

Transcript of conference call on Thousand Cankers Disease, 6/26/14, 2:00 PM EDT

Welcome everyone to this call about Thousand Cankers Disease in Indiana. I'm Liz Jackson, Executive Director of the national Walnut Council and Indiana Forestry and Woodland Owners Association (IFWOA), also with the Hardwood Tree Improvement & Regeneration Center (HTIRC) at Purdue University.

This call was prompted by the announcement last Thursday by the IN Department of Natural Resources (IN-DNR) that thousand cankers disease (TCD) was discovered in Indiana. The purpose of this call is to talk about what that announcement means to you and answer any questions you might have. I want to note that I am recording this call, and plan to type up the discussion and post it online.

Joining us today is Phil Marshall, he's the state entomologist and director of the Division of Entomology and Plant Pathology in IN-DNR. His division coordinates monitoring and control of current and future pests and is also the regulatory authority.

Also joining us is Dr. Matthew Ginzel, associate professor of entomology at Purdue University.

Phil, would you to go ahead like to start?

Phil Marshall: Thank you. Again, we have found the fungus of Thousand Cankers Disease here in Indiana. And again the fungus was found from a new insect, this weevil *Stenomimus pallidus*, we have no common name for it, just the Latin name. The weevil was collected from a girdled trap tree there in Brown County on Yellowwood State Forest (YSF) in that project that Matt (*Ginzel*) and Jenny Juzwik have worked on starting in 2011. And I got the report from Jenny last December that she had detected the fungus from the weevil, that they were checking everything and gave me that information. I told her at that time to have the culture sent to a gentleman by the name of Ned Tisserat. He's the one that first identified the fungus and I wanted that confirmation from him.

And then the weevils were identified by Dr Sharon Reed in Missouri, University of Missouri, and I had those weevils that were still in the collections from the site in Brown County sent back to me so that I could turn those into USDA-APHIS. We have an identifier at Purdue University that we use for Emerald Ash Borer. And we took those in, just gave them to him, didn't tell him what they were, and asked him to ID them. And he did and confirmed the ID that Dr Sharon Reed had given. So I wanted two confirmations on both the fungus and the insect, which I have. That made me feel comfortable with taking regulatory action.

We also had to wait, because Jenny had found this fungus out of one site in Indiana. However there were 11 additional sites in Indiana that had the weevil, and she had to test all the weevils from the other 11 sites to see if any of those were carrying the *Geosmithia morbida*. Which took several months to do, didn't finish that up till late March or early April (2014). At the same time, Matt and Jenny and their studies had collected ambrosia beetles out of there. And there was two species that came out quite

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numerous. She tested those, because preliminarily she had a test come positive under DNA off of one of the ambrosia beetles, but that turned out to be negative, it was a false positive. Which would have added some more concern with another insect carrying, but that was negative, all the ambrosia beetles, the two species that were tested, there were several hundred of those, were all negative for the fungus. And they only tested those ambrosia beetles from the Brown County site. So at that point we have the Geosmithia fungus confirmed from the state of Indiana off a new insect. Part of the question now is what its role is, but it's there. And with that information, the fact that all other eleven sites were negative for Geosmithia, then I could develop what action I need to take on a regulatory basis.

I had the option of doing nothing, I could go ahead and quarantine the entire county of Brown County or a part of the county, or we could go in and destroy this plantation, which is a 10 acre plantation totally owned by the Division of Forestry. It was planted in 1974 and 1977, interplanted with autumn olive. The autumn olive is still there, unfortunately, but helpful because it hides what we are doing in the way of traps so nobody bothers anything. So with that we totally own it, the one thing we could do is destroy the plantation. And my other option was I have the authority to regulate an area of Indiana that I can define. So we defined the 10 acre black walnut plantation as the quarantined area. That gave us the opportunity under my rules, I need to follow up with surveys and evaluations of the site for the organisms. So that helped out by having that part there to bring Jenny and Matt back in to do further studies in addition to the surveys that we have in the plantation for walnut twig beetle.

