Tuesday, June 13 – Morning Session

Bill Wesela

APHIS-PPQ Response to Thousand Cankers Disease 2010-2017

In August 2010, the Plant Protection and Quarantine (PPQ) leadership team explored options to respond to TCD that ranged from not being involved at all to enacting a full regulatory program. The leadership team decided that PPQ would not take regulatory action on TCD but would continue to provide technical support as resources allowed in three areas – survey, traps and lures, and treatments. In making the decision, the leadership team considered what effect a regulatory response might have on TCD and agreed that, given the possible extent of TCD and the lack of effective regulatory controls, a regulatory response would have little impact on the spread of the disease and that resources would be better devoted to providing technical assistance. PPQ also decided to pursue a unified USDA response through a strategic partnership with the Forest Service.

Bill Luppold

Current Walnut Resource and Market Situation

After heavy rates of harvests that extended from the 1950s to the early 1970s, the walnut resource has increased by 210 percent since 1977. Walnut is one of the premier domestic hardwood species that has historically held the highest prices in lumber and log markets. Still, walnut lumber prices have declined in inflation adjusted or real terms since the early 1970s. However, real walnut stumpage prices have remained relatively constant between 1970 and 2000 and have increased by 150 percent since 2000. Exports of walnut lumber and logs have always been an important component of the overall market for this species but the importance of these markets has increased in the 21st century. Today it is estimated that 75 to 80 percent of the walnut timber cut is exported in some manner. While the traditional export markets for this species has been Western Europe, Japan, Canada and Korea; China is now the most important market for walnut lumber and logs.

Tuesday, June 13 - Afternoon Session

Debra Martin

Program Manager (State Plant Regulatory Officer or SPRO) for the Office of Plant Industry Services within the VA Dept. of Ag. and Consumer Services (VDACS).

I will be providing a TCD Regulatory /Survey Status Update for the state of Virginia, covering the first find, 5 years of survey data since, and the status of our quarantine and our thoughts/concerns about the survey results.

Scott Schirmer

Nursery and Northern Field Office Section Manager, State Plant Regulatory Official, Illinois.

Approximately 12% of Illinois is forested, totaling 4.4 million acres. Of that forested land, 53% consists of oak and hickory, with black walnut included in the hickory family. Based on these numbers, we estimated that nearly 2.3 million acres may contain black walnut, and are thereby potentially susceptible to TCD. This does not include cultivated walnut stands nor urban trees. At the time Illinois was considering regulations surrounding TCD in 2010-2011, Illinois ranked 5th in the US with regard to volume of black walnut growing stock on timberland with a saw timber volume of 855 million board feet, and had a 144 million board feet per year annual net growth. 15.6 million board feet were harvested per year with a $13.1 million value in paid stumpage, or $18.3 million value paid at mill price.

With >2000 miles of interstate highway, >34,000 miles of other highways, 3 coast to coast interstates, 2 of the largest rail gateways, >1000 miles of navigable waterways, and >100 sawmills in Illinois, the wood product and outdoor recreation industries, and the ecological value of black walnut, it was felt that introduction and spread of TCD was a significant risk warranting justification of an external regulation to prevent its introduction into Illinois.

G. morbiba was confirmed in Illinois in 2016 at 4 individual locations in 5 counties through destructive survey and log rearing operations. These detections were associated with non-WTB beetle species. WTB has not been found, and TCD has not been found infecting host material to date. Surveys are on-going and partial results have been reported thusfar with no positives, but operations will continue in 2017, and ideally beyond, to hopefully provide better resolution into the status of TCD in Illinois for regulatory, management, and education and outreach purposes.

Mike Bryan

Regulatory/Survey Status of TCD in Michigan

The Michigan Department of Agriculture and Rural Development (MDARD) promulgated a state exterior against the causal agents of Thousand Cankers Disease (TCD) of walnut in May 2010. TCD is not known to occur in Michigan and the quarantine is designed to protect the state's walnut resources. The Michigan TCD quarantine prohibits the movement of walnut nursery stock, logs, lumber and hardwood firewood (regulated articles) from infested states into Michigan. The current quarantine considers regulated articles from non-infested states that transit infested states to have been exposed to TCD and therefore prohibits those articles as well.

The Michigan TCD quarantine is under review and a draft revision is being considered which would allow shipment of regulated articles from non-infested counties in infested states into Michigan provide the origin state has an active TCD survey program. The proposed revision would also allow entry of regulated articles from non-infested counties that transit infected states and would allow heat treated hardwood firewood from all areas.

MDARD has conducted surveys to assure the absence of walnut twig beetle. Ongoing surveys performed under contract by Michigan State University have resulted in negative survey results for walnut twig beetle. Michigan DNR also conducts surveys on state lands, which have also demonstrated an absence of this insect.

