

Current Trends in Urban Forest Diagnostics

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[00:00:00] Intro music playing

Tom Smiley: [00:00:13] Welcome to the ISA Science of Arboriculture Podcast series. This is Dr. Tom Smiley at the Bartlett Tree Research Laboratory host of this podcast series, which is brought to you by the International Society of Arboriculture and the Bartlett Tree Expert company.

Today's talk is by Dr. Sara Ruark who is a technical advisor for the Davey Tree Expert Company. And she's based in Houston, Texas. Her talk is on Current Trends in Urban Forest Diagnostics, based on her literature review of diagnostic tools and mobile apps.

Sarah Ruark: [00:00:55] Hi thank you for checking out my talk today. I'm going to be discussing [00:01:00] the current trends and urban forest diagnostics, and I appreciate you taking the time to click on the link.

So my name is Sarah Ruark. I currently work for the Davey Institute, which is the research arm of the Davey Tree Expert Company. I have a master's degree in plant pathology, and if all goes well in a few months from this recording, I will have defended my dissertation for my PhD in plant pathology as well.

This talk is based on some research that we did for ISA on tree diagnostic practices. And a version of that research was published as an article in *Arborist News* in April 2020. That work was done by myself and some of my colleagues at the Davey Institute. So if you would like more in-depth information or to check out that article, you can just look forward in April 2020 edition of *Arborist News*.

So before we get too deep into the [00:02:00] current trends in diagnostics, I wanted to do a little bit of background on how diagnostics are done. Diagnostics is really just a series of steps you do to figure out what's wrong with the plant. So the first step is to identify the plant. This is important because you need to know what's normal for the plant, what it should look like, maybe that gives you some information about the growing conditions it prefers, and that can also be useful information.

The second step is to check for symptoms and signs. So you're trying to notice what's unusual about the plant, what looks like it is going wrong. And you're also looking for evidence of any pathogens or insects that might be contributing to the problem.

The third step is to observe patterns either on the individual plant or more generally in the landscape around the plant. You know, are multiple species affected or the, just one plant, that kind of thing.

[00:03:00] The fourth step is probably the most important, which is to analyze the history and environmental conditions. That'll usually give you a lot of the answers you're looking for and what's gone wrong. Particularly in cases where construction or major site changes have taken place.

The fifth step is to conduct tests or essays, and that's where the majority of the focus of the talk will be on the different tests and essays that are available. And kind of where that area of research is going these days.

Then luckily if you've completed all five steps successfully, step six, you determine the cause and you're able to make a diagnosis.

The next thing we need to consider are what's causing the problem This can be either abiotic or biotic. So I wanted to talk a little bit about the differences between the two. Abiotic problems are your non-infectious issues. [00:04:00] Typically this is going to cause a uniform symptom across a plant, or maybe even multiple species.

This is caused by things in the environment, like drought or herbicide damage or frost damage. You can see why it would affect multiple species. In contrast, the biotic agents are infectious. These are your fungi, insect pests and things like that. Typically these are going to have non-uniform symptoms on a plant and be restricted to a single species because most infectious organisms have a preferred host.

Examples of symptoms you would see in a biotic infection are cankers, leaf spots, or branch die back. But as you are aware, branch die back could also occur from an antibiotic condition. So symptoms alone, it can be difficult to distinguish sometimes what's happening.

Now we'll get into the methodology of the review that [00:05:00] we did for ISA. This was a literature search. We were looking for scientific articles that have been published in journals on the topic of tree diagnostics. We searched two databases, which just pull all of what it thinks are the relevant papers from every journal in the world, almost. And we use the web of science database and Google Scholar.

The topics we searched for, we tried to keep it very general. So we searched for "tree", "urban forest", or "forestry diagnostics" in general. However, we did exclude any results that were pertaining to fruit or nut trees, because those tended to be projects focused on like surveying a large orchard or something that's a little less useful when say you're looking at a specimen tree in an urban environment.

We also did a digital application search. So as these were looking for apps for this, we just use the Google play store and [00:06:00] included both free and paid apps.

