

Genome Insider S2 Episode 7: THE Bioenergy Tree

ALISON: Hey! I'm Alison Takemura, and this is Genome Insider, a podcast of the US Department of Energy Joint Genome Institute, or JGI.

Last month, I was trapped in my apartment for a week because of terrible air quality. It hung like a dirty curtain over the former gold rush town that I live in, on the outskirts of Denver, Colorado. And I did not want to tango with that air.

It had come from the West Coast, plumes of smoke, blown on the wind, from the raging wildfires in California: smoke from trees burned in conflagrations that include the River complex fire, the Caldor fire, and the Dixie fire, the second-largest fire (for now) in California history. And because the sooty, acrid smoke wantonly disrespects state borders, I could not go outside. Unfortunately, I think we'll be seeing more days that make it hard to breathe. Because this situation is a symptom of climate change.

The Intergovernmental Panel on Climate Change released a report on August 9th. And it documents how we're dealing with hotter summers, deeper droughts, and thus bigger fires and befouled air. Not to mention, a warming climate is also causing more catastrophic hurricanes and flooding. The report says that these effects are the 'unequivocal' result of burning fossil fuels and releasing greenhouse gases.

U.S. President Joe Biden also drew attention to climate change on September 7 as he visited New York and New Jersey in the wake of Hurricane Ida.

JOE: Climate change poses an existential threat to our lives to our economy. And the threat is here, it's not going to get any better. The question is, can it get worse? We can stop it from getting worse. And so folks, we got to listen to the scientists, and the economists, and the national security experts. They all tell us this is code red. The nation and the world are in peril. That's not hyperbole. That is a fact. They've been warning us the extreme weather would get more extreme over the decades. And we're living it in real time now.

A plantation of poplar trees. (David Gilbert)

So, how are we rising to this challenge? The US Department of Energy is funding research to help humanity course-correct and facilitate a humongous energy transition to clean and renewable energy sources.

And this episode is about one of those sources: a tree called poplar. DOE researchers are growing poplar in field sites in California, Oregon, and Tennessee, and they're studying the traits that'll make poplar trees a commercial biofuel feedstock in this decade, to fuel jet planes and ships, and even create new materials.

So, why poplar?

JERRY: It's the fastest growing temperate tree species in the Northern Hemisphere.

ALISON: That's Gerald, or Jerry, Tuskan (Gerald with a G, Jerry with a J). He's a plant biologist and geneticist at Oak Ridge National Lab in Tennessee. He's director of one of the four DOE Bioenergy Research Centers: The Center for Bioenergy Innovation, which is led by Oak Ridge National Lab.

Gerald "Jerry" Tuskan, plant biologist and geneticist at Oak Ridge National Laboratory, studies poplar as a bioenergy feedstock. (Jason Richards)

And Jerry has devoted his career to the investigation of poplar as a sustainable substitute for fossil fuels. He and his team are making huge strides in understanding poplar genes and using them to craft poplar varieties that are better for making biofuel. We'll get to that work later in the episode, but first, a little more on poplar.

JERRY: The US Department of Energy in the '70s designated it as its primary woody energy crop, and people have been working on it since then.

ALISON: I should clarify one confusing thing about poplars. Poplars, which are found in Europe, Asia, and North America, all belong to one genus, called *Populus*. And there are many species within *Populus*; they're called black cottonwood, lombardy poplar, white poplar, balsam poplar, eastern poplar — the list goes on. And these different species have different traits — bigger, smaller, weaker, stronger, shorter, fatter — you get the idea.

The neat thing about poplars is that many of them can be crossed together — in other words, they can hybridize and have offspring, and those can have offspring themselves. And these hybrids with combined traits are actually the ones that are the most interesting candidates to make into biofuel feedstocks.

But DOE's plunge into poplar research started with one *Populus* species: and that is black cottonwood.

Researchers from Oak Ridge National Laboratory and Duke University sample root microbiomes from poplar (*Populus trichocarpa*) at a natural field site in Longview, Wash. The poplar trees in the foreground are roughly 5 to 10 years old, and older ones in the background are estimated to be 60 to 80 years old. (Wellington Muchero)

ALISON: Black cottonwoods are among the largest trees in the Pacific Northwest. They can get to 80 feet tall — that's like a 6- or 7-story building — and they have elongated ovate to heart-shaped leaves: dark emerald on one side, silvery on the other. The leaves turn golden in the fall, and they flutter dramatically in the breeze. Their seeds also help explain why black cottonwoods have that word 'cotton' in their name: their seeds get released in cottony tufts of white fibers.