Wednesday, June 14, - Morning Session

Steven J. Seybold

Host Selection Behavior and Impact of Walnut Twig Beetle in California

The walnut twig beetle, Pityophthorus juglandis Blackman, is a phloeophagous insect that vectors the pathogenic fungus, Geosmithia morbida. The beetle and fungus colonize the phloem of walnut, Juglans spp., and wingnut, Pterocarya spp., resulting in localized necrosis. Together, the beetle and fungus cause thousand cankers disease (TCD), which is fatal to these trees in the Juglandaceae. The beetle has expanded its range dramatically in the USA and has recently been introduced and expanded its range in Italy.

Since the host selection behavior of P. juglandis likely determines the impact and progression of TCD in various host trees, that behavior has been investigated in northern California by measuring and comparing rates of landing and gallery initiation by P. juglandis adults on intact branches and branch sections of walnut. Landing rates were assessed on branches of five host species native to North America (J. californica, J. cinerea, J. hindsii, J. major, J. microcarpa) and three non-native hosts (J. ailantifolia, J. regia and Pterocarya stenoptera). Host species significantly affected the landing rate of both male and female P. juglandis whether or not branches were infested. In several of these field assays, southern California black walnut, J. californica, elicited the highest landing rates among all uninfested trap branches or branch sections. Beetles showed a preference for landing on the underside of branches. Similar studies have been conducted with a native population of P. juglandis in southeastern New Mexico. Landing rates of P. juglandis are also being measured and compared near Davis among northern California black walnut, J. hindsii, and an ensemble of native and non-native riparian hardwood trees outside of the Juglandaceae.

The frequency, severity, and progression of TCD over time and space was surveyed in areas with native and planted walnut trees in California. Seven survey locations were selected in six counties spanning latitudes from 39.745° to 34.099° (680 km) and longitudes from –121.978° to –118.195° (350 km) (Butte, Los Angeles, Solano, Sutter, Tulare, and Yolo Cos.). Elevation of the locations ranged from 23 to 122 m. The survey included all walnut species native to western North America (J. californica, J. hindsii, J. major, J. microcarpa) as well as English walnut, J. regia, the species used in commercial walnut production. Disease-related symptoms and signs such as crown condition; number of P. juglandis entrance/emergence holes; and total number of sap stains on the main stem and branches were recorded and integrated into models. The greatest frequency and fastest progression of symptoms (years until 5% of same symptom cohorts die) occurred in the two Juglans species native to California (J. californica and J. hindsii). Symptoms progressed more slowly in J. regia and in J. major (the ancestral host of P. juglandis) than in any other species. Over the course of the study, there were also significant increases in rates of tree mortality for most species and locations. Cumulative TCD-associated mortality in native stands of J. californica and J. hindsii was approximately 10% by the end of the survey. Juglans californica at the USDA ARS National Clonal Germplasm Repository (Solano Co.) had both the highest cumulative level of mortality (43%) and nearly the highest cumulative rate of increase in mortality (from 5.5 to 43% or 9.4% y-1 from 2010-2014). The cumulative levels of mortality for J. regia across three locations were similar and less than 5%. The survey revealed that TCD is present with a relatively severe impact throughout the entire latitudinal and longitudinal range that was evaluated in California.

Yigen Chen

Walnut Twig Beetle Flight Capacity and Behavior in Response to Weather Effects

Yigen Chen[1,5], Aubree M. Kees[2], Brian H. Aukema[2], Robert C. Venette[3], and Steven J. Seybold[4]

[1]University of California, Davis, CA; [2]University of Minnesota, St. Paul, MN; [3]USDA FS, St. Paul, MN; [4]USDA FS, Davis, CA; [5]Current address: E&J Gallo Winery, Modesto, CA

The walnut twig beetle (WTB), *Pityophthorus juglandis*, vectors the fungus *Geosmithia morbida*, and both organisms play a role in thousand cankers disease of walnut. To better predict its future range expansion and to provide guidance for detection trapping, we examined seasonal aspects of the flight habits of WTB. Studies on a flight mill in the laboratory revealed that WTB is a weak flyer, and the maximum flight distance unaided by wind is approximately 3.6 km in 24 h-few individuals. Flight distance, flight velocity, and total flight time did not differ between the two sexes of WTB, although males were larger than females. Proportion of beetles that initiated flight declined with beetle age (< 5% at five days after emergence). How WTB flight patterns vary with weather fluctuations has been poorly understood. Therefore, we sampled flying adults weekly, diurnally, and bihourly over various periods of time with 12-unit black plastic multiple funnel traps baited with male-produced aggregation pheromone In California (USA). We then examined the flight pattern with temperature, humidity, barometric pressure, and wind speed. Our results indicated that weather variables collectively and interactively affected WTB flight.