In general, we could kind of classify the results we got from this search into several different diagnostic practices, which, and I'll go through a few of those. The first set were the computational practices. Here, the researchers are trying to define a set of rules that you would basically be checking a box. "Yes. It's this kind of tree. Yes. It's growing in this environment. Yes. I see the symptom has spend this many days of rain this year" to determine if you're falling in the set of parameters. That would cause it indicate a specific disease or pest issue.

The second, most common we found was the Eliza practices. This is a molecular test that's used to detect a pathogen or pest proteins using antibodies. And I'm going to discuss this in more detail later in the talk. The next molecular technique that we found a lot of papers on [00:07:00] was the PCR technique, which instead of detecting pest proteins, it's going to detect nucleic acids or DNA. And I'm going to, we'll discuss that more later as well

A lot of papers coming out on remote sensing. This is where you're trying to assess the forest or trees health either from a satellite or drone and the images that they're able to capture. They're also papers on nutrition sampling. This was comparing the level of elements in a symptomatic tissue to normal tissue and the different ways of collecting and analyzing that data.

There was also quite a few papers on the topic of risks, risk assessment, trying to determine the presence of internal decay in standing trees and where it's located.

The majority of the results were in three areas: the plant nutrition analysis, the risk assessment techniques and the molecular laboratory tests. Those are the ones [00:08:00] I'm going to go into more detail on today. I'm going to start with the plant nutrition analysis, which includes Orville fluorescence and remote sensing.

In chlorophyll fluorescence researchers are measuring the photosynthetic efficiency of a plant or a leaf. This is based on the idea that when plants are under stress, they're going to have decreased metabolism or be less efficient in their photosynthesis. Research has also established a link between nitrogen and chlorophyll content, so it can also be used as a measurement of plant nutrition. This is typically measured with a thermometer that's where you're seeing a lot of the research is either in the equipment or the way to interpret the results.

And remote sensing, as I mentioned, you're using drone or satellite data to assess health or growth. This could collect either visible or other spectral data that's outside the visible [00:09:00] spectrum. And here's where we're seeing a lot of research and that's because the technological capabilities are constantly improving, at a pretty rapid rate.

I pulled one example, just to show the type of research that's being done in this area. Now, this paper is not included in the survey that we conducted, because we did that last year. And I thought was the title of my talk is current trends, so let me try to find something that was published in 2020, just to keep it current. So this was actually published this year.

The title is "Street Tree Health from Space and Evaluation Using Worldview Three Aata and the Washington DC Street Tree Spatial Database". This work was done by researchers from West Virginia University, the University of Illinois, and the urban forestry division in Washington DC.

What they were interested in doing was to use satellite spectral data to determine the relative health of trees in Washington, DC. [00:10:00] They looked at different spectral data that was available from the satellite, so that's what this worldview three data is. And also looking at different image timings, so either from the beginning of the season, mid-season or late season, just before senescence. And also looking at how the data looked from different tree species and on trees that they had already established were under varying health conditions.

Their conclusion from the paper was that the satellite data is good enough to prioritize which trees you should go inspect in person. So you can imagine if you're trying to take care of, you know, of all the street trees in a large city, like Washington DC, it would be helpful to be able to prioritize, like these are the ones that you really need to go check out in person see what's going on with them.

They also propose that future research could take images that happened over successive years and monitor an individual tree or decline over a longer [00:11:00] period of time. That's kind of the direction that type of research is going on. Just one example.

We'll now go on to the second major area of results, which were the risk assessment techniques, which is our tomography, either sonic or electrical. So if you're not familiar with tomography, I'll begin by explaining a little bit that sonic tomography is utilizing sound waves to detect decay or cavities. It's based on the speed of sound traveling through healthier decayed wood. With the underlying factor being sound is traveling faster, through healthy wood than decayed.

In contrast the electrical impedance or resistance tomography uses an electrical current in a similar way. In this case, the electrical resistance is decreased indicate wood. So more moisture means a higher

conductivity, and it can be more sensitive to detect early decay compared to [00:12:00] sonic demography. I didn't pull a specific paper to discuss here because these papers tend to be a little difficult to synthesize down to something that's convenient to explain in a PowerPoint.

In general, the research in this area tends not to be in generating the sonic or electrical waves or current to send through the tree and more on the computer side of the algorithms used to interpret the data you've obtained and give you the best image.