But I also have at any time, of any concern, we can go in and destroy the entire stand. We can go in and cut it down and burn it. Although its 40 years old almost, these are young pole sized trees and there's no evidence of any decline or dieback in any of the walnut trees in the stand other than the ones we are girdling right now. We looked at the stand in January. I was in the stand with my staff, I was looking at these crowns of the trees for internode length, dieback, sprouting, any symptoms I could see. And all the crowns were healthy. I saw good internode length, no stunting, no dieback. We have the normal suppressed branches in tree crowns in the lower part of the crown, we have the normal suppressed trees in there. But even the suppressed trees were not showing a large amount or even a small amount of epicormic sprouting and trying to survive. And now with full leaf-on my staff is in there every week taking samples out of our traps. And they check the trees looking for any symptoms and they say everything is healthy. They have not found anything in the stand indicating any tree is infected with disease. So from that standpoint we have that safeguard right now.

In addition to what we've got in the way of our traps in there, we have 18 walnut twig beetle traps in the site. I'll let Matt talk about more about the study that they are doing because they have done that directly. The other thing that I am going to do, is have on our state forest lands that surrounds basically $\frac{3}{4}$ of this plantation, the other $\frac{1}{4}$ is private land and a crop field. I'm going to locate all black walnut in the forest away from this stand, so we know where all black walnut trees are at least within 10 chains away from

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the stand first. So we know where everything is. And we can monitor those trees over the summer.

So that's where we stand with it right now. So for the landowners there's no need to sell your black walnut trees, there's not any immediate risk to anything. Walnut in the rest of Brown County can come out of the harbors, there's no restrictions on that. They can pass through the county there's no restrictions on that. So there's not any concern on our part because of this situation. And of course we control the plantation and the size of the trees. There's no planned harvest on any of those trees at all.

Now other states, the impact will be from other states to our timber industry. They will consider us, just like I do the other states, that any walnut coming from Indiana will have to be certified not from this plantation. And that will be work for my staff to do. We'll be able to handle that. The other restriction, the worst, the most restrictions come out of Missouri. Missouri does not allow anything in or through their state. And I've checked with them. There's some situations where they'll allow if we have lumber coming out of our sawmills, its bark free and kiln dried, that can go out. The other one, we could work out arrangements to take logs to Missouri from Indiana if they're fumigated. But the other states, I think Ohio will work just the way we worked when Ohio found TCD. I had them certify all logs coming to our mills did not come from Butler County, Ohio. Ohio requires that any logs coming out of Butler County to be fumigated before they can come out of the county. So, that's where we are right now.

In addition to what we're doing at that site, we have, I think, an additional 90 to 100 walnut twig beetle traps around Indiana with quite a few of them focused on the Ohio border from the Ohio river on up north almost to Richmond, to be up against where it's located in Ohio. But the other ones are around high-risk places, our sawmills, veneer mills. We keep traps around those to see if we can find anything. The other work that we do is visual surveys. My staff does a survey in the wintertime in cities, to locate walnut, especially cities that have some of the sawmills. Then in the summer in August they will go back through and revisit those trees for symptoms. And our other visual surveys, we have gypsy moth trap tenders, have all their traps set now, and they are out monitoring their traps. We taught them to look for a walnut tree in the vicinity of their gypsy moth traps and report that tree to us and say whether it is healthy or there's indication of dieback or decline. From those reports then my staff will follow up any tree that they report is declining or dead. So that adds quite a bit more background information. That's where we are right now with the situation here.

We can field some questions now.

Question: The Geosmithia that was sent to Ned Tisserat, did he confirm on his genetics where it potentially originated from or if it's actually a fungus that might be native to Indiana?

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Marshall: No, he was just having him confirm it by cultural appearances, not by DNA. Jenny was doing the DNA testing on that and I'm trying to think who she sent it off to. Matt do you remember if she did?

Ginzel: You're asking if she asked anyone to confirm that? I know she used it by both plating and molecular markers. And it was verified by Ned Tisserat at Colorado. That is was morbida. The fungal isolate was identified as morbida based on the morphology and also the microscopic characteristics of it. It was subsequently verified by Tisserat at Ft. Collins and then DNA from that fungus was then isolated and amplified using techniques that are established and the fungal isolate was extracted and the sequence was 99% identical to the G. morbida isolate that is in Genbank now. The two sequences that were obtained from the PCR cloning and from the amplification were then sequenced and those were 99-100% identities to accession numbers that are already in Genbank. We're pretty certain that its morbida. Accession numbers that were in Genbank were from California and from Colorado. So we can't really state where it came from we only know that it has very close similarity to the fungi that were isolated both in California and in Colorado.