In summary, most WTB flight occurred at 1) temperatures of ca. 26–27 °C; 2) light intensities of less than 2000 lux; 3) barometric pressures around 755–757 mbar; and 4) wind speeds between 1 and 4 km/h. Since WTB is a weak flyer, human- and wind-aided dispersal might be the major method of spread.

Matthew Ginzel

Behavioral responses of Pityophthorus juglandis (Coleoptera: Curculionidae: Scolytinae) to volatiles of black walnut and Geosmithia morbida, the causal agent of Thousand Cankers Disease

Thousand cankers disease (TCD) is a pest complex formed by the association of the walnut twig beetle (WTB), Pityophthorus juglandis Blackman (Coleoptera: Curculionidae: Scolytinae), with the fungal pathogen Geosmithia morbida Kolařík, Freeland, Utley and Tisserat. Current monitoring and detection efforts for WTB rely on a pheromone lure that is effective over a limited distance. Influences of plant- and fungal-derived volatile organic compounds that may facilitate host location within this system remain poorly understood. In this study, we test the hypothesis that adult beetles are attracted to volatiles of black walnut and G. morbida. We characterized the VOCs emitted from the leaves and stems of twelve genotypes of black walnut and found that adult WTB were more attracted to some genotypes than others. Gas-chromatography-mass spectrometry analysis revealed that three compounds (i.e., camphene, α-pinene and cymene) were more highly represented in the volatile profile of the most attractive genotype when compared to the least attractive. Moreover, girdling the most attractive genotype resulted in an increase in camphene and α-pinene emissions, and a decrease in the amount of cymene and limonene released. In a field experiment, the addition of limonene to pheromone-baited traps repelled adult beetles, suggesting that this compound could be used to manipulate populations of WTB. Finally, through behavioral bioassays, we demonstrate for the first time that adult WTB are attracted to volatiles of G. morbida. These findings suggest that, in addition to aggregation pheromones, dispersing adult beetles potentially use host plant and fungal volatiles to locate suitable host trees.

Robert Venette

Walnut Twig Beetle cold tolerance and colonization

Andrea Hefty1, Robert C. Venette2*, Brian Aukema1, Mark Coggeshall3,4, James McKenna4, and Steven J. Seybold5.

1 Department of Entomology, University of Minnesota, St. Paul, MN, 2 USDA Forest Service Northern Research Station, St. Paul, MN, 3 The Center for Agroforestry, University of Missouri, Columbia, MO, 4 USDA Forest Service, Hardwood Tree Improvement & Regeneration Center, West Lafayette, IN, 5 USDA Forest Service Pacific Southwest Research Station, Davis, CA.

The walnut twig beetle, Pityophthorus juglandis Blackman, plays a central role in the dynamics of thousand cankers disease on Juglans and Pterocarya spp. Although the beetle has spread from its native range in northern Mexico and the southwestern United States through parts of the United States and Europe, projections of future spread and impact have been hampered by insufficient knowledge about its basic population ecology, particularly its cold tolerance and host usage. One objective of the study was to identify cold temperatures that might be lethal to overwintering walnut twig beetle and determine whether cold might constrain its distribution within the geographic range of eastern black walnut, Juglans nigra. We used contact thermocouple thermometry to measure the supercooling points (i.e., the temperature responsible for the onset of freezing) of adults and larvae and lower lethal temperatures of adult walnut twig beetle from a population from northern California. Supercooling points ranged from -12.2°C to -25.0 °C for adults; lower lethal temperatures of adults ranged from -14 °C to -23 °C. Parts of the native geographic range of eastern black walnut may be too cold currently for this insect to persist. The second objective of this research was to determine potential limits to, and variation within, the host range of walnut twig beetle. Colonization and reproduction by P. juglandis were studied in no-choice laboratory experiments with 11 Juglans spp., one Pterocarya sp., and two Carya spp. over two years, and we found that all but the Carya spp. were hosts. Considerable variation in quality occurred among hosts, so the availability of quality hosts could constrain the future distribution of walnut twig beetle. Breeding for host resistance to walnut twig beetle appears to be a viable management option. In addition, information on cold tolerance and host utilization provides a useful backdrop to evaluate the need for measures (e.g., quarantines) to prevent future spread of this insect.

