**Missouri Chapter**
The Missouri Chapter of the Walnut Council had its spring meeting on Friday and Saturday, May 10 & 11 at member’s properties in central Missouri. On Friday, the 32 attendees carpoolied to the Fred Crouse property. Management of mainly white oak was discussed along with the past management history and plans for the future.

Then the group visited the Jeremy Wilson property, which has been managed intensively for the last few years. The tract had been environmentally harvested and this was followed by a timber stand improvement practice. One of Jeremy’s goals for the tract is to manage for high quality white oak and to harvest a tree per acre per year. He has his own equipment and has harvested the tract marketing the lower quality trees and removing most of the pole material, some of which has been sold as firewood. He is also trying to sell the remaining small wood and cull logs for biofuel.

From that point the group drove to Scott Brundage’s property. Scott has about 20 acres under a deep proof fence (most of the time). He is cooperating with HTIRC on a plantation of walnut selections from several states. Each year the trees are pruned lightly and evaluated for forest, diseases, nut production and growth.

Scott also has several thousand pitch/loblolly hybrids growing on the property for potential pine straw production. There is also a plantation of walnut selections from several states. Each of these selections has been managed intensively for the last few years. The management history and plans for the future.

On Saturday, 44 attendees from Missouri, Minnesota and Iowa met at the property of Mike and Nancy Gentzsch in Callaway County. The morning session involved the selection, grading and evaluating walnut trees for marketing. During lunch a representative from the American Chestnut Foundation discussed research being conducted with chestnut trees, including how to plant and grow them. The afternoon session demonstrated pruning, spacing, and decision-making for thinning the plantation for maximum timber growth. The fall field day will be October 5th in Greene County.

Submitted by Susan Greene

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**Indiana Chapter Report**
The Indiana Chapter met on April 20th at the Bill Rodecker property in central Indiana with 34 in attendance. The sessions were led by Lenny Farlee (Purdue University extension), and Phil O’Connor (Indiana Division of Forestry). The 10 year old planting featured over 2,000 walnut trees with some single and double row white pine borders. The site has a gradient in soil quality and is scheduled for thinning and pruning soon. The trees represent genetic tests of select seedlings versus bed run seedling, sprouted seed from a single improved tree, and a progeny test of walnut showing the effects of diminishing soil quality.

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**Bark and Ambrosia Beetles and Their Associated Fungi Colonizing Stressed Walnut in Missouri and Indiana**

By Dr. Sharon Reed and Dr. James English, post-doctoral fellow and professor of the University of Missouri Plant Sciences Division, Dr. Jennifer Juvalk, forest pathologist of the Forest Service Northern Research Station, and Dr. Matt Grinzel, associate professor of the Purdue Department of Entomology

Thousand cankers disease (TCD) is a growing threat to black walnut, an economically and ecologically important tree in the eastern US. Trees in the earliest stages of TCD do not display symptoms. In later stages, leaves of the canopy become yellow, wilt, and ultimately die, yet remain attached to their branches. Eventually, all branches in the canopy may die, leading to tree death.

TCD is caused by the fungus, Geosmithia morbida that is carried by walnut twig beetles (Pityophthorus juglandis) attracted to walnut trees. After landing on a tree, the beetles bore into the inner bark to create egg galleries. The fungus spreads from galleries and creates small bark cankers. When trees are attacked by a large number of beetles, the fungal cankers can coalesce and lead to development of the disease (Figure 1).

**Declining walnut trees with TCD symptoms were first described in the 1900’s in the southwestern US, within the native range of walnut twig beetle. However, the role of the beetles in tree decline was not known at first and decline was blamed on drought. Then in 2009, Colorado scientists made the connection between walnut twig beetles, the pathogenic fungus, and declining black walnut trees. Since that time, the walnut twig beetle and Geosmithia morbida have been detected outside of their native southwestern range, with known occurrences in nine western states and three eastern states, including Tennessee, Pennsylvania, and Virginia. G. morbida infected trees have also been detected in North Carolina but not the beetle, and walnut twig beetles have only been collected from insect traps located at an Ohio mill.**

In addition to walnut twig beetles, there are many other types of bark- and wood-boring beetles that create egg galleries in black walnut trees. Most of these beetles carry fungi on their bodies, some of which can be pathogenic to trees. Researchers at the University of Missouri, Purdue University and the Forest Service have teamed up to investigate which bark and ambrosia beetles attack black walnut in the Midwest and to characterize the types of fungi that they carry with them. Bark beetles create egg galleries in the inner bark and ambrosia beetles create egg galleries in the wood. These beetles are close relatives of the walnut twig beetle.

