to read these guidelines completely prior to initiating a trapping program.

Purpose of Trapping

The aggregation pheromone lure (available from ISCA Technologies, Inc., 1230 Spring St., Riverside, CA 92507, www.iscatech.com, 951-686-5008 or joey.palomera@iscatech.com) can be used, together with a trap, to detect an incipient population of WTB, or, in areas where TCD or WTB has been recently discovered, to delimit a known population.

Where to Locate Traps

For detection of WTB across a large area, such as a state-wide survey, a much lower density of traps is more feasible than the higher density needed to assess the extent of a known population. Costs of trapping (e.g., materials, labor, and travel) are important factors to consider in determining the number of baited traps that can be deployed.

Sites previously identified to have black walnut trees with unexplained dieback would be logical locations for trap placement in a detection survey program. Wood waste utilization sites, firewood lots, and saw or veneer mill sites with walnut logs and branches are also appropriate locations. In a delimitation survey to determine the extent of a population, a grid system may be useful for systematic placement of a larger number of traps.

Regardless of purpose, baited traps can be used near walnut trees in various types of sites, including residential areas, parklands, and roadways in urban areas; walnut plantations, arboreta, or orchards; and bottom-land or riparian forests in rural areas.

When to Trap

Ideally, pheromone-baited traps can be deployed whenever WTB are active; i.e., from March through November when maximum ambient air temperatures exceed 65° F. More likely, however, limited resources will require that state agencies involved in trapping limit trap deployment to a shorter time period. Two possible scenarios are:

1) Trapping for about six weeks from either late August through mid-October or from late April through mid-June. Starting dates in the spring will be earlier for more southerly states.
2) Trapping for three weeks in May/June and three weeks in September/October.

Materials and Supplies Needed

Materials and supplies needed for each trap (multiple funnel trap, lure, and antifreeze) are given in Table 3 on page 6 of Appendix 8. Additional items needed for hanging traps from poles are also included.

Installing Traps

The recommended approach to placing a trap on a site is to locate it about 9 to 15 ft. from the main stem of suspect walnut tree, 5 to 10 ft. from the live branches of that tree's crown, and hang the trap on a 10-ft pole so that the top of the trap is located about 9 ft. above the ground. Do not hang traps directly on trees as this may bring the tree under attack by WTB. Detailed installation instructions are provided in Appendix 8. Once hung, check the trap to ensure that it is hanging vertically and all funnels are fully separated.

Maintaining and Servicing the Traps in the Field

Routine checks and servicing of traps and collection cups – Traps should be checked every 7 to 14 days. Ensure trap is upright, not damaged or broken, and rainwater has not diluted the antifreeze or caused cup contents to overflow. If the latter has happened, remove trap catch as soon as possible. It is critical to use antifreeze that does not contain ethanol (=ethyl alcohol) or ethylene glycol in the trap cup. See Appendix 8 for details.

Lure replacement – Lures should be replaced approximately every 2 to 3 months. Each lure lasts for 2 months when the air temperature is 86° F constantly over a 24 hour period, so the replacement schedule will vary by season and location.

Collection of Trap Catches

Inspect the trap collection cups every 7 to 14 days to determine if beetles are present. Bag and preserve the catch as instructed in Appendix 8. Bag labels (written with pencil, not ink) should include the following information: Trapping site name/identification (County, Municipality, or other); trap number for that site; date trap period began; date sample was collected; and trap collector's name, agency affiliation and contact information (telephone number and email address at a minimum).

Upon return to the office, place catches in freezer (e.g., -10º F) for a minimum of 72 hours. After this freeze treatment, beetle samples may be shipped in a crush-proof box or directly delivered to the designated individual responsible for screening the sample in that state for WTB.

Determining Presence of WTB or *Geosmithia morbida*

Ideally, each state will have individuals or laboratories with sufficient expertise to process branch samples from suspect trees, including isolation of fungi from the samples and bark removal to obtain any potential WTB. These individuals or laboratories may also have the expertise to identify *Geosmithia morbida* or WTB. If so, this screening and identification is sufficient for confirmation. If not, screening aids to help identify both organisms are included in Appendix 8 and 10 (WTB) and Appendix 12 (*G. morbida*). Pure sub-cultures of suspect *G. morbida* cultures also may be sent to a regional lab with such expertise. Detection of *G. morbida* DNA on WTB and "by-catch" insects from trap catches using molecular methods can also be performed by such labs (Appendix 13). Suspect WTB that cannot be confirmed in-house may be placed in small, screw-top glass vials filled half way with 70% ethanol and shipped to a designated identifier (see Appendix 14).

If WTB are identified in trap catch (es) at site(s) not previously reported to have TCD, the trap catch collector for each site (or other trained individual) should return to the site(s) and inspect black walnut trees in the vicinity of the trap(s) yielding the WTB. Branches exhibiting symptoms of TCD, including branches with apparently healthy foliage but with WTB-sized entrance or emergence holes, should be collected and submitted to a plant diagnostic lab to assay for presence of *G. morbida* from bark cankers. In cases where the population of WTB is low, branches infected with *G. morbida* have been difficult to obtain in the field, so it may be more efficient to apply the pheromone-baited walnut branch technique to lure live WTB (Appendix 11). These beetles inoculate the fungus into the phloem of the branch section, which can be analyzed subsequently for *Geosmithia* in the laboratory (Appendix 12). Cankers may not be observed in the laboratory on the bait branches submitted, but isolation of *G. morbida* from

WTB found in galleries or from walls of the galleries is possible. TCD is confirmed by both the capture of the WTB and the isolation of the pathogen.