Paul Rugman-Jones

Phylogeography of walnut twig beetle in North America

Paul Rugman-Jones & Richard Stouthamer, Department of Entomology, University of California Riverside, CA

Walnut twig beetle (WTB), *Pityophthorus juglandis,* has a long (but sparse) collection history in southwestern areas of North America (collected in 1896, New Mexico; 1907, Arizona; 1959, California; 1960, Mexico), but historically, this beetle has not been considered a major pest of walnut trees. However, since the early 1990's, the range of WTB has increased rapidly, and the beetle is now widespread in all western states of the U.S. This range expansion has been accompanied by the emergence of a deadly plant disease, Thousand Cankers Disease (TCD). TCD results from aggressive feeding by WTB, accompanied by inoculation of its feeding galleries with a pathogenic fungus, *Geosmithia morbida*, carried by the beetle. WTB and TCD have now crossed the Great Plains, establishing in several eastern states, and threatening economically (> $500 billion) and ecologically valuable native stands of eastern black walnut, *Juglans nigra*. Mitochondrial DNA sequences have revealed two clearly divergent genetic lineages among North American populations of WTB: one with likely phylogeographic (native) origins in the Madrean Sky Island region of southern Arizona and New Mexico; the other with likely phylogeographic origins in the neighboring Gila National Forest region of western New Mexico. Despite their proximity, there is very little evidence of the WTB lineages mixing between these regions. Indeed, geographic overlap of the two lineages is only common in parts of Colorado and Utah. Ribosomal DNA sequences corroborate the mitochondrial lineages, but also reveal evidence of potential hybridization between them in all areas except those identified as being the most likely native origins. These findings: have implications for the status of WTB as a single species; help reconstruct its recent range expansion; and, may provide some insight into the emergence of TCD.

Melanie Moore

Development of a specific PCR Assay for detecting Geosmithia morbida on scolytine insects

Per its initial description, thousand cankers disease (TCD) results from branch and stem death of eastern black walnut (Juglans nigra) caused by large numbers of Geosmithia morbida (Gm) cankers that develop around galleries of the walnut twig beetle (Pityophthorus juglandis) (WTB). The fungus is considered a symbiont of the WTB and the beetle an efficient vector of the plant pathogenic fungus. Gm, however, has more recently been detected on other scolytine insects (bark-colonizing weevils, ambrosia beetles) based on both isolation and molecular assays. The published reports of the latter assay method are based on polymerase chain reaction (PCR) of DNA extracted from macerated beetles using a Gm specific primer (Geo3) paired with ITS4. DNA sequencing, however, is required because the primers can amplify Trichoderma as well as Gm. Since the disease is likely to be detected in new locations, there is a need for an efficient Gm-specific assay for insects so that states and municipalities can detect early on if the TCD fungus may be in their area. We developed a PCR-based assay with primers targeting the β-tubulin gene. It is both highly specific for Geosmithia morbida and able to detect very small concentrations of DNA. Recently, it has been used operationally for assaying WTB's and other scolytine insects from trap catches and/or emerged from trap logs in five states. The PCR assay has detected Gm DNA on a number of these insects, while culturing methods have detected viable Gm on them only rarely. The presence of Gm on these insects in non-TCD areas gives evidence that Geosmithia morbida may have been cryptically present in the eastern forests already. Since the assay only detects the presence or absence of Gm DNA, we are not able to assess fungus viability on the assayed specimen nor the potential for a specific insect to successfully transmit the pathogen to a healthy walnut. Additional research will be needed to assess the level of viable propagules of Gm on these scolytines and to study their potential ability to colonize healthy black walnut trees in sufficient numbers to cause damage.