Our research team aims to improve TCD detection in the Midwest and elucidate the possible roles of other beetles and fungi in the disease. Knowing which bark and ambrosia beetles attack black walnut in the native range will aid surveyors in differentiating between walnut twig beetle and other species. Also, knowing which fungi are carried by beetles will determine if we need to focus on other beetle species that might carry G. morbida or other tree pathogens.

For this investigation of beetles and associated fungi, our team was faced with the question of how to collect enough beetles to study. We decided to take advantage of the fact that bark and ambrosia beetles are attracted to trees that are stressed and declining in vigor. Consequently, researchers created declining “trap” trees. During 2011, twelve trap-tree locations were selected in Missouri and fifteen study sites in Indiana. At each location in Missouri, four black walnut trees were girdled and treated with glyphosate or pickrin to induce decline. At each study site in Indiana, two to four black walnut trees were stressed by girdling. Trap trees were left standing for three months to give beetles time to attack. Afterwards, two 12-inch long stem and four branch pieces were removed from the trees and placed in beetle emergence buckets (Figure 2).

**HTIRC select seedling plantation in central Indiana, photo by Jim McKenna, US Forest Service**

**Figure 1—Walnut twig beetle egg galleries and coalescing cankers on a walnut branch (Photo by Ned Tisserat, Colorado State University, bugwood.org)**

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Bark and Ambrosia Beetles

Our emergence bucket traps consisted of a five-gallon plastic bucket with most of the bottom cut out with an attached funnel and a transparent collection jar. Logs were end coated with wax and suspended from the lid of the bucket. After adult beetles emerged from logs, they were attracted to light emanating from below the bucket and fell into the collection container. Our team found the risk of beetles and the fungi on their bodies desiccating by placing a moistened paper towel in each collection cup. More than 35,000 bark and ambrosia beetles were collected as they emerged from the logs. Each beetle was identified based on its body shape and its patterns of stripes, ridges, and punctures. On the basis of these traits, beetles collected from Missouri and Indiana were sorted into 19 species, with 18 of those species collected in Indiana and nine in Indiana. Several species of bark-inhabiting weevils were also collected in our study, but are tentatively considered to be minor or inconsequential to walnut health.

No walnut twig beetles were captured at any of the trap-tree locations. However, a close relative, Pityophthorus lautus, was collected at a single trap tree location in Indiana. Unfortunately, a close relative, Pityophthorus lautus, was collected at a single trap tree location in Indiana. Fortunately, a single trap tree location in Indiana. Fortunately, a single trap tree location in Indiana was sorted into 19 species, with 18 of those species collected in Indiana and nine in Indiana. Several species of bark-inhabiting weevils were also collected in our study, but are tentatively considered to be minor or inconsequential to walnut health.

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No walnut twig beetles were captured at any of the trap-tree locations. However, a close relative, Pityophthorus lautus, was collected in a single trap tree location in Indiana. Although not collected in Missouri during this survey, P. lautus beetles have been collected previously throughout Missouri. Fortunately, P. lautus beetles are not a black walnut pest and there is no evidence that this beetle can transmit G. morbida.

The number of beetles collected varied considerably among the trap tree locations in Missouri and Indiana (Figure 3A and 3B). For example, researchers collected as few as eight beetles from a managed plantation but collected over 2,000 beetles from an abandoned plantation located in the middle of a forest. One reason for some trap-tree locations having more beetles than other sites could be differing amounts of breeding material (nearby dead trees, wood debris) available to beetles for egg laying.