WELLINGTON: It looks like it's snowing. (ALISON: even though it's spring.) I love that feeling where I feel like I'm driving through a snowstorm....

ALISON: ...when it's actually seeds instead of snow. That's Wellington Muchero, a plant biologist and quantitative geneticist who works with Jerry at Oak Ridge National Lab studying black cottonwood and other poplars. He says they're truly astonishing trees.

WELLINGTON: One thing that always amazes me about the black cottonwoods is how fast they grow. I remember just last year, we established one of our newest field sites.

ALISON : Jerry and Wellington have multiple field sites in California and Oregon, and their newest site is in Davis, California. There, they had planted 1300 different genotypes or genetically distinct black cottonwood trees.

WELLINGTON: And when we put them out in the ground, they were probably like, a foot tall. And within two months, they were six feet tall.

ALISON: In two months!

WELLINGTON: And then, by the time we went back this year, after one growing season, most of them were close to 15 feet tall. So they are amazingly, super fast growing trees. And I just wonder what makes them so, so productive.

ALISON: As part of the DOE's efforts to study this vertical speed racer of trees, the Joint Genome Institute sequenced black cottonwood and published the genome in the journal Science in 2006. It was the first tree genome ever to be sequenced. The paper has been cited more than thousands of times, and the month of this episode's release actually marks the publication's 15th anniversary. Happy anniversary, sequenced poplar genome!

So, in the last 15 years, what have scientists been able to uncover about these fast-growing trees? What breakthroughs have they made to help turn this DOE Energy Tree into a bioenergy resource and part of the bioeconomy? What is a bioeconomy you might ask? Well, it's the use of biology and biotechnology for fuel, materials, and products. And poplar will have a part to play.

Stay tuned to find out more.

Wellington Muchero, a plant biologist and quantitative geneticist at Oak Ridge National Laboratory, has mapped poplar traits that will make the tree a better source of biofuel and bioproducts. (Carlos Jones)

WELLINGTON: The major problems that we face in terms of enabling the bioeconomy revolves around the fact that black cottonwoods are living things. So they have naturally evolved

tendencies that can either inhibit or hinder our ability to make fuels. And they are also susceptible to problems like pathogens, drought stress, nutrient limitation, that prevent optimal production of that biofuels feedstock.

So even though poplar is highly remarkable in the way it grows, there are still some of these major challenges that we face when we deploy a field filled with poplar trees, many things can happen to those. Fortunately, for us, most of those things are genetically controlled. So with the studies that we are doing, we are actually trying to find solutions to those challenges based on genome sequencing technologies. So the genome enabled discoveries that we are making, allow us to solve some of the inherent challenges that we face as we deploy these feedstocks in fields and natural environments.

ALISON: Wellington, Jerry, and their team at the DOE Center for Bioenergy Innovation are hunting for the best adaptations that evolution has forged into poplar tree genomes.

WELLINGTON: Being able to figure out what evolution has done over millions of years, in the short period of time that we have achieved is really, really remarkable for me. So it's like we are replaying evolution over millions of years. And we are figuring out what mutations happened to make black cottonwood what it is today. What makes it tolerate disease, what makes it tolerate drought, and we are seeing those things today, and we can engineer those exact same behaviors in a period of months. So what evolution did over millions of years, we are seeing and reproducing in a period of months.

ALISON: Their goal is to then take all of those best traits that they've pinpointed and can reproduce, and put them into the poplar trees of tomorrow.

The story of one of these traits starts with a fungus. This is not just any old fungus, but a mycorrhizal fungus — 'myco' being Greek for fungus, and 'rhizal' being Greek for root. Like the name suggests, they can cozy up to the roots, and even infiltrate them. And that can be great for plants, including poplar, because the mycorrhizal fungus can help gather more nutrients from the soil and bring them back to the plant.

Fruiting bodies of *Laccaria bicolor*. The ectomycorrhizal fungus can funnel nutrients to poplar — if the species get along. (Courtesy of D. Vairelles and as seen in the Nature publication of the *L. bicolor* genome)

The fungus in this episode's spotlight is called *Laccaria bicolor*. Its genus name, *Laccaria*, comes from Latin and translates to lacquer or shiny paint because the mushroom fruiting bodies it makes are rather shiny. *Laccaria* is an ecto- (or outer) mycorrhizal fungus, and in the soil, it forms a kind of white net around root cells and makes structures that come out of the roots and look like curious little antlers. Weird? Yes. Good for poplar? Absolutely.