Jennifer Juzwik

Relative virulence of canker-causing fungi on Juglans nigra in Indiana and Ohio

Mortality of eastern black walnut (Juglans nigra) is reported to occur as a result of coalescence of an enormous number of bark cankers caused by Geosmithia morbida. The fungus is introduced to the host by its insect vector, the walnut twig beetle (WTB) (Pityophthorus juglandis), that attacks and colonizes the host. It is important to understand the role of each of these biotic agents in development of branch dieback and tree death of what is now known as thousand cankers disease (TCD) so effective and cost-efficient control methods can be devised. Other native, canker-causing fungi of J. nigra, also have been found colonizing TCD-symptomatic trees. Field experiments were established in 2014 and 2015 to determine: 1) whether multiple inoculations with G. morbida in the absence of WTB can lead to branch death over two growing seasons, and 2) the relative virulence of G. morbida compared to other known or putative canker pathogens of J. nigra within its native range and in the absence of WTB. Small masses of G. morbida-colonized agar slurry and of sterile agar were placed in each of 50 holes (1 per 12 cm2 bark area) drilled to the cambium on multiple branches of five J. nigra in a Brown County, IN, plantation. Bark canker areas associated with each inoculated hole were significantly greater for both of the two G. morbida isolates used compared to controls at both 3 and 15 months after treatment. However, significantly smaller canker areas were found for branches harvested at 15 months compared to those cut at 3 months post-inoculation. Callus wound tissue produced by the host limited canker expansion and, in some cases, was closing the wound. No fungus-inoculated branches died prior to harvest. Experiments on relative virulence of several known or putative canker pathogens were initiated in June 2015 in J. nigra in three parks in Butler County, OH, and on additional J. nigra in the IN plantation. Aliquots of aqueous suspensions of spores or of sterile distilled water were placed in 24 to 42 drilled branch holes (depending on branch diameter) at the same density used in 2014 study. Inocula of locally-obtained isolates of Fusarium solani, Diplodia seriata, and G. morbida were used in both locations. In addition, Botryosphaeria dothidea was added to the IN study. Four (OH) or five (IN) branches per tree (10 in IN; 9 in OH) were selected and randomized to receive one of the treatments. Details on branch condition were recorded in September 2015 and in mid-June and early September 2016. Several branches died in each location over the two growing seasons; however, none had been inoculated with G. morbida. Branches were harvested on the final monitoring date, bark carefully removed to expose any cankers around inoculation points, and data on canker size recorded. No differences were found in percentages of cankered inoculation points on a branch across all treatments within a location. Mean canker sizes (cm2) in IN were smallest for control and G. morbida-inoculated branches and largest for B. dothidea branches. In OH, the smallest mean canker sizes were found for control and D. seriata branches compared to those for cankers associated with F. solani isolates (species complex 6 and 25, per M. Kasson, West Virginia University) and G. morbida. No evidence of canker coalescence was observed for any of the G. morbida-inoculated branches. In summary, numerous G. morbida inoculations did not result in branch death on J. nigra in the two eastern USA study sites. Furthermore, G. morbida was found to be a very weak pathogen based on size of cankers and host callus response observed 15 months after inoculation.

Jane Stewart

Investigation into virulence and pathogenicity mechanisms of different genetic clusters of Geosmithia morbida

Jane Stewart, Rachael Sitz, Jorge Ibarra Caballero

Colorado State University, Department of Bioagricultural Sciences and Pest Management, Fort Collins, CO

Geosmithia morbida is well documented as the causal agent of thousand cankers disease of black walnut trees. However, it is currently not known if G. morbida strains differ in virulence, how interactions with co-occurring pathogens contribute to disease severity, or the molecular basis of pathogenicity. This presentation will discuss research that investigated virulence of genetically distinct G. morbida strains that were collected in Colorado, effects of co-inoculation with Fusarium solani (FSSC 6) and examined pathogenicity factors in G. morbida. We inoculated adult walnut trees with eight strains of G. morbida representing recently identified genetic clusters, and also co-inoculated these with F. solani. We found varying degrees of virulence, although differences were not related to genetic groupings. Furthermore, the degree of disease contribution from the co-inoculation with F. solani did not yield a synergistic response; virulence of G. morbida isolates co-inoculated with F. solani were not significantly different. Lastly, the transcriptomes of G. morbida growing on mycelium and on black walnut after 7 days were compared to identify putative pathogenicity factors. In preliminary results, of the total 7,971 expressed transcripts, 276 were differentially expressed between mycelium collected from potato dextrose agar and mycelium collected from black walnut. Possible pathogenicity factors included transcripts found only in mycelium collected from black walnut, which included ABC transporters, peroxidases, peptidases, and endo-1,3-β-glucosidases. Further work will identify genes expressed on black walnut at different time internals in comparison to non-pathogenic Geosmithia species.

Denita Hadziabdic

The things we know and don't know about what we don't know about TCD (or, I'm pretty certain I need a drink)

The plant pathogenic fungus, Geosmithia morbida, vectored by the walnut twig beetle (WTB), Pityophthorus juglandis, has been associated with a disease complex of walnuts, Juglans spp., known as thousand cankers disease (TCD). Infected trees initially display wilting and yellowing foliage, branch dieback and eventually tree mortality within 3–4 years after symptoms develop. As the WTB and the pathogen move within the phloem and spread throughout the tree, multiple dark brown- to- black cankers form, coalesce, and girdle twigs and branches, hence the name "thousand cankers" to describe the disease. TCD was originally described from the western U.S. and now has expanded to the native, eastern range of black walnut in the U.S. TCD has recently been discovered in northwestern Italy on both black and native J. regia, English walnut. Due to pathogen movement and possible global distribution, there is a critical need to understand

genetic diversity and population structure of both the fungal pathogen, G. morbida, and its vector, WTB. We used microsatellite loci to investigate population structure and spatial distribution of G. morbida and WTB in the U.S. and Italy. Our results indicated high genetic diversity among G. morbida and WTB subpopulations with evidence of gene flow, presence of population structure, and a significant correlation between geographic and genetic distance. Analysis of molecular variance (AMOVA) indicated that most of the genetic variation was attributed to individual variation rather than divergence across sampling localities. Our results support the hypothesis that G. morbida and WTB were disseminated to different regions multiple times from multiple sources. Understanding the genetic composition, host-pathogen-vector interactions and demography of this complex can provide insight into future predictions of TCD occurrences.