Question: If this state forest is the point of entry to be found in Indiana, are there campsites where firewood could have been brought in, or any theories along those lines as of now?

Marshall: No, there's no campsites near this plantation. There's a private residence across the road from it, we could look at that. There's a low grade sawmill nearby, but their percentage of walnut moving into that mill is about 10% of their business or less, and they only do it from a local area, they are a low grade mill. So we don't have any point of entry at all here. What I think we have is a new situation. Do we have Geosmithia native in our environment perhaps? And this role of this weevil. The weevil in the literature indicates that it attacks wounded areas on walnut, oak and hickory. So it has other hosts besides walnut. And we have some other questions to worry about regulatory wise in the future.

Question: You said that only one weevil was found to have this fungus on it? No other weevils in that area?

Marshall: Only those weevils, there was only the three in that plantation. All the weevils from all other plantations in Indiana were negative for it.

Ginzel: So in that year, if I can add to it, in that year we reared out from the girdled stems. We girdled 4 black walnuts at 15 sites throughout Indiana. We recovered the weevil from twelve of those sites and 435 adult weevils were obtained from 12 sites in Indiana. And the Geosmithia was only recovered from 3 of those individuals. So the 3 individuals emerged from 2 trees that were growing in Yellowwood (*state forest*).

Question: I'm not familiar with that particular weevil. When I hear of weevils I think of cotton weevil, alfalfa weevil, and so on, that is more foliar feeders. Is this particular weevil one that as an adult is going to work its way, eat its way in and underneath the

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bark, like the ambrosia beetles or the walnut twig beetle, or do you see it as a secondary insect that opportunistically entered into a hole and got the spores on its body?

Marshall: I think we have to look at it from both ways right now. When you say it comes into a weakened area from the literature then you think of it as not a primary pathogen entering into the tree – an insect is a pathogen just like a fungus by definition – but they are not primary in there. But then again, it could be, we really don't know.

This weevil is about the size of the walnut twig beetle, it's tiny. And given the weevil, because it's got a snout to it. Matt, you can correct me on this, probably the most similar it's like some of the grain weevils, those elongate grain weevils. It's not the typical weevil that you think of like you get from off of (?), the stuff you get off pine, the more rounded, these are more elongate flat. It's light brown or reddish brown in color.

I think it's still probably a weak pathogen in there. Like Matt said, we only have it out of 3 insects out of 2 trees and there was 435 other weevils checked. So we really don't know. The other question to bring then, is it possible that *Geosmithia* is naturally occurring in the inner bark of black walnut? That's a question I would have. Is it naturally occurring? Because if it was there and that tissue was weak and then the weevil went in and got it on its body and then we collected it when it came out. So we really don't know whether it went in with the fungus or came out with the fungus.

Ginzel: All we know at this point is that it came out with the fungus and we caught it from an emergence trap from sections of those main stems of the girdled trees. There's not a whole lot known about this beetle beyond what Phil mentioned. It's found under the bark of wounded hickories, black walnut and also some oak species. Dead oaks.

Question: A PowerPoint presentation said that there were nine of these individuals captured in Missouri in that 2011 study. Were these also examined for *Geosmithia*?

Ginzel: Yes.

Question: On the two stressed trees, I would assume with the planting in 1974, 1976, that they're somewhere around 7 -14" DBH, Are you considering taking down one of them where you can more thoroughly examine the crown, the 1" to 1-1/2" branches up there, or are you relying strictly on traps?

Marshall: The planting is probably 6-10" (*dbh*). We might have a rare one that might run 14". Matt, why don't you go ahead and tell them about the two studies going in there?

Ginzel: In that Yellowwood site for this year, we have girdled an additional 6 trees. We have also felled a few trees and as Phil mentioned a lot of these trees are suppressed. And we looked up in the crowns and didn't see any evidence of dieback or presence of beetles or beetle activity.

Then the other objective of our study down there is to determine the ability of the weevil derived isolate, the *G. morbida* isolate that we got from 2011, to cause cankers on black

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walnuts there in the site. So we're taking that very isolate that we got from the site and inoculating branches, large branches of black walnut there in the site. And then to follow any symptoms that might result from the inoculations of those branches over time. So that's 2-3 years. So that's part of the rationale for not destroying all the trees at that site, so we can understand. We don't know the pathology of this or how the trees respond to an inoculum of morbida.