I'm going to spend the most time in the third area, which are the molecular laboratory tests, because this is my area of expertise. These tests tend to either detect pathogen or insect proteins or DNA. So we'll begin with the protein essays. I mentioned in the beginning the Eliza test that stands for the enzyme linked immunosorbent assay. So you can see why we call it a Eliza instead of that mouthful. And this is [00:13:00] using antibodies to detect pathogen proteins. It does require a specialized lab equipment to evaluate the results. In this image we have this is called a 96 well plate. So each of these circles is a well that's holding liquid and you put your sample in the wells and when it is present and detected, you get this yellow color. So that means the pathogen was in the sample, but not in the ones with no color.

To explain it in a little more detail. Here's what happens. So you begin with the capture antibody. These antibodies are actually generated in mammals, even though we're detecting in plant problems. So these are things like rats or rabbits. Luckily all of these antibodies are available commercially, so you don't have to deal with any of that. You just call up a company and say, I need antibodies to detect, you know, whatever pathogen you're interested in.

You coat these wells with this capture antibody and that ..your sample, which is going to contain proteins of your pathogen or [00:14:00] pest of interest represented by this blue circle.

So if the pathogen protein is present, it's going to stick to the capture antibody that's at the bottom of the well. And then you add another set of antibodies on top that will also detect your pathogen. So you've kind of made a sandwich where your pathogen has been detected in the middle. And so if these detection antibodies have found your pathogen to bind to that's when you get the color change reaction.

There are a few Eliza tests available for tree problems, including tests for the pine wilt nematode, our blight, bacterial leaf scorch and Dutch Elm disease, as well as many, many viruses. And all of these molecular tests are going to have a lot of options if you're trying to detect a virus because we have no other way of confirming the presence of a virus in a plant, or even in people or animals without doing these molecular tests.

[00:15:00] Another type of protein essay that you may have seen are these strip tests. These get different names depending on the manufacturer. So you may have heard of an immuno strip or an agra strip. It's nice about these are, these are really fast tests that don't require any technical expertise. Anyone could do these and you can get results in 10 to 15 minutes.

They're relatively low cost at \$10 to \$20 per sample, but there are very few tests available for trees with this. Most of the work in this area has been for agricultural purposes, but there are tests to determine by top throat down to genus, but not species, fire blight, and again, many viruses. So the nice thing about these is they're really easy to interpret. They pretty much work like a pregnancy test and it is a similar concept, where you would have grinded your sample and a buffer that they send with the sticks and then stick with the stick in there and let the liquid work its way up. For a positive test. You would get [00:16:00] two bars

and a negative test. You get one bar that's just confirming this test worked, but your pathogen wasn't detected.

We're now going to move to the molecular test that detect DNA. So I mentioned the PCR or the polymerase chain reaction. And you may have been hearing about this one too, in the news lately, because it's been mentioned quite frequently with the current pandemic. The nice thing about PCR is it can detect very low levels of infection, but the problem is it takes a pretty high level of technical expertise. And some very specialized lab equipment because it requires cycling your sample and the reagents between different temperatures very rapidly for kind of a long period of time. This is a PCR primer recognition. I'm going to try to explain this in a kind of straightforward way. We'll see how it goes.

You can think of DNA as a [00:17:00] series of letters because the molecules that compose DNA can be broken down into four different letters: A, G, T, and C. For PCR to work, what you're going to do is you're going to know the sequence of what you're trying to detect and design a small fragment of DNA to recognize that.

When it doesn't recognize what's in the sample, you're getting no reaction. If it does recognize the sample, and it does this by complimentary pairing these four letters, you're going to get a reaction. And that's when you start getting this chain reaction and is making a lot of copies of the DNA to increase it to a detectable level.

The more old fashioned way of detecting that this has happened is by running the DNA on a gel. So a gel, you can kind of think of like Jello and you put it in an electrically, charged water bath, or buffer bath. And DNA's [00:18:00] negatively charged and you pull it down towards the positive charge and the different fragments you've created with your primers are going to be different sizes so they're going to move at different rates and that's how you can determine if your pathogen is present. This is kind of falling out of favor now for more rapid way, because this can take several hours. And so that's called a real-time PCR, which will just detect as the chain reaction it will detect that the DNA is accumulating in your sample and give you a readout on a screen that that has occurred.