Wednesday, June 14, - Afternoon Session

Romina Gazis

Making Thousand Cankers Disease diagnostics simpler, faster and cost-efficient

Thousands Cankers Disease (TCD) is often difficult to diagnose due to the absence of external signs and the non-specific symptoms in the host. Although early detection of TCD is critical to delimit quarantine zones, it is far more important to detect the pathogen/vector presence in wood products that are in-transit as cargo or as packaging material. Timber trade in the USA is extensive and the movement of TCD complex members across our borders can have detrimental impacts in the nut and timber industry at a global scale. Current methods for TCD diagnosis require the identification of both complex members (*Geosmithia morbida* and *Pityophthorus juglandis*) through morphological examination or through standard DNA barcoding. While the use of DNA barcodes has significantly facilitated the identification of TCD members, especially by circumventing the need of taxonomic expertise, these methods require the axenic isolation of the organisms. The latter is especially difficult for *G. morbida* due to its slow growth and co-existence with many other wood-associated fungi. Using species-specific microsatellite regions, we developed a rapid molecular protocol for the simultaneous detection of *G. morbida* and *P. juglandis* directly from infected wood material. To demonstrate the utility of the method, we tested samples collected in localities with different TCD incidence levels: California (high incidence), Tennessee (low incidence), and Missouri (no incidence). A total of 1600 drill cores were taken from 40 trees at each location. Results confirmed that California samples had the highest incidence of the TCD organisms (85%, 34/40) compared to Tennessee (42.5%, 17/40) and neither organism was detected in Missouri. We demonstrated that the protocol has a high sensitivity and specificity, and it significantly reduces the sample-processing time, making it a powerful tool for the detection of TCD.

Karandeep Chahal

TCD research in Tennessee: Geosmithia morbida virulence, alternative vectors and associated Geosmithia species

Fungal pathogen, *Geosmithia morbida* along with its vector, *Pityophthorus juglandis* are the causal agents of Thousand Cankers Disease complex in black walnut (*Juglans nigra*). Tree mortality has been more severe in the western states than in the eastern states. To test the hypothesis that *G. morbida* isolates from the western and eastern states differ in virulence, I am comparing 5 isolates from 5 haplotypes (n=25) for differences in virulence. Results from my preliminary experiment showed significant differences in virulence among *G. morbida* genetic clades. My up-coming results will be available to determine if virulence is related to each haplogroup of the fungus. In the eastern U.S., *G. morbida* has also been detected on other insect species. To better access the potential for alternative beetles to vector the fungus in Tennessee, wood boring beetles were collected in black walnut canopies using ethanol-lured traps. Out of sixteen analyzed insect species, *G. morbida* was detected on eleven beetle species. Beetles detected with *G. morbida* and collected in high numbers were *Cnestus mutilatus*, *Dryoxylon onoharaense*, *Monarthrum fasciatum*, *M. mali*, *Xyleborinus saxesenii*, and *Xylosandrus crassiusculus*, (Coleoptera: Curculionidae: Scolytinae), *Stenomimus pallidus* (Coleoptera: Curculionidae: Cossoninae), *Xylobiops basilaris* (Coleoptera: Bostrichidae) My findings raise the awareness of the potential threat imposed by alternative vectors that may contribute to the spread of *G. morbida* within the native range of black walnut. While exploring alternative vectors of *G. morbida*, genetically distinct species of *Geosmithia* were detected to be associated with bark and ambrosia beetles in Tennessee. Forty-five isolates of Geosmithia species were obtained with 34 isolates grouped within the "*G. pallida*" species complex and coincided to previously-reported, but not formally-described species. Four other isolates from "*G. pallida*" species complex did not cluster within any pre-described genetic clade and these isolates may represent a novel clade. Another 11 isolates were identified as *G. obscura*, *Gesomithia* sp. 10, and *Geosmithia* sp. 21. Some of our isolates have a close genetic relationship with *Geosmithia* sp. 41; the pathogen causing Foamy Bark Canker disease on coast live oaks in California. This report raises awareness of a potential emergent threat to oaks in native forests.

Keith Woeste

Genetics of *Geosmithia morbida*: How pathogen genetics can help us to understand an epidemic.