WTB in trap catches

Screening and Identification of WTB in Trap Catches

All trap catch collections received by the designated screening individual or laboratory should be stored in a freezer until processed. The suggested steps for screening catch samples are listed below:

1. Carefully examine folded filter and any other material included with the sample (e.g., leaves or large insects) or collection bag.
2. Sort out small insects (less than 3 mm long) from the larger insects.
3. Examine all small insects with a stereo dissection microscope (40 to 60 X power) and use Appendix 8 or 10 to focus on and sort through the small beetles.
4. Divide the above into either:
    a. Obviously not a bark beetle or not WTB-like → discard (or save if needed for a separate study)
    b. If potentially a WTB → proceed to next step.
5. Record number of potential WTBs found on Datasheet for Screening Individual (Appendix 13)
6. Transfer suspect insects to small screw-cap vial that is half full with 70% ethanol.
7. Label vial (paper labels written with pencil) with state, site identifier, trap number, date sample was collected (or date range for trapping), name of the collector and name of the screener
8. Photocopy trap catch data sheet with recorded numbers
9. Deliver or mail labeled vials with specimens plus appropriate datasheet(s) to the designated state or regional identifier (also see Appendix 14).

State/Regional Identifier Report

1. Examine submitted vials with suspect WTB
2. Record determination of WTB and, if desired, record determination of other beetles (Appendix 15 – Identified WTB Trap Catch datasheet)
3. If WTB found, notify the State Plant Regulatory Official.

Collecting and Isolating Geosmithia morbida

In addition to collecting samples of potential *Geosmithia*-infected walnut branches from the crowns or stem sprouts of trees, freshly cut, healthy walnut branch sections can be placed in the field to lure in the WTB. These smooth-barked branch sections (18 inches long x 1-2 inch diameter, ~45 cm x 3-6 cm) can be baited with the WTB pheromone lure and suspended by wire from the funnel trapping pole or from a separate pole. When left in the field for 2-4 weeks during the peak flight period (May-June or Aug. – Oct.), WTB will find and colonize the branch

section. When sufficient entrance holes have been observed on the trap branch sections (10-20 holes), the branch sections can be harvested from the field and handled subsequently like a branch sample taken from a tree suspected to have TCD. In areas with low population densities of WTB, the branch sections may need to be left in the field longer to accumulate sufficient WTB entrance holes.

For those who do not have the capacity to identify *G. morbida* or if screening is inconclusive (see Appendix 12), samples may be sent to a member laboratory of the National Plant Diagnostic Network. Locations of these laboratories 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 72 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 from the identification of submitted samples will be reported 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 or trapping plan. By collaborating with stakeholders and sharing these survey guidelines, more trees can be surveyed with the limited resources available. List of Appendices

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Appendix 1 – USFS Pest Alert: Thousand Cankers Disease, February 2013 (NA-PR-02-10). http://na.fs.fed.us/pubs/palerts/cankers_disease/thousand_cankers_disease_screen_res.pdf

Appendix 2 – Graves, A.D., Coleman, T.W., Flint, M.L., and Seybold, S.J. 2009. Walnut twig beetle and thousand cankers disease: Field identification guide, UC-IPM Website Publication, 2 pp., Nov. 21, 2009, http://www.ipm.ucdavis.edu/PDF/MISC/thousand_cankers_field_guide.pdf

Appendix 3 – Thousand Cankers Disease Survey Decision Tree

Appendix 4 – Walnut Twig Beetle Trap Result Reporting Spreadsheet

Appendix 5 – Key Identification Features of *Juglans* species

Appendix 6 – ID Guide for *Juglans nigra* (black walnut)

Appendix 7 – TCD Sample Collection Datasheet

Appendix 8 – Seybold, S.J., Dallara, P.L., Hishinuma, S.M., and Flint, M. L. 2013. Detecting and identifying the walnut twig beetle: Monitoring guidelines for the invasive vector of thousand cankers disease of walnut, University of California Agriculture and Natural Resources, Statewide Integrated Pest Management Program, 13 pp. **http://ipm.ucanr.edu/PDF/PESTNOTES/WTB_trapping.pdf**

Appendix 9 – Seybold, S.J., Dallara, P.L., Hishinuma, S.M., and Flint, M. L. 2013. Quick guide: Installing, maintaining, and servicing walnut twig beetle pheromone-baited traps, University of California Agriculture and Natural Resources, Statewide Integrated Pest Management Program, 2 pp., March 2013, **http://ipm.ucanr.edu/PDF/MISC/WTBtrapping_quickguide.pdf**

Appendix 10 – A Screening Aid for the Identification of the Walnut Twig Beetle, *Pityophthorus juglandis* Blackman

Appendix 11 – A Field Technique for Detecting *Geosmithia morbida* with Walnut Branches baited with the Aggregation Pheromone of the Walnut Twig Beetle, *Pityophthorus juglandis*

Appendix 12 – Isolation and Morphological Identification of *Geosmithia morbida*

Appendix 13 – Molecular Assay for the Detection and Identification of *Geosmithia morbida* on Insects on Fungal Cultures

Appendix 14 – Names and Contact Information for Expert Identifiers of *Pityophthorus juglandis* by USDA Forest Region

Appendix 15 – Datasheet for WTB Trap Catch Screening Individuals

Appendix 16 – Datasheet for Identified Walnut Twig Beetles Obtained from Trap Catch Samples