There are a lot of protocols available for PCR. I would say, this is the state-of-the-art in diagnostics and where I believe all future diagnostics are going to be headed this way. Agriculture is already way down this road. I think if you're in a position where you're running a diagnostic lab, this needs to be a capability that you're working towards having.

Some of the many protocols available, [00:19:00] this is just a sampling are distinguishing among the ...[.]...species. So, you know exactly which ...[.]... your dealer with detecting Oak wilt, verticillium wilt, wood, decay fungi, special in conifers, dripping rot or drippy blight, the red Oak boar, which I believe is to distinguish between biotypes... horse woody type viruses, and so many more and more all the time. And there's just papers coming out all the time with new PCR protocols for different pathogen or pest.

The other component obviously as I just said, all of these PCR tests are based on developing primers that recognize the DNA sequence, but how do we know what the DNA sequence is? That's where the genome sequencing comes in.

This is the upstream tool you use to determine the nucleic acid sequence or the DNA sequence of an organism. Then you use this to develop a DNA based essays like PCR. The [00:20:00] very old fashioned way, but quite cheap and reasonable to do these days for genome sequencing relies on fluorescently tagging.

Again, we have the four different letters here: A, G, T, C. You can tag each one with a different fluorescent label and the machine and a computer just read that out and then give you the sequence. So you do see a lot of papers coming out where a lab has sequenced a particular pathogen or pest. And then a few years down the road, you start to see the PCR protocols come out based on the sequence that was developed.

An exciting area of research when it comes to PCR, as I mentioned, it requires use in the lab because you're heating your sample up and down many, many times over a pretty long period of time. And in agriculture. And now I see coming and to forestry situation is trying to find a way to move that to the field so that you could at the point of [00:21:00] where you're sampling, go ahead and run a PCR test confirm pathogen or pest is present.

This article was entitled the "Institute Processing and Efficient Environmental Detection or eye Speed of Tree Pests, and Pathogen Using Point of View Real-Time PCR". This way research came out of Canada. It's an exciting concept that you would be able to use this very sensitive and accurate detection method right on site. The majority of this paper is focused on getting all the reagents and different components to do this test to be field stable, meaning you could store them at room temperature because in most cases, these things need to be frozen or kept on ice to make it all portable. So you could carry it in a backpack, for example.

And they also used a commercially available thermal cycler. So this is the equipment that's adjusting the temperature. In this version of this equipment, it reports the results directly to your cell phone. You could have it really within [00:22:00] a few minutes of taking a sample in the field and they tested this both with the foliar fungal pathogen and an insect.

I think this is the way this kind of technology is going to be used moving forward. I think this will become more and more common. I expect in the next couple decades, PCR will be done in the field regular basis.

Now just to switch a little bit for the web-based apps that we looked at for this review, they mostly fell in a couple of different categories, the most common being: identification and keys. So these tend to be, I think usable by a lot of different people and even people without much experience, because a lot of times they're based on pictures.

On this one, you can see it's like, what are you dealing with? An ant, an aphid, a beetle. And you work your way through the key, and then it tells you exactly what you have. These do tend to be region specific. So that is a [00:23:00] problem that not all places have a good ID or key app that they could use for their specific region.

The other group of web based apps tend to be diseased or pest specific. This one here is for Emerald Ash borer and Ash tree identification. This really is more for a homeowner type situation where they don't even know what kind of tree they have. It starting out with the key to figure out, Oh yeah, you probably have an Ash tree. You may need to be concerned about Emerald ash boar.

But you know, apps are always getting added and taken up from these different stores all the time. It's hard, I think, to keep an up to date list as best to just go and try to search for what you're looking for. It may exist. I think there's a lot of opportunity here to expand the number of apps that are available. But there's probably a kind of high input cost to develop these.

So that's what I had talked to you about today, and I thank you very much for your attention.

Tom Smiley: This [00:24:00] concludes Dr. Sarah Ruark's talk on Current Trends and Urban Forest Diagnostics. This talk was originally presented at the 2020 ISA Virtual Conference.

The views and information expressed are those of the presenter. If you would like additional information on tree diagnostics, you can visit the ISA Web Store. There are numerous books on this topic. Please join us next month for another presentation and the ISA Science of Arboriculture Podcast series.