Genetics first contributed to TCD research by helping to answer the question—what is the fungus causing TCD? The answer was found by Tisserat and colleagues in 2011 using genetics (sequencing) and classical mycology. It is important to know what fungus is causing a disease because we need to know if we face a new enemy or one we have experience with, and because related fungi sometimes have similar epidemiology, similar pathology, and similar methods of control. After identifying G. morbida as new, the next step was to understand its genetic diversity and structure. Diversity is genetic variability and structure is the relationship between variability and the environment, most especially the host. We have data showing diversity is low -- of dozens of ITS sequences submitted to GenBank, all are 100% match. The genetic structure of Gm is consistent with human movement of clonal lineages, not habitat or vector or host specialization. In short, Gm seems to be a Juglandaceae generalist, although not all Juglandaceae have been shown to be susceptible (Pecan). I could not determine if new reports in Europe or reports on new hosts indicate genetic specialization because I haven't seen data for these isolates. There are no previous reports of specialization (strong race-host associations). P. juglandis seems the most common vector but it is also possible the fungus will begin to evolve specialization on vectors too. The main factor limiting Gm evolution is its asexual lifestyle. The complete genome of G. morbida has been studied in some detail (In Press). Most Geosmithia are not pathogens, so how GM became one is not clear and has been called a "black swan" event. There are now two Geosmithia species that appear pathogenic. There are at least 3 genetic types of Gm based on SNPs and SSRs, but genetic type was not associated with increased virulence. Some isolates did appear to cause larger cankers, but the reason for differences in virulence is unknown and may not be genetic—it seems clear the epidemic is not caused by a single clone outbreak but movement by a generalist onto new hosts in new environments. That pattern may make Gm harder to control than other types of pathogens.

Mark V. Coggeshall

Results from a Thousand Cankers Disease screening project

As part of a USDA Specialty Crops Research Initiative (SCRI) project, a study was conducted to evaluate potential levels of host tolerance in black walnut (Juglans nigra) inoculated with two isolates of Geosmithia morbida (Gm), the fungal pathogen that causes Thousand Cankers Disease (TCD). In the eastern U.S., field observations strongly suggest that disease development is also related to water stress. As a result, the decision was made to subject all trees to one of two drought stress treatments. Black walnut clones from the University of Missouri (MU) breeding program, plus representative clones originally acquired by the USDA Forest Service in Carbondale, IL were artificially inoculated in a poly-house structure located at the University of Tennessee/Knoxville in June 2016, using two Gm isolates from Ohio and Tennessee. The extent of canker development (length, width, depth) at each inoculation point was evaluated in December 2016. All data were analyzed using the GLIMMX ANOVA procedure in SAS. For canker length, significant differences were detected among clones and Gm strains, but not water stress, nor any interactions associated with stress. For canker width, there were significant differences among clones and water stress, but not Gm strain. Again, there were no significant interactions. Overall, water stress was not a significant factor for canker length. However, water stress did effect canker width, with unstressed trees exhibiting greater wider canker development, which suggests that 1) the water stress treatment used was inadequate in terms of impacting Gm canker development, or 2) inoculation studies using containerized trees will ultimately need to be repeated in the field to fully understand the impact of drought stress on TCD development in the eastern U.S.

Ron Mack

Vacuum & Steam Treatment for TDC Insures Safe Movement of Black Walnut Logs

Effective treatments for causal organisms of thousand cankers disease on quality black walnut logs are currently lacking. Fumigation with methyl bromide is uncertain, and conventional heat treatment would likely degrade logs due to moisture loss and subsequent checking. Vacuum and steam in combination was evaluated using two distinct treatment regimes (56C/30 min.; 60C/60 min. exposure to outer sapwood) on J. nigra logs (21.5 - 42 cm dia.) that were artificially inoculated with Geosmithia morbida in an effort to kill the fungus in colonized wood. For each treatment regime, there were 5 replicates of paired logs, with the last 56C/30 replicate containing one additional log. The average treatment time and energy use for 56C/30 min. exposure was 4 hours and 16 minutes (48.92 kwh), and 5 hours and 8 minutes (49.85 kwh) for 60C/60 min. exposure. For assay, a 30 cm section of the log was removed from each end before and after treatment for pathology and P. juglandis emergence. Isolations consisted of removing 4 wood chips from the margins of sixteen inner bark cankers on both the pre and post treatment log sections and plating on ¼ strength potato dextrose agar amended with streptomycin and chloramphenicol. Pathogen re-inoculation rate on the 22 pre-isolation logs sections was 9.94%, and post- treatment pathogen re-isolation rate was 0.28%. There were no P. juglandis recovered from emergence barrels on post-treatment log sections, and the percent of P. juglandis collected from pre-treatment log sections that tested positive for G. morbida was 17.5%. Results indicate that vacuum and steam has great potential as a quarantine treatment for TCD causal organisms in black walnut logs, paving the way for safe domestic and international movement in commerce.