JERRY: We've known for a long time that *Laccaria*, a mycorrhizal fungus, also sequenced by JGI, gives a positive impact on growth.

WELLINGTON: You can get two to three times more biomass yield, just by introducing that microbe in your field. So it's huge benefits in terms of plant production. The really big challenge that we face is that interaction is highly species specific. So that fungus could be there and you plant a genotype or a poplar species and they will ignore each other — like, nothing is happening. Poplar goes its way, Laccaria goes its way.

ALISON: But some poplars are much more receptive, like Black cottonwood, whose species name is *Populus trichocarpa* — 'trichocarpa,' by the way, means hairy fruit, and it's an homage to its cottony, fluffy seeds.

WELLINGTON: *Populus trichocarpa* and *Laccaria* behaves really nicely together, they form a healthy relationship. *Populus trichocarpa* grows nice, big and strong.

ALISON: But some poplars don't form that relationship. For example, Eastern cottonwood or *Populus deltoides* — *deltoides* from the Greek letter 'delta', whose capitalized form looks like a triangle. And *Populus deltoides* has triangular, or heart-shaped leaves. The poplar *Deltoides* interacts with *Laccaria* in the exact opposite way as black cottonwood, *Populus trichocarpa*. *Deltoides* actually sees *Laccaria* as a pathogen.

Jerry and Wellington examine the roots of a poplar tree (*Populus trichocarpa*) in a research plantation near Placerville, Calif. (David Gilbert)

WELLINGTON: So when it finds *Laccaria* in nature, it fights it off and produces defense compounds to actually fight it off. So you don't get the benefits. And we are really interested in identifying that. How do we regulate? How do we make them play nice together? You know, how do we tell the *deltoides* that this is actually a good friend for you, you shouldn't be fighting with. And so that's always been a really, really difficult question in the science field.

ALISON: Scientists had long thought that engineering this symbiotic interaction between fungus and host plant — well, it was so complex, that you wouldn't be able to just shoehorn in one gene to broker this new relationship; you'd have to engineer entire metabolic pathways.

WELLINGTON: You have to bring the gene that recognizes the fungus, you have to bring genes that transport nutrients between the plant and the fungus. And you have to.. all kinds of things that people were thinking that you need to have in order to engineer this.

ALISON: Ugh, such a heavy lift! But the team was in for a huge surprise, because it turned out that how a poplar species reacted to *Laccaria* was regulated by just one gene.

WELLINGTON: People did not believe what we were talking about.

If we took that one gene, then put it into *deltoides*, which normally doesn't work well with *Laccaria*, they play with each other very happily now with just one gene. And so we were going

away from this notion that you need entire pathways.

ALISON: Wellington and his team now knew that this one gene in poplar brokers this relationship with a fungus. But they wondered, what would happen if they put the gene in a totally different kind of bioenergy plant?

WELLINGTON : The next step that we took was even crazier, was, can we engineer grass to interact with *Laccaria bicolor*? In grasses in general.. nature has never engineered a grass that interacts with ectomycorrhizal fungi — that we know of at least. Then we took again, that one gene, put it in switchgrass.

ALISON: And they saw what they had hoped for: that now switchgrass was suddenly, happily interacting with *Laccaria bicolor*.

WELLINGTON: And then when we do transcriptome profiling—

ALISON: i.e. sequencing the gene messenger RNA transcripts to see which genes are expressed—

WELLINGTON: ...instead of seeing defense initiation, we actually see transport mechanism, that they're exchanging nutrients, they're exchanging carbon, and that's something that we would have never expected.

ALISON: These results are wild! Wellington and his team had made friends out of complex organisms that had evolved to ignore each other.

So far, these experiments with poplar trees, the fungus *Laccaria*, and switchgrass have only been done in the lab. So the next experiment will be to test them out in the field.

Jerry Tuskan and a field crew assess drought-induced leaf senescence and tree height at a hybrid poplar (*P. trichocarpa* x *P. deltoides*) field site in Boardman, Ore. (Courtesy of Wellington Muchero)

But this story is just one of many breakthroughs that Jerry, Wellington, and their team have made in order to make poplar a better source of biofuel. They've discovered several other genes that unlock impressive traits. For example, they found a gene that prevents poplar's leaves from yellowing during drought conditions. Another gene, if deleted, makes poplar, incredibly, grow bigger. And yet a third gene makes poplar produce less lignin, which is a complex polymer in plants that helps keep them standing upright, but it doesn't break down as easily into fuel. Poplar with less lignin is easier to handle, like a porcupine with fewer quills. All these traits, or phenotypes, make poplar a more enticing biofuel feedstock. So now, Jerry and Wellington want to take all of these traits and breed them into one poplar. But—

JERRY: You can't do traditional breeding, the generation intervals are just too long to make

much progress.

ALISON: Poplar doesn't reproduce until it's at least 7 years old — sometimes, if it's a late bloomer — haha — not until it's 15. But because scientists have been studying and sequencing poplar for more than a decade, they now have the genome sequences of 2,000 potential poplar parents.

JERRY: And so we're applying advanced computer algorithms to predict parent performance. And we've begun making crosses based on those predictions.

ALISON: They're modeling the attributes of their projected seedlings, hypothetical baby poplars.

JERRY: And so within the next 12 months, we'll plant out the progeny set. And we'll see if we were able to predict the favorable combinations. And if we are, then we're off to the races.

ALISON: Another recent development might help catapult their efforts forward.

JERRY : One of our collaborators at the University of Georgia has been able to develop an early flowering poplar, so it flowers within 30 days inside a test tube.

ALISON : Isn't that amazing? Just a month, instead of 7 years! That development will allow the researchers to test new combinations of progeny all the more quickly.

And hopefully, that means that we are hurtling toward a future where poplar has a place in our energy portfolio.

Poplar (*Populus trichocarpa*) at a field site in Sand Lake, Wash. (Courtesy of Jerry Tuskan)

JULIE: We think that there's a path forward with poplar to create fuels in a very sustainable and inexpensive way.

ALISON: That's Julie Mitchell, who oversees the BioSciences division of Oak Ridge National Laboratory, the division that Jerry and Wellington work in.

JULIE: My name is Julie Mitchell, and I am the director of Biosciences at Oak Ridge National Laboratory.

ALISON: And I asked her about how poplar biofuels are going to be used.

JULIE: The sectors we'll most likely target with biofuels are going to be aviation fuel, so highly refined jet fuel, as well as marine fuel,

ALISON: or fuel for ships

JULIE: which is closer to diesel, and looking for cost effective ways of turning biomass into those two types of fuels. We'll focus less on the car sector since there's a big push toward electrification, right? And really focus on you know, kind of these large industrial needs for biofuels in aviation and shipping.

ALISON: Are biofuels already getting into these areas? Julie had an answer that surprised me.

JULIE: If you've ever flown through LAX (ALISON: the airport in Los Angeles, California) actually and and got on a plane there, then the plane that you left in had biofuels in it.

ALISON: I'm actually from LA, and I had no idea. Apparently, United Airlines buys biofuel from a company called World Energy in Paramount, CA. The biofuel is made from agricultural and cooking waste, and it gets added to the general supply of fuel that goes into all of the planes. One day, poplar-derived fuel might be added to the mix.

But besides being a source of biofuels, poplars are also fixing CO₂, helping to pull the greenhouse gas out of the atmosphere. And, Julie says, another application that Oak Ridge National Lab is developing is grinding up poplar and mixing it with other materials to create new 3D-printed composites. These materials could be tuned for hardness and flexibility, so that they can be tailored to different manufacturing applications, like printing molds for making automobiles and boats .

So to recap, Jerry and Wellington are working on making poplar trees more drought tolerant, fungus friendly, and less lignin-producing, so that they can be used in these exciting ways: to sequester carbon, produce sustainable fuels, and create new materials.

JULIE: All of this will improve the quality of life for Americans and people worldwide, if we can pull this off. And especially, if we can start to mitigate some of these dramatic climate change effects that we've been seeing in recent summers. We're clearly past a tipping point. We need solutions that we can implement in the short term, and poplar really is a solution that we can implement in the shorter term, I believe.

ALISON: How long is the shorter term?

JULIE: I think the shorter term is five years, right? I mean, you know, the problem with plants, though, or trees in particular, is they have a long growth cycle. But again, the second you get something viable into the ground, you're already beginning to address the problem.

ALISON: With everything researchers have uncovered, it sounds like poplar could soon be a popular bioenergy solution — helping to mitigate our climate crisis.

This episode was directed and produced by me, Alison Takemura, with editorial and technical assistance from Massie Ballon, Ashleigh Papp, and David Gilbert.

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If you haven't heard Oak Ridge National Lab's podcast, check it out! It's called *The Sound of Science*. Hosts Jenny and Morgan do a wonderful job of humanizing ORNL's science stories, which span plutonium-fueled space travel to a geneticist's passion for chocolate.

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That's it for now. See ya next time!