But the low frequency of morbida on this weevil suggests really at the very least just a casual relationship between the fungus and the beetle. And also if you think about Thousand Cankers Disease it takes thousands of individual inoculations on the part of the beetle to introduce enough fungus to kill a tree. We only collected 400 and some throughout the course of the year in our 15 sites in Indiana, and it was only found on 12 of those sites. So it may not be capable of vectoring enough fungus to actually cause some detrimental effect on tree health. So at this point there's not real reason for any alarm, as Phil mentioned.

Marshall: I just want to mention too, on those inoculation studies, to understand the pathogenicity of this isolate and the role inside, those branches are treated with insecticide.

Ginzel: Yes, they're treated with permethrin.

Marshall: And they are also being caged, aren't they Matt?

Ginzel: Yes.

Marshall: So we're trying to not let anything come to that twig and get in there and then get back out, manage that. Jenny wants to see and we want to understand what is this fungus doing in the tree.

Ginzel: We want to protect those inoculation points from any other insects so that they can't become incidental vectors from those inoculation sites.

Marshall: Another thing on the trap trees that Matt mentioned this year in addition that is different than the previous study, is when they take the bolts out, part of the bolt goes into the insect cage and the other bolt will be cultured, dissected.

Ginzel: So we're taking the first 16' of the main stem think of it as 4-4' sections. One section we will rear insects from and the other section will be evaluated for cankers.

Question: My understanding in the 2011 sampling the stresses that were introduced there was to girdle the tree and then harvest both branches and bole wood. So all of the stressed trees have been destroyed, correct?

Ginzel: Yes. So not only were they girdled, we treated those girdles with Glyphosate, with Roundup.

Marshall: Now the original stressed trees, I don't know if the ones the weevils came, they were still in the stand, they were laying on the ground in there.

Ginzel: Yes that's true.

Marshall: They were broken down, but they were still in there. They weren't ground up or anything after that.

Ginzel: Right, but we didn't leave any of the girdled trees standing. The main stem and portions of the branches were taken and put into emergence buckets and reared in my lab. Those insects were reared out in a confined place in my lab, a rearing room.

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Question: We have a single plantation, cut out probably 100 trees that were various diameters from 2" -3". A lot of those trees are 12' long that we have piled up in a woodpile and are just using it for firewood. Is this a hazard?

Marshall: Well, I think it is. I'm trying to remember if Steve Seybold had seen any of those twig beetles attacking those trees and then coming back out of it. I don't remember him saying anything along those lines in California or whether they even have had an opportunity to study those. The only thing I know is they have taken some of the infected trees that had beetles in them and they were getting thousands out of them. The bolts, the firewood bolts. If you want to be on a safeguard, the principle that I put through to my state parks people and property people to understand is the killing area of a tree is the cambium - that bark, cambium, and sapwood interface. If you manage that and don't spread that around, you're going to help prevent the spread of the killing insects and diseases. So from that standpoint I'd go ahead and just burn them and not leave them for firewood over a long period of time.

There are further resources available at the [national TCD website](#) or at the [IN DNR division of entomology and plant pathology website](#).

Ginzel: I want to add I have put together what's called a "Hot News" story for our entomology extension group that's going to be on our [Purdue Extension Entomology web page](#), it will be out on Monday (*June 30*). It will be a short summary of our findings from 2011.

Marshall: One other thought, as we work through this study, with Matt, Jenny and my crews with the traps in there, any tree that is not part of the study that starts to show any symptoms, that tree will be dissected. We'll take care of it. If we feel there's any risk it's coming out, coming in to study to find out what is going on with it.

Ginzel: The other thing to mention, Phil's crew has put out a lot of pheromone traps in that planting so if the beetle is in that plantation they should pick it up in those pheromone traps this flight season. The beetle has a flight season mid-June to mid-July and another one in the fall and I think they even baited them with bolts of walnut and the pheromone so if it's there we should be able to pick it up.

Marshall: The survey with the twig beetle, we're working with Steve Seybold on that.

Ginzel: There's a commercially available pheromone lure that's effective at capturing or attracting those beetles over a short distance.

Thank you to our speakers for taking the time to tell us more about this situation. And thank you callers for participating.