Albert (Bud) Mayfield

Phytosanitary wood treatments for TCD and nursery stock colonization by the walnut twig beetle

Jackson Audley, Scott W. Myers, Adam Taylor, William E. Klingeman, Stephen W. Fraedrich, Paul Merten, Robert Camp, Albert E. Mayfield III

Thousand cankers disease, caused by the walnut twig beetle (Pityophthorus juglandis, or WTB) and an associated fungal pathogen (Geosmithia morbida), threatens the health and commercial use of eastern black walnut (Juglans nigra L.), one of the most economically valuable tree species in the United States. Effective phytosanitary measures are needed to reduce the possibility of spreading this insect and pathogen through the movement of infested wood or plants. When stem-heating infested walnut bolts, G. morbida did not survive in logs exposed to treatments in which minimum temperatures were 48°C or higher, and mean WTB emergence decreased steadily to zero as treatment minimum temperature increased from 36 to 52°C. Methyl brominde fumigation at 80 mg/L for 24h at 5°C prevented WTB emergence from infested bolts; an effective schedule for G. morbida is still being evaluated. Wood (bark present) treated with steam heat, fumigation, or kiln drying was recolonized by WTB when baited with pheromone under high beetle pressure, but risk of wood recolonization was low when a pheromone attractant was absent. Bark treatments with permethrin prohibited colonization by WTB. Beetles caged directly onto nursery stock bored into the stems, but in the field WTB only attempted to colonize seedlings that were baited with a pheromone lure and there was no evidence of successful progeny development in nursery stock.

Albert (Bud) Mayfield

Susceptibility of the walnut twig beetle to pathogenic fungi

Louela A. Castrillo, John D. Vandenberg, Michael H. Griggs, Adam Taylor, Jennifer Juzwik, Robert Camp, Bryan Mudder, and Albert E. Mayfield II

Thousand cankers disease (TCD), caused by the walnut twig beetle (WTB), Pityophthorus juglandis, and its associated fungal symbiont, Geosmithia morbida, is a disease of economic and ecological concern on eastern black walnut, Juglans nigra. Because advanced development of TCD requires multiple WTB generations and numerous beetle attacks, control strategies that reduce beetle attacks and brood production, without completely eliminating infestation, could significantly benefit tree health and survival. We evaluated the use of entomopathogenic fungi Beauveria bassiana and Metarhizium brunneum against the WTB. Laboratory and field studies showed that WTB adults are susceptible to commercial strains B. bassiana GHA and M. brunneum F52. Exposure of beetles to sprayed walnut bolts resulted in reduced brood production, primarily due to the death of parental adults from fungal infection prior to egg laying. Spraying walnut bolts with B. bassiana GHA and M. brunneum F52 prior to field exposure to natural WTB populations reduced emergence of next generation adults by up to 98% and 96%, respectively, due to a combination of fewer beetle attacks and mortality among those that tunneled. These results demonstrate the potential use of entomopathogenic fungi in the integrated management of TCD in walnut trees.

Bill Wesela

TCD Survey Guidelines

Since 2010, Plant Protection and Quarantine (PPQ) has worked closely with the Forest Service to support the development of, and continue to maintain annually, the survey guidelines that include how to use traps, information on how to identify walnut twig beetle and Geosmithia morbida, and resources for identification assistance. We also have funded surveys in 20 states since 2010, which supports survey of walnut trees and walnut twig beetle trapping in urban, residential, and industrial settings.

Thursday, June 15, - Morning Session

Matthew Ginzel

Integrated Pest Management of Thousand Cankers Disease in Black Walnut Plantations

Thousand cankers disease (TCD) is a pest complex formed by the association of the walnut twig beetle (WTB), *Pityophthorus juglandis* Blackman (Coleoptera: Curculionidae: Scolytinae), with the fungal pathogen *Geosmithia morbida* Kolařík, Freeland, Utley and Tisserat. This disease has caused the widespread death of walnut trees (*Juglans* sp.) throughout the western United States, and more recently it has been detected within in the eastern U.S. – the native range of black walnut. The long-term protection of the walnut resource at risk to TCD requires the integration of multiple tactics, and there is a critical need for more effective methods for managing the disease in plantations of high-value trees. This project aims to systematically evaluate components of an integrated pest management strategy to mitigate the severity of Thousand Cankers Disease (TCD) in plantations of eastern black walnut (*J. nigra*). This approach relies on pheromone-baited traps for monitoring, along with trap trees and the conservative use of insecticides to reduce walnut twig beetle (WTB) populations and protect high-value crop trees.