Most beetles collected in Missouri and Indiana were species of ambrosia beetles that have been introduced to the United States, and therefore are considered “exotic.” Very commonly, exotic beetles arrive on infested wood or wood products imported from countries worldwide. The small size of these beetles makes them very difficult to detect on imported products during port inspections. The majority of exotic beetle species arrive in solid wood packing materials from U.S. trading partners in other countries.

The three most abundant exotic species that were collected from black walnut included the granulate ambrosia beetle (Xylosandrus crassiusculus), the black stem borer (Xylotrupes gideon), and the fruit-tree pinhole borer (Xyloxytus saxicolda). These three beetles are known agricultural pests. In addition, the granulate ambrosia beetle and the black stem borer have been reported to attack and kill stressed black walnut trees, regardless of age. In some cases, young black walnuts attacked by black stem borers will resprout. Young trees attacked by these species may not appear to be stressed. Tree death and dieback are thought to be the result of pathogenic fungi introduced to woody tissues when beetles create egg galleries.

Our team knew that exotic and native ambrosia beetles carry many types of fungi on their bodies but no one had described the full diversity of fungi associated with these beetles. The first step in sorting this out was to isolate fungi from beetles collected from trap-tree logs. A simple procedure was used to isolate fungi from individual beetles selected from each of the four most abundant species. Each selected beetle was ground into small pieces that were spread onto a nutrient agar growth medium that supports growth of G. morbida and many other kinds of fungi. Each type of fungus found growing on medium was recorded and purified. Rather than identifying each fungus visually using taxonomic keys, the organisms were identified by molecular methods, using the DNA code unique to each species.

Geosmithia morbida was not among the fungi isolated from 257 beetles; however, a close relative, G. pallida, was recovered from two types of ambrosia beetles, the granulate ambrosia beetle and the fruit-tree pin-hole borer. The association of Geosmithia fungi with ambrosia beetles is not unusual and has been described previously in Europe, Central America, and the U.S. However, G. morbida was not detected, other pathogens were commonly associated with sampled beetles. Fusarium solani and multiple Phaeoacremonium species were isolated frequently from beetles. These pathogens are known for their ability to attack many species of woody plants, including nut- bearing trees that have been weakened by drought, flood, and freeze damage.

Other than Geosmithia pallida, more than 80 types of fungi were recovered from the bodies of bark and ambrosia beetles. These fungi normally occupy many different habitats such as dead and living plant material, soil, insect guts, and parasitized insects and fungi. Most types of fungi occurred on just a few beetles; however, some fungal types were collected from nearly half or more of beetles belonging to a particular species. These fungal types included Aspergillus, Fusarium, Penicillium, and Phaeoacremonium species. It is uncertain why some types of fungi are associated with very few others.

The number of fungi found in this survey is likely a conservative estimate of the number of fungi carried by beetles. One reason for this is that not all fungi are capable of growing on the nutrient medium used in the study. In addition, some fungi go undetected because they grow slowly or they are suppressed by colonizing chemicals produced by other fungi. A different molecular method, also using DNA, is being used by our research team to detect fungi that otherwise would not be detected.

Our survey demonstrated that stressed black walnut trees are an attractive habitat for many types of beetles. These beetles are similar in size and appearance to the walnut twig beetle and who provided oversight, equipment, vehicles, and research space. Also, we extend thanks to research technicians, Megan Shawgo of the Missouri Plant Science Division and Matt Paschen and the library staff of the Purdue University Entomology Department for collecting wood, rearing and collecting emerged insects, sorting beetles and isolating fungi. Another special thanks to MDC forest pathologist Simeon Wright, Walnut Council member Harlan Palm, Hardwood Tree Improvement and Regeneration Center (HTIRC) research scientist Jim McKenna, HTIRC extension forester Lenny Farlee, USDA HTIRC forest manager Brian Beheler, MU technician Jim Lockiefer, USFS biological aide Keith Brown and numerous MDC Forestry Division employees for assistance in site selection and field work. Finally, a thank you is extended to the George O. White State Forest Nursery for donating black walnut seedlings for testing pathogenicity of the fungal isolates.

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Author contact: Dr. Sharon Reed, reedsh@missouri.edu