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Society and Policy Influences on Biotechnology Risk Assessment for Restoration of Threatened Forest Tree Species

Transcripts of the International Conference, April 2021

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ORGANIZERS

Dr. Douglass F. Jacobs
Department of Forestry and Natural Resources, Purdue University,
West Lafayette, Indiana

Dr. R. Kasten Dumroese
USDA Forest Service, Rocky Mountain Research Station,
Moscow, Idaho

COVER: A white ash (*Fraxinus americana*) killed by the emerald ash borer (*Agrilus planipennis*), an invasive species that has decimated tens of million native ash trees across the United States. Courtesy photo by Douglass F. Jacobs, Purdue University.

Executive Summary

This novel conference integrated input from key scientists, regulators, and stakeholders toward forming broad consensus on the state of knowledge concerning the use of biotechnology to restore tree species threatened by introduced insects and diseases. The conference included three keynote presentations and additional presentations from 15 invited scientists, three federal regulatory agencies, and three nonprofit stakeholders working in the biotechnology, ecology, and social science areas of threatened forest tree species restoration. The conference identified knowledge gaps and key research and policy/regulatory issues that must be overcome to achieve restoration of threatened tree species. Participants also prioritized effective means for improved education and outreach in genetic engineering methods, benefits, and risks, and understanding of public opinion and concerns. Subsequent discussions among all participants formed the basis of a multi-authored synthesis article.

Five key conclusions were identified as a result of our conference: (1) unprecedented potential and momentum exists for restoration of threatened forest trees; (2) a sense of urgency to restore threatened forest trees is being driven by global incentives for tree planting, forest restoration, and maintaining biodiversity; (3) society is increasingly open to using biotechnology for restoration of threatened forest trees; (4) policy is likely to follow public opinion, provided that risk-benefits are favorable; and (5) the massive costs and scale will logically limit the pursuit of successful restoration to a few species in specific regions.

The two-day conference, originally scheduled for April 2020, but postponed because of the COVID-19 virus (SARS-CoV-2), was held virtually on April 21 and 22, 2021. Presentations were recorded, transcribed, and printed here.

Transcriptions

Recorded presentations were uploaded to YouTube and closed captioning transcripts were automatically generated. These transcripts were edited by two independent editors to ensure appropriate sentence structure, clarity, and conversational cohesiveness, and edited a third time to confirm technical rigor. Text in brackets [ ] was added by the editors to improve clarity.

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Data Archive

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POTENTIAL FOR BIOTECHNOLOGY TO AID IN RESTORATION OF THREATENED FOREST TREE SPECIES

KEYNOTE PRESENTATION

Biological, Regulatory, and Market Conditions Affecting Forest Health Improvement With Recombinant Biotechnology: Constraints of Today and Visions of a Smart Tomorrow

Steven H. Strauss

Distinguished Professor of Forest Biotechnology
Department of Forest Ecosystems
Oregon State University

Thank you, Kas [Dumroese] and Doug [Jacobs], for the chance to speak. I'm honored to be a keynote in this symposium. It is fascinating what you've put together, really looking forward to it. I have a lot I want to cover so I'm going to move pretty fast. I just mainly want to say, I think I have a little bit of a reputation, or maybe a lot of one, for being a crazy advocate of modern biotech and recombinant biotech. I really feel like it's an important tool that can be added to breeding, particularly given the speed, the severity, and the novelty of forest health challenges. It's really the freedom to operate, and the freedom to use it, that we don't have today and that's what I want. This is not better than breeding: As I always teach when I teach ag biotech, breeding is the foundation of everything, and transgenic gene editing is an add-on that can help in certain cases, and I feel that strongly here. Just wanted to make that little bit of a disclaimer.

Today I want to talk about the biological and social constraints and then give a little bit of a sense of what a smarter way to do things could be. The USDA [United States Department of Agriculture] is just putting into place this thing called SECURE [Sustainable, Ecological, Consistent, Uniform, Responsible, Efficient rule]. I think it's a hell of a good start, and so I'll tell you about that as well as, you know, my broader vision. I wasn't sure who was going to be on the call and how much knowledge they had, so just quickly, [here's] what recombinant biotechnology is. Recombinant biotechnology encompasses what we call genetic engineering and gene editing. All the genomics we do these days are impossible without recombinant techniques. By recombinant biotech, we mean modifying DNA out of an organism and in some way putting it back in to modify or change it. You may remember this slide here, it just shows you got to get it in some sort of vector, biological or physical. You have to find rare cells that have a permanent, stable modification and then get them back into organisms. Often this last part is the hardest, and I'll talk about the challenges of actually doing large-scale, efficient genetic transformation.

So, let's start with what I call breeding, pathosystem, and global environmental change complexity. First, you know things have become so urgent that I often feel pessimistic that any kind of host resistance breeding is almost useless because you just don't have enough time but, obviously it is part of a general strategy to try and make more resilient forests. We have extremely diverse and very extremely slow-to-reproduce genotypes and forests, which means that whenever we have a few genes oligogenic, whether they be recombinant or natural, they're getting more rare than common. It is really hard even if we have a dominant gene action, as you have for the chestnut, the OxO gene—as I'm sure you'll hear about soon from Bill [William] Powell—it takes a long time so that's sobering, and we need to keep that in mind. A good little fix is the cool ability to make

things flower rapidly through transgenic or speed-breeding kinds of approaches, as you might also hear about with chestnut and because that's also dominant gene action. If we could get transformation to be more routine and robust, this could be a very powerful way to speed conventional breeding; and again, if we could transform many genes and genotypes, this problem of having to take rare genes and get them out into large diverse populations could be much more reduced, and as you'll hear, that's a gigantic constraint.

So, just a little bit about speed-breeding transgenic approaches; I do a lot of work with eucalyptus and companies around the world. I worked with Ove Nilsson, who's also an author on this paper about FT overexpression induces precocious flowering and normal reproductive development, [a paper from] many years ago. This study showed that you could speed up the reproductive cycle in poplar and eucalyptus, with the technique working better in poplar. In eucalyptus you can produce little trees in the greenhouse that when you look closely are packed with floral buds, and when you study them over time, they produce very normal pollen grains and seeds. This is operational now for apple breeding, plum breeding, and probably some others I don't know about. If we could do this routinely with our forest trees, this could be a very powerful way to speed conventional breeding.

I grew up and went to school in the East Coast and pretty much all of the Eastern deciduous forest is not planted, so if we're gonna be planting things that have new resistances we need a huge major investment, a big social engineering, as well as all the silviculture involved. We're really concerned about pathogen evolution. We want diversified solutions with high stability. I love the model of what's going on in chestnut of trying to combine genomic selection, transgenic methods, and new kinds of transgenes and cis genes—that's what we need to do. We need to put this all together and of course it's very challenging, and so one of the take-home messages for me is that this means that society needs an appetite for experimentation, for learning as we go, commonly called "adaptive management." I think that's the paradigm in all of forest research really; we never know what we're doing. When you consider a life cycle of a tree in genetics or otherwise, the problem is that it's simply not allowed in the recombinant era and until things are declared safe by a very cumbersome regulatory process. We need a revolution, and I'll talk about that a little bit on the social side.

A second biological constraint, transformation and regeneration, species and genotypes are extremely variable in response—the routine and reliable methods for something comparable to *Arabidopsis* in planting methods, or even standard leaf disk methods in tobacco and Solanaceae, remain elusive pretty much for all of our trees. That's because of this very complex area of science and technology where you're trying to understand the interaction between getting DNA in and to recombine into the genome and getting those cells to regenerate. Then the complicated stress and developmental responses that are occurring as you're trying to do that. The bottom line is you need very specialized laboratories. You see that in agriculture, there's a number of transformation service laboratories around the country that have that kind of crafty people. If you have a transformation lab you know you depend on them to try to get things done. This of course requires you to have the expertise in regulatory compliance. A lot of what's been done in the private sector is essentially unavailable, or what they do as secrets are secrets, and that's not going to fly for the future for the big challenges.

So, one of the things that is helpful is as knowledge of developmental biology explodes, which it has been for about two decades, we've been identifying some of the key genes that enable transformation and regeneration. We're starting to use those to enhance transformation, and so one of the hopes I have for this is what I call dev genes. People call them morphogenetic regulators; the folks at Pioneer Hybrid, now Cortesva, have really pioneered it for monocots, but there

have been some tremendous advances just the last couple of years in bringing this over to dicots. Discovering new kinds of genes using an implant that is a nonsterile *in vivo* kind of environment, gives me hope that something great is going to happen. You can see stuff like this in these papers, where you have these embryos bursting out due to induction of these genes, telling you, "hey, I'm transgenic because I have a fluorescent reporter," like you see here. Then in a more recent paper, UC [University of California] Davis folks and folks from a biotech company use this GRF GIF chimera to really speed up transformation in monocots. Here you see wheat but also there's really significant benefits in citrus and grapevine. This gives me hope that maybe in our dicot trees and maybe even in our conifers, we might have some more robust solutions over time. Essentially, we need to know what are the best sets of genes, how to express them, and how to get rid of unwanted genes. There's no tree I'm aware of in the world where this technology has been put to work and proven. We've been trying to do it, and it's hard. The notion that you're going to throw these genes on and it's going to be a miracle simply is not true. There's a whole lot of R&D work to be done if this is going to be an important solution.

Moving on to the ability to do recombinant biotech in contrast to breeding is all based on mechanistic knowledge of how genes work, and how pathogens and trees interact. We mostly don't have that detailed scientific knowledge, but fast and recombinant methods are one of the very most powerful ways to sort of get and demonstrate and improve that knowledge, so more freedom to operate would actually give us better science. I work with a number of people who are doing microbiome or pathology science who need transgenic or gene-editing verification to know they're really doing what they think they're doing. I just want to make clear that this is the key limitation, and the National Academy report said that as well for wildfires even for plantation forests, and recombinant builds on that knowledge.

Moving on to the social aspect, a very brief comment on investments. Society obviously decides what kind of science it's going to do and what it's going to permit, and so I've been watching the biotech GMO [genetically modified organism] controversy of the late 1990s and early 2000s. The USDA used to fund a lot of that, but stopped funding, so their USDA NIFA [National Institute of Food and Agriculture], a plant breeding sort of a priority area, and it's all about non-non types of breeding. And it's been clear, I've talked to some of the heads there, and it's because they don't believe you can get through the social barriers, so why should you invest in it. Monsanto [now Bayer] can but public-sector researchers just can't, so why should we give money? That's been the attitude, so a lot of the scientific development we need and I just talked about has not happened. There's been more funding from the National Science Foundation on how to develop transformation methods and understand them better than there has been from USDA this year, so it's kind of amazing. They're allowing targeted gene editing, and that probably has to do with a secure method where very simple, secure regulatory innovations can be allowed as conventional breeding. It is very limited, but for at least 20 years the USDA, the main funder of applied crop and forest research, has not touched transgenic anything really apart from basic science, so investment is a critical thing that we need to change if we're going to have freedom to operate to do great things on a broad scale.

A second item I want to talk about in a little more depth is the regulatory side. If you talk to legal scholars about regulations, the notion that regulations lag behind science—that's kind of a, "of course it does," and the notion that it lags behind by decades is, "of course it does" because it's all tied up in politics and public perception. Science is moving forward in the biotech area, it's been, you know, what I would call flagrant for years and years. I've written about it, in a policy essay and Science in 2003, similar things with respect to the global situation and nature biotech in

2009, a similar kind of thing more with a biofuels emphasis in Bioscience in 2010, with various plant breeder and legal or economic scholars collaborating on a lot of these, and I've kept trying. I worked with a lot of crop breeders back in 2005, as the genomics world was happening, and we understood more about the flux and the variability and the mutations and the rearrangements during conventional breeding. We could begin to say, "hey, a lot of what we do in transgenics looks actually very similar," and we should have that as a sort of a grass standard. Which it basically isn't, by the way. Then, in 2015, we started talking about the urgency of it given forest health issues and talking a little bit about the market obstacles, which I'll cover separately. The National Academy has been saying the same thing in different ways for about 30 years. The most recent statement, that it should be the trait and its novelty and risk and not the method, was said again in 2016 and with a more modern perspective, so it's really frustrating. The key issue with regulation is the legal presumption of guilt because you use the biotech method. So, gene editing is getting some various exceptions around the world to different degrees including in the U.S.A., but if you look at the secure system it's only the simplest, cleanest, most natural types of edits. It's a very narrow piece of what gene-editing technology can do, and what guilt means is zero tolerance. The presumption of guilt is for any gene flow in research and breeding trials, and so the adaptive experimental management with the diversity of genes, cis genes, and gene-edited materials transgenes is essentially impossible. You have to have genes with momentous import and obvious effects in the lab, and that will be something you go forward with, like the OxO gene in chestnut. I'm sure we'll hear more about it, yet there's a tremendous safety built into the whole system because breeding trials tend to be small and condensed, and so compared to the environments into which the little bit of pollen and seed flow happens is very small. In conventional breeding of all kinds, we simply accept that in almost every single case it is without any regulation at all, yet of course the need for solutions is immense and growing; conventional breeding with trees is just hard work, it's really slow and hard to keep up, so it's been argued many times by ethicists, legal scholars, and philosophers that this kind of system is labeled precautionary.

In the European system, it is actually the opposite. It takes away critical options to deal with a very existential threat to our forests. Just in case you're wondering, you know, if you drop the rDNA trigger, are we going to unleash all these horrible things on the world? No, you're not. There are many options for legal constraints on the things you can do based on the traits in their novelty based on critical coexisting considerations, and I just put up a couple here that are probably obvious to all of you. For things where there's questions, like there's a protein that might be an allergic threat, there's no reason you can't do an early conference with the FDA and find out if you can do adaptive management with it or not, if it's such a severe threat. Then you could go forward, and that kind of thing is actually allowed under the secure system where you can propose a project, look into the science of it, and the USDA will let you go forward without years and years of field trial results that in some cases are just showing the obvious. Obviously, we also have a legal system in the U.S. where anybody can sue anybody. If you create a nuisance, there's another way to sort of curb the bad actors, apart from having this regulatory trigger. And then critical for all this is having coexistence thresholds that are workable, that allow people to get along, and we don't really have that now. Those need to be in the market system as well as in our regulatory system, and some countries like Japan and the food system have that and are much easier to avoid international trade issues. Others have very difficult systems, like in Europe, that are essentially zero from a practical point of view, so we have to have that down, it needs to come from a very high level.

Moving on to markets, perhaps the biggest problem right now. So, what we have in forest, we have this thing and I put it in quotes because I

don't think it's really "green," it's a certification system that was intended, was created with the best of intentions and reduces some bad things but also does a lot of bad things, in my view. One of them is it's a complete preclusion of biotech. So, it's a big deal because it's not like organic food, which is sort of a niche; in the larger world food market it affects lots of huge areas of forest, almost all planted forests around the world, and the FSC [Forest Stewardship Council] started this. Greenpeace was a key creator, you know, they're against all GMOs no matter what in agriculture or forestry, where one of their major principles is genetically modified trees are prohibited period. It's kind of like a "Thou shalt not kill"; it's not a detail, it's a fundamental principle, so very, very hard to change. So, since they did it, it spread around to really all the certification systems that I know of, all the major ones around the world, where you can't even do research, you can't check out a genetically modified tree in a small scale to see if it gives you benefits or if it has problems—it's just "no." So, I've been frustrated by this, as you've seen I've written about it since 2001. I spent some time at Oxford and with some scientists there you may know—the very famous Jeff Burley, one of the great forest geneticists of the previous generation, worked with us—which is just to say this is scientifically absurd, that there's a ban on research, and then again that [policy] paper I mentioned earlier raised that question again, and really nothing had happened at all. In fact, it had gotten worse between 2001 and 2015. My colleagues Armand Sagin and Adam Costanza said this is just so ridiculous; can we do anything as scientists to try to move the dial on this. And so, what we did is, we created this petition, really just because we didn't know what else to do, basically petitioning these certifiers to allow scientific research on certified lands or on associated lands. They put together a website and a committee of scientists and some literature—this is based at Oregon State [University]. This [slide] is a field trial of genetically modified poplars that I've done for a number of years here in Oregon. The Alliance for Science, that you may know is heavily funded by the Gates Foundation and is very involved in biotech outreach for Africa and the developing world, agreed to host it and do the social media for this. I went around and with the other scientists working on it and got the American Society of Plant Biologists to endorse it and help us to publicize it. Then triple AAAs [American Association for the Advancement of Science] did the same thing; they didn't endorse it but they agreed to help us publicize it and got the word out to tens of thousands of scientists. So, this is obviously a very nerdy narrow issue; this is not like, "all GMOs are bad, don't eat it and you'll feel healthier"; this is about a narrow issue, so we didn't know if we would just get me and my mother to sign it or more. But we were pleased to see that 1161 forest scientists and stakeholders signed it. The majority of them were PhDs, and we managed to get a paper published in Science magazine, not a paper but a letter to the editor. And there's the group of scientists, fairly robust international groups, some of which I consider the finest forest biotechnologists on the globe, to kind of say this is absurd and it must change. Science magazine itself decided it was newsworthy and had a little news article about it at about the same time.

So, what's happened, I'll just summarize quickly what's in that letter, what's in the petition: Forest health crises are huge; growing customized biotech tools can clearly help; extensive research and field trials show that these are not scary crazy trees, they're just trees with a new trait, a lot in common with breeding in terms of the variability that you see. Gene editing is more precise, we have enough research already, than conventional breeding if you want to make specific changes. We need local site-specific research though to see if they make sense in particular situations—that means putting trees on land which is mostly certified. The ban contradicts the long-standing scientific opinion from the National Academies all over the world that it's the trait not the method that matters. So, you can look at it more there. So, happily this petition did actually prompt FSC to take a more serious look. They just ignored it for decades, but what they're

doing right now is they're looking at only allowing what's called "associated use of GMOs," that is, a company that is growing GMOs on some other lands, maybe other private lands where they rent, it could not put any product in their materials at all. It has to be completely segregated. Now they're thinking of allowing these companies to at least, you know, still have certification on some lands and products but then in other places be able to use GMOs. And that's only as far as it goes, so the vast majority of lands would remain prohibited for GMOs. I will go a little quicker here, and I'll just say that I've been critiquing. So, as part of this review they've hired an environmental journalist who knows nothing about biotech. He's not a bad journalist, I've read his stuff, but nothing [about biotech]. We've had a bit of going back and forth where it's just been a whitewash. It's been really scientifically absurd, so there's clearly no real look at this by FSC at all. They're just using this, I think, as an excuse to go on doing essentially what they're doing. I don't really have time to go through SECURE, and those of you who don't do biotech, we can talk about this maybe later in the meeting because I think the details really matter.

Here's a wonderful paper if you'd like to read about it, I'm sure you all read the European Food and Feed Law Review, but it's actually very readable and you know some key provisions like I said. Techniques now are the triggers; it's actually a broader network than the USDA had before but it's much less in that it's more scientifically based compared to their planned pest sequence and vector thing. It's based on test risk now, it's based on the trait not based on the method, and as I said, simple edits and simple deletions and things like that are sort of pre-exempted. But they have this regulatory status review, which I think is really encouraging, and apart from the fact that you can self-exempt they have this mechanism of action decisions. So, after decades of scientists saying it's not each event that really matters in terms of the bigger biological impacts, it's what you're doing, it's how the gene works, it's the organism, it's the trait that it affects, so you can get essentially deregulation of categories of stuff that can apply say to all of *Castanea* or all of *Pinus*. In theory we don't know exactly how it's going to work. In the long run there are concerns with it and how it's going to affect trade when you have exempted edited varieties that can't be tracked—that could create a lot of chaos so there's a lot of stuff to be worked out about this.

To finish, these are just statements of what I've been saying. Obviously huge forest health problems have significant potential for recombinant biotech to help. We've seen some cases already, and we know it's not just in theory. Biological and social constraints are huge. We're hardly investing in the biological side, as I mentioned, and the social constraints are easing a little bit with SECURE, so we'll see what really happens in practice. EPA [U.S. Environmental Protection Agency] are the ones that really matter with respect to pest resistance traits, and we don't know what's going to happen there though there's some talk. Hopefully Chris [Christian Vieglais] will tell us about what's going on later in terms of aligning with SECURE somewhat. Last slide. So, I think the USDA is really, from my point of view, a tremendous move in the right direction. It has a tough role, given it is coming into a national and global system which is method and event obsessed, and so it's going to be a tough road. Essentially what we have here is an ethical question. What is precaution? The way we've codified it in our rules and market obstacles is scientifically absurd. We've known that for decades but now that we have this incredibly stressed world, it's just looking, uh, tragically absurd. I think we need to do something about it, so I'll end right there. Thank you very much.

KEYNOTE PRESENTATION

Tree Breeding: A Necessary Complement to Genetic Engineering

C. Dana Nelson

Research Geneticist
Southern Research Station
U.S. Department of Agriculture, Forest Service

Co-Director
Forest Health Research and Education Center
University of Kentucky

I am going to discuss the role of tree breeding in genetic engineering and genetic engineering in tree breeding. I hope this will be a nice follow-on to Steve's [Strauss] presentation and hit at some of what Richard [Sniezko] was saying [during the previous question and answer session], or at least a give an extra perspective on that. As an overview, I'll have a few introductory slides and comments. I have put out the major purposes for genetic engineering and tree improvement; why we would want to use genetic engineering; try to be developing a generalized approach for using genetic engineering and tree improvement; take a look at American chestnut as a case study for the application of genetic engineering in tree improvement for the purpose of species restoration, the major topic of the workshop; and then end with a couple conclusions. The intro will have a couple preliminaries on biotech and tree improvement; it will look more specifically at genetic engineering, looking at the three major components to move genetic engineering forward, as Steve really hit on in the first presentation. Technology capability, regulatory approval, and societal acceptance are all needed to make progress. Then I'll touch on what I call the JDN (Jacobs, Dalgleish, Nelson 2013) model for species restoration. We saw a preview of this in Doug's [Douglass Jacobs] opening remarks, where we worked with Doug and Harmony del Gleashon developing this model, and it has a technology, ecology, and society phase to it, pointing toward what's needed for successful restoration.

Genetic engineering, as Steve [Strauss] pointed out, is any technique that uses recombinant synthesizer amplified nucleic acids to modify a genome, also referred to as genetic modification, and it produces what is most commonly termed as genetically modified organisms. Couple notions here on adding a gene from an unrelated species: It can be a transgene making transgenic plants and so this is, you know, a common application. You don't have the gene in your species, so you get one from another. There's also the concept of the cis gene, which is a gene added from the same or a closely related species. This could be an allelic variant within a species or just a related gene from a related species. These are pretty cisgenic plants, still engineered, but something to note here is that the cis gene addition could be achieved by breeding. This is important with respect to the SECURE rule, the new rule that APHIS is using on regulating genetic engineering and, of course, genome editing primarily being enabled now with the CRISPR-Cas9 technology. This is very nice; it allows for site-directed modification whereas previous types of genetic engineering basically are going to be randomly directed modifications. With genetic engineering, of course, as mentioned we have regulation. Steve got to take us through that a bit and all of the issues in history behind that. I'm just going to say that APHIS [United States Department of Agriculture, Animal and Plant Health Inspection Service] stands there to protect agriculture from plant pests, their old rule basically

regulated genetic engineered plants if the DNA from a plant pest was used and/or incorporated into the GMO [genetically modified organism]. The new rule, or the SECURE rule, went into effect last year. It says that GMO can be regulated if it imposes a plant pest risk. I think Steve stated that it, you know, all GMOs, would be regulated that they'd looked at or evaluated, but you'd have much more freedom to operate if you could show up-front that the new trait didn't have any elevated risk. GMOs are not regulated under APHIS, under SECURE, if the same modification could be made with breeding, so again this is where the cis gene concept comes into play.

The [U.S.] Food and Drug Administration and the [U.S.] Environmental Protection Agency also play regulating roles with genetic engineered plants. Tree breeding, the way I'm defining it and talking about it here, is one aspect of the larger tree improvement enterprise. Tree breeding involves improving trees through breeding per se, so this implies multiple generations or cycles; and these cycles of course have a crossing, testing, and selecting phase; and those typically need to be repeated a time or two or three to move the trait to a level that's effective for application. Breeding objectives need to be defined for the intended product, so keep in mind what does this product need to do and need to be to be successful in the field. What traits need to be improved, what is the performance and diversity levels required, what method of propagation can be used, and then most, you know, quite importantly is what is the intended silvicultural system. Biotech, broadly speaking, provides lots of very valuable tools to tree improvement. Primarily for maintaining, assessing, and creating genetic variation, and of course genetic variation is the raw material for any tree improvement. Without genetic variation there's nowhere to go forward with any form of tree improvement, including tree breeding. The clonal propagation technologies provide us with the ability to maintain or conserve and even exploit genetic variation. The molecular marker technologies have allowed us to assess, monitor, and manage genetic variation, and of course genetic engineering is providing the opportunity to create or to add and enhance genetic variation. So, a very important added tool to the tree improvement tool chest. Another point with respect to biotech and tree improvement, there's often been sort of a bit of an antagonistic stance, kind of within the, say the forest genetics community, and there's kind of this misperception that's quite common that you know on the surface biotech is fast and it allows for the use or, you know, improvement of novel traits versus tree breeding, which is slow and sort of limits you to old traits. Again, we had a little bit of discussion with Richard's [Sniezko] question earlier that kind of hits on this. In reality, to be useful, biotech must be integrated with tree improvement, so taking a synergistic approach, you know, we need to ask what is the problem, how can tree improvement using any and all available tools help solve this problem. We have some tools, some fantastic tools, and of course there's lots of R&D needed to really advance these tools to the point where they can be readily available in all of the species that need work, but clearly we need this integrated to really gain the synergy of genetic engineering being integrated with tree breeding. With genetic engineering we, of course, have these three major areas: within technology capability, you know it's not easy in trees but possible; regulatory approval, not easy especially in trees but possibly possible; and on societal acceptance, it's not easy for any but trees for restoration could be different. How does genetic engineering fit with restoration?

Here's one model for successful restoration that, as I mentioned, Doug and Harmony and I published in New Phytologist (2013). I call it the JDN model. It describes the need for three spheres to work, to grow and converge, for restoration to be successful. And so genetic engineering clearly fits in the technology and society spheres, as mentioned earlier and as Steve pointed out. Within that respect, my presentation will see that the testing phase within the generalized approach to be developed will address silviculture in the ecology sphere, so growing and converging these spheres

lead to increased probability of success for restoration. Part two is purposes of genetic engineering and tree improvement, so the whole focus of this workshop is really on this first major purpose, species rescue and restoration, but there are two other important possible purposes for genetic engineering and tree improvement. The second one I have here is value-added trait addition or enhancement and that's probably, over the years, received the most attention within the four within forestry, and of course within agriculture as well. Then within forestry, there's this concept that would be possible with genetic engineering to consider: domesticating high-value hardwoods for various applications in forestry and certainly in wood production. So, on species rescue and restoration, as mentioned by both Doug and Steve, there's a whole list of very important forest species that are under pressure from invasive, non-native pests and pathogens, of course as well as climate change, and how they can respond to these threats. If we can engineer resistance to diseases or insects where it's not present, or as was indicated, is present at such a low frequency or at a low level of effect, you know that engineering may be an important tool to bring the species back through improving its survival and reproduction. In this case we're going to be developing self-sustaining and evolvable populations, so seedlings would be deployed, followed by natural regeneration. Here the transgene is released into the environment, so this is kind of, this is very much a unique situation that species rescue and restoration brings to the forefront with genetic engineering.

Value-added trait addition is another important purpose typically applied to plantation species of commercial interest. Trait service side tolerance are, you know, resistance and tolerance to biotic and abiotic stresses. Wood properties are often important, and of course more recently the idea of increasing carbon capture and sequestration potential of trees, looking specifically at photosynthetic efficiency, rooting depth, and sort of characteristics of this nature. Deployment in this case is very much different than in species rescue; it's typically, you know, sterile clones, so sterility is engineered along with the trait of interest. Sterile clones are planted to confine the transgene. Possibly full-sib families as seedlings could be planted if there was management techniques for limiting transgenes or if the reviews suggested that there wouldn't be an enhanced risk for a transgene release. Hard to know, but certainly, again, a seedling deployment is typically much more efficient in forestry and to foresters. Then the third idea with respect to a purpose of genetic engineering is the idea of domesticating the high-value hardwoods. These are typically later successional species domestication genes, which are known in various plants; they could be modified or engineered in trees and high-value hardwoods. They could promote early and sustained survival and growth in plantation or orchard-like settings. These would no doubt be clonally deployed, probably as sterile clones. They could be grafted into an orchard and managed for high-value wood production. Of course, this would provide for confining the transgene. With those three kinds of major purposes of genetic engineering and tree improvement, I wanted you to know there is a kind of a generalized approach on how we would utilize genetic engineering. As an overview, the general approach basically has three major stages or phases, you know, initially just getting the technology up and running is a big thing for any species; the second phase with technology in hand is what I call selecting, testing. Selecting, this is where you're working with candidate genes. For your trait transgenic-specific transgenic events, you've got to look at the gene expression and the trait performance. Then moving into the third phase is what I call crossing, testing, and selecting, and then finally increasing. Here's where the transgenic events are incorporated into the breeding program for the purpose of producing transgenic positive planting stock for end-use development. The first phase then is, of course, developing a technology transformation system—how do you have a system where you can reliably get the trees in or make the modifications to the genome. Recombinant DNA work, tissue culture,

is a key technology for the transformation system, initiation growth rate, plant regeneration, conversion to plants—we saw that indicated clearly in Steve's presentation.

Another opportunity with working with the breeding program at this developmental stage is obviously to be able to screen genotypes from the breeding program for their ability in tissue culture and transformation. So, a lot of diversity there if that can be screened, you know, you can get to this point where you can develop workhorse lines that would, that will be efficient in the approach to move the genetic engineering into the tree breeding program. You clearly want to sample relevant genetic diversity for your intended end use at this stage. Early flowering could be an important trait to incorporate, and certainly developing environmental conditions for early flowering will be important. And, you know, whether you're using genetic engineered events or not, that's something that's really needed in tree breeding, and certainly utilizing elite performers at this stage would be important as well. The middle phase here is when we have workhorse lines available through the first phase, testing candidate genes for gene expression, testing transgenic events for trait performance, and this is what I refer to as phase one of the testing. I would relate this to the clinical trials for vaccine and drug development in that they have a four-phase system in clinical trials; it's very analogous to what we should be looking at as we move through a genetic engineering program in trees. This stage, we're really at the phase one testing. We need to move as soon as possible into a wider [exploration], into testing the transgenic event in wider samples of genotypes and environments. The most very effective way of doing that is crossing the transgenic events into a wider sample of genotypes to produce what's referred to as a T1 transgenic one generation. This was written about and published with Newhouse et al. in 2014, and testing T1 seedlings are very convenient because oftentimes you'll have segregates, where you'll have TG transgene positive, transgene negative plants, from the same genetic background. These can then be tested under a sample of conditions, and this is effective, really phase two testing. The third phase of this generalized approach is the specific strategy, which is going to depend on the purpose of the genetic engineering and the end use of the plants. There's basically two goals in this phase: one is to further test the transgenic events or events in more genotypes, and as you're doing it, you'd be diluting the founder effect of that workhorse line. It's really important to make sure that as we get closer and closer to field deployment, we're diluting out any residual founder effect that would come along with the transgenic event. We're incorporating the selected transgenic events into the breeding program with additional generations of crossing, testing, and selecting in the T2 and T3. If this is needed for, again, the purpose and end use, with respect to species rescue and restoration, this is going to be a required phase. This does provide then plant experimental material for phase three testing, so these are much larger trials with larger genetic diversity, sampled in more environments, and able to look under more environmental conditions. I'd point out that the phase one to three would typically be done during regulatory review. Phase four then is sort of the first bit of testing done in the non-regulated period. To get to phase four, you basically are needing to intercross the selections from the last transgenic generation to get to a point where you can select for homozygous transgenic genotypes, preferably that would also have high breeding value for other traits of interest. The seed orchard would then produce transgenic positive planting stock for deployment.

Putting all that together, sort of into one page that is a generalized approach. The tree breeding program sort of overrides the whole approach—it contributes materials and information, it does crossing in testing and selecting and increasing, so those important components are handled by the tree breeding program as the genetic engineering is moved through this sequential stage of technology, development, selecting, testing, selecting, and then crossing, to get to the increasing stage. It's important to have really strong tree and plant

genome information regarding this, and of course ultimately you're coming through to the silviculture and ecological aspects of this as we get the plans for deployment to the field.

The last section on the talk is a case study on American chestnut. Coming up on the end I'll touch briefly on this as I know Bill [William Powell] is to follow, and Andy's [Andy Newhouse] talking, and so I know there'll be plenty additional discussion on this. [Chestnut is] a foundational species widely distributed in the Appalachian region, extirpated by the '50s; you know, breeding approaches over the decades have been used. The American Chestnut Foundation now has a revised approach on their backcross breeding program that emphasizes recurrent selection with genomics or genomic prediction. The genetic engineering approaches were brought along by primarily University of Georgia and SUNY ESF [State University of New York College of Environmental Science and Forestry] through technology development. The SUNY ESF program now has the Darling 58 OxO transgenic, which is in a single genotype, and they are advancing that. Their goal is to advance it to the T5 generation where intercrosses can be made to recover homozygous transgenic parents. Jared Westbrook sort of published this effort and the rationale behind it in 2019, and you're really, again, you know, diluting that founder effect getting to high effective population size and low average inbreeding. Regulatory and societal APHIS petition to deregulate Darling 58 is in progress. The environmental impact statement is being prepared by APHIS, there's been support voiced now by some environmental groups, citizen scientists are involved, public interest remains high. This was just put out in Nature Biotechnology, where the Sierra Club has come onboard suggesting that genetically modified chestnut is a good thing as it can lead to restoration, and the silviculture and ecology aspects of this, of course, are underway as well; lots of, you know, collaborative work underway with the Forest Service and others. American chestnut appears to be approaching the JDN convergence for restoration success, as these pieces over the years and years and years have finally grown and converged to the point where we're really setting the stage for success in this particular case. In conclusion, genetic engineering is an important tool for tree improvement; tree breeding is needed for successful genetic engineering. Ultimately, it's about smart breeding with biotech tools and the breeding objectives well-defined. What are the target traits and environments? What are the required diversity and performance levels? Then, how are you going to propagate and deploy these new trees? And what's the silvicultural system?

KEYNOTE PRESENTATION

The American Chestnut: How Biotechnology Can Be Applied to Conservation

William A. Powell

Professor
College of Environmental Science and Forestry
State University of New York (SUNY)

Director
American Chestnut Research and Restoration Program

What I want to do is present how biotechnology could be applied to conservation using the chestnut project as an example. Our research is part of what we call the three BUR collaboration with The American Chestnut Foundation (TACF). The three BUR stands for breeding, biotechnology, and biocontrol united for restoration. I just want to point that out that we actually like the full toolbox approach to restoration of the American chestnut. I will try to touch on some society and policy influences as I go through the talk, but this is pretty much my general talk that I've done before with a few things added to it. First, you got to start by saying why American chestnut is the poster child. Why is it important? Well, because American chestnut once was a very common tree in the Eastern forest, in some places it was probably one of the most common trees. This is a picture along the Blue Ridge Parkway in North Carolina about a hundred years ago, probably one out of every four trees. This scene would have been an American chestnut; we know they were very abundant. This is one of the rare pictures; I just love this picture of a chestnut forest. You can see the chestnut trees by the white tinge on the tops of trees. Those are the catkins, the male flowers, of the American chestnut, and people used to describe this is almost like seeing snow in June because the tops of the trees would all be white. Of course, we've lost all that because of a pathogen introduced a little over a century ago that basically wiped out all the large American chestnut trees.

What are the values of American chestnut? It has an agricultural value, a social historical value, a wood products value, and also just has an environmental value. It's considered to be a keystone species, or a foundational species. All these values kind of point to why the public likes the American chestnuts, and they like it for different reasons and therefore, when we actually produce a resistant tree and start getting these trees out, we actually might be putting out a group of different kinds of trees for different purposes. There have been many efforts to stop the blight. Back in history they started off with using fungicides. They did not work. Sanitary methods where they removed infected trees did not work; they even tried to replace the chestnut with timber-type Chinese chestnut and planted thousands, if not tens of thousands. Those have not really survived. They even tried mutagenesis where they treat nuts with gamma or X-rays, and this was a popular technique back in the 1970s. We planted tens of thousands of those trees but very few of those are left today. Hypovirulence or biocontrol, that's actually how I got my start with the American chestnut, looked very promising and actually works fairly well in Europe with the European chestnut but not here in the United States for a lot of reasons, which I'm not going to go into right now.

They have made what's called "super donors" of the fungus that can donate the viruses that cause hypovirulence very readily, and we're actually

going to probably be using those in our breeding programs to keep mother trees alive. There's been a lot of species hybrid breeding going on; there's been the backcross breeding of hybrids with TACF and still going on right now; there's adding genes for resistance to genetic engineering, which we are doing at ESF; and we are actually starting to try to develop our techniques for gene editing at ESF. I'm going to mostly focus on these last ones in this talk. You can actually buy and grow chestnut hybrids, some are very common, but, and I want to make sure that people know that I'm not against breeding or making hybrids or anything like that, hybrids are okay but they have certain purposes. Mainly for managed systems such as ornamentals or crops; they're not as great for restoration and the reason why, at least with chestnut, is because the different species really evolved in quite different environments in different parts of the globe. They're not exactly the same. Hybrids were made actually before the 1920s to try to improve the agricultural value of chestnuts and after that to try to increase resistance. You can actually buy these all over the place. One of the most popular ones right now I think is the Dunstan chestnut; it's a hybrid between American and Chinese, but if you look at these chestnuts and the nuts that they produce, they're actually quite different. The American chestnut is much smaller, about the size of a dime to a nickel size, where these other ones are quite large, almost silver dollar size. This is important for restoration. The reason why is because not all wildlife are going to eat those large nuts, such as these mallards who are chowing down some chestnuts. Also, it turns out that the most susceptible chestnut trees, the American chestnut and the European chestnut, are the two timber-type trees, where the other resistant chestnut trees in Asia are more of a, we kind of consider them, an orchard type or much shorter trees. Therefore, if you want to restore the American chestnut you have to have something that's going to be able to reach the canopy in the Eastern forest, because it will not flower [without] light.

These hybrids oftentimes will have reduced growth or sometimes intermediate traits throughout the tree. The other thing with hybrids is that as they breed, you often lose the traits that you built into them because they're quantitative and they get diluted out in subsequent generations. There are also problems often with male sterility, actually that's not a problem in agriculture, sometimes you want male sterility, but in a wild situation you really don't. There are things like cracked bark where if you cross certain species you get this unusual condition. Internal kernel breakdown is another one where you cross a certain European hybrid with Chinese and about 40 percent of the nuts just disintegrate within the shells. So again, all these things are manageable, can be prevented, if you're under a managed system but might not be great for a restoration tree. Now the American Chestnut Foundation actually went and did a much better project than just hybridization. They actually took a hybrid and then they backcrossed it through several generations to make a more Americanized tree. The idea here is that you're getting rid of all those unwanted traits through different generations yet maintaining the resistance from the Asian species. The goal initially was for 1/16th Chinese chestnut genome to be maintained with the three—what they thought were only three resistance loci now have found out there's actually many more than that. They found out that there's actually resistance loci on all 12 chromosomes. The resistance seems to be linked to how much Chinese genome is in there, so it's been very challenging. It still can be done but it takes a long time, a lot of trees, to get where you want to be. Okay, this next slide I'm going to show you is one that I use with the public quite often, and it's actually the most common slide that I use, and people really like it in the general public. It describes why genetic engineering is useful for restoration, and it's going to compare genetic engineering to hybrid breeding. So, you know the different traits that we need for a restoration tree, such as tree height. Chestnut, we know, has around 30,000

genes approximately from sequencing, we know that, then a hybrid would have about 30,000 of those gene pairs donated from the Chinese chestnut and 30,000 from the American chestnut. The backcross if the perfect situation came out, we still have about 1/16th Chinese gene alleles. To help the public understand this, I like to use this book example. So, let's say this book describes the genome of the American chestnut, and the words in the book represent the genes of the genome. Okay, so that means even under the best situation in the backcross breeding, you still have around 10 pages or 1,800 words written about Chinese chestnut. Is that a problem? Actually, some of it's what we want, we want to have the words to say "make me more resistant" obviously. But along with that, you also can get the words that say "make me shorter," "make larger nuts," and all these other traits that are not adapted to the Eastern forest. So, the trick here is to make sure you get the genes you want in that 1800 words and not the ones you don't want. Let's compare that to genetic engineering. Again, starting with the same book, describe an American chestnut, and I'm gonna pull out a passage from that book. This patch I like because it's written by Henry Thoreau, it says, "It was very exciting at that season to roam the thin boundless chestnut woods of Lincoln," so with genetic engineering or gene editing later, basically, you're making very small changes only adding one, two, or three words to that book. Okay, so everything else in the book is exactly the same; so now, and the whole book's going to be the same, and it's going to read, this passage is going to say. "It was very exciting at that season to roam the thin boundless, light-tolerant chestnut woods of Lincoln." Okay, so everything else is the same. Now the neat thing about genetic engineering compared to hybridization, it's also gentler on the genome, and there's been several papers coming out recently looking at genomes of plants that have been bred compared to the ones that have been transformed, and so there's less mutations with genetic engineering than hybridization. You end up retaining a hundred percent of the American chestnut alleles, and you're just adding back tolerance. Why is that important? Because if you're trying to put it back into the forest, you don't want to be missing any of the traits that allow it to be adapted to the forest. So, what traits do you want to pick? Well, I like to take a mechanistic approach, you can take a genetic approach, we do that some also, but I like to look at it mechanistically. I like this model that was produced by Fred Hubbard a long time ago describing how the fungus actually attacks the tree, and what it will do over here on your left is it will enter through a wound. It colonizes that wound just like a saprophyte would. The tree responds by forming a ligand lignin, lignified zone. This is the same whether it's American or Chinese chestnut. Both do the same thing. Then you have a wound paradigm that forms, and if you're in the resistant Chinese chestnut, that wound *Peridermium* completely forms and that fungus is walled off, that area is eventually slopped off as the tree grows. But in a susceptible American chestnut the fungus changes in a way that it forms these mycelial fans, and it breaks through that lignified barrier forming a canker, which eventually girdles a tree, killing everything above it.

Okay, so what is the fungus doing that allows it to break out in the American chestnut, and you know, can we stop that by adding certain genes? Well, people have been looking at how the host pathogen interacts for a long time. This is a paper from 1978. Back in 1978, that was before I even went to college, I was just an airman in the Air Force, but this paper describes oxalic acid and polygalacturonate as two key virulence factors in the fungus. Now oxalic acid, what it does is basically lowers the pH at the margin of a canker to 2.8. It also will inhibit lignin formation. It's toxic to the cells and can even trigger apoptosis or cell death, and it's not the typical type of cell death so it actually is providing food for the fungus. This can be countered with an enzyme called oxalate oxidase that removes that toxin. Other things that we're looking at right now is this polygalacturonate, which degrades pectin in the cell walls and releases the nutrients to the fungus. Basically, plants have also developed a defense against

this called polygalacturonate inhibited proteins, PGIPs, and basically that protects the protein. Now working with this is a challenge because actually there are many polygalacturonates in the fungus as well as many PGIPs in the plant, and you got to match them up just right to get the inhibition that you need. And actually, this is something I think some bioinformatics people should look at as you know what's different in the PGIPs of American chestnut versus Chinese chestnut. Maybe you can find ones that actually work as resistance genes. Now, one other thing that we're looking at is how can we stop the fungus's ability to break through the American chestnut lignified zone but not the Chinese chestnut? We came across a gene called a lactase-like gene in our studies. Lactase helps form lignin as well as produces flavonoids, which are accent antioxidants, and the interesting thing about this particular gene is that the amino acid sequence of the enzyme is exactly the same in American and Chinese chestnut, no difference whatsoever. But there is a difference in the expression and a difference in the motor region, and so this particular gene we think might be a good target for CRISPR, where we can just go in there and maybe change the promoter and change expression and maybe get more resistance. So, I'm going to talk mainly about this oxalate oxidase. So, oxalate oxidase, the one we used originally, comes from wheat, it's ubiquitous in plants and fungi, it's a non-gluten enzyme so it's non-allergen. This is just a list of some crop plants that also have oxalate oxidases, so it's very common. There's a lot of wild plants that also have oxalate oxidases and again, I said it's in fungi and bacteria and mosses, it's out there in the world. This is important because, for the public to know that we're not introducing something new into the environment. Now we haven't detected any oxalate oxidize activity in chestnut leaves or stems, but as we start looking at the genomes of these species we did find that there is a germ-like protein, GLP, that possibly could be an oxalate oxidase in Chinese chestnut, and you know, matches pretty well with the domains of an oxalate oxidase. Again no one has described this in a chestnut, but there is a paper out there where people are looking at gall wasp resistance, and they actually think they might have found a GLP that might be an oxalate oxidase in the buds. So, it might just be that chestnut has this gene, it's just not expressed in the right place.

Now why is that important? Well, it's important because of all these new rules that are coming up. This is one that I hope Chris [Christine Vieglais] will mention, this is a proposed rule where this kind of matched the SECURE rule. It also has this part in here where it says genes that could have otherwise been created through conventional breeding. So, if we happen to use this one from Chinese chestnut, would that not be regulated? So, how does this work. Basically, it detoxifies the oxalic acids and it's not a pesticide, it does not kill the fungus, so there's no cytoactivity. Since the fungus survives there's actually less selective pressure to overcome this, so it should be very stable, but we're not relying on that alone. We are looking at other genes, the examples I just mentioned, the PGIP in lactase case you stack with the oxalate oxidase and that can be done in a couple ways. It can be done through genetic engineering, through gene editing, or through breeding. How do we test for resistance? So far we've pretty much been testing with a small stem assay; this is a very severe assay. The wild type American chestnuts will usually get girdled and die in a few weeks. The American chestnut that we have genetically engineered with OxO survives, shown here.

We've also developed a rapid breeding method where we can take the chestnut seedlings, put them in under high light conditions, and actually get pollen in less than 11 months. If we were doing this out in the field, it would take anywhere from 3 to 7 years to do this breeding. This is based on the pollen, so it's male introduction, which is actually good because then you don't have to worry about always stacking up the same chloroplasts and mitochondria, the cytoplasmic genomes, meaning they tend to diversify through the mother. So, we can take these and outcross it to survive in American chestnut trees and get the offspring

to inherit the resistance, as shown here on the left compared to the non-transgenic American chestnut on the right. The Chinese chestnut, I do want to say that these are not super trees. Just like the Chinese chestnut they can get damaged from chestnut blight, such as the Chinese chestnut on the left and our OxO in the middle, but it's much better than the wild type American chestnut on the right, which has a killing sunken canker that kills everything above it. We tried to see if there's any natural cankers out in our field plots. I just took these about a week or so ago, it's hard to find them, but these might mean we have to get some students out there to see if we can isolate the fungus from these. But this might be what they look like in the natural setting. Worst one, over to the right, kind of looks like cruddy bark, if anybody is familiar with cruddy bark on chestnut. Okay, so this leads us to a hypothesis in that, does the oxide oxidase just add to the natural resistance of the chestnut tree? I mean all resistance is quantitative you know, there's many genes involved in resistance, and American chestnut already has resistance genes in there, and we know that if you look at Chinese chestnut there's actually a range of resistance. It's not just one type of resistance, and they can fall anywhere in there and you can kind of distinguish them by how much damage occurs in the cankers. Likewise, the American chestnut probably has a range of susceptibility with some dying faster than others. So, what we've done with the oxide oxidase initially with our LS1 line, we add the oxidized oxidase, bump it up to being resistant. But that's also, you know, is it additive to all the genes that are already there so if we were to start with a more susceptible line and add the oxide oxidase, would you have a tree with more damage in it; or, if you started with one that was more resistant, would you have one that have less damage? When you add the oxide oxidase, so we're actually going to test that right now. We are up to our T3 generation, and we're going to start a common garden this summer where we're looking at T3s different genetic backgrounds and seeing if there's a variation in the amount of damage that occurs on these trees.

Now along these same lines, we want to actually cross these and we're doing this with some of the backcross trees from The American Chestnut Foundation. The idea there is, can we actually stack the resistance together? We know we can do this breeding because we've sequenced the Darling 58 and LS1 genomes. We found out that the insert of the OxO is actually on chromosome 7 in the upper end. This is distant from a resistant QTL on chromosome 7, which is in the middle to the bottom part so they should segregate independently. All the other QTLs for resistance are on different chromosomes. Even genes that we know, such as lactase-like gene is on chromosome 1. So, all these things should segregate independently, and so we should have a fairly simple breeding program with this. Okay, so we're taking our transgenic trees—our original ones are clonal, something we never want to put out in the forest as a restoration tree—we've taken those and outcrossed them to regionally adapted mother trees. We're trying to get them to the whole range of the American chestnut, so they'd be locally adapted. Approximately half of the offspring will inherit the light resistance or tolerance. We have an easy oxide oxidase assay that allows us to distinguish which ones have the gene, which ones don't. All these offsprings will have different complements of genes from their respective parents, and the idea is to go out one to three generations for a horticulture distribution, which we're already now at the third generation in our greenhouses right now. And three to five generations for more of forest restoration. Okay, and to do this we're trying to get citizen scientists involved. Once we have this deregulated, we've actually developed methods so we can collect pollen from rapid breeding and put them on slides. We can freeze them, store them, and we can actually ship them out to people to cross with their own local trees. We actually made up a workshop that people can go see and watch and learn how to pollinate chestnut trees. Now one thing I really want to point out that's really neat about genetic engineering, that's different than other types of breeding, is that if you have one of your hemizygous chestnut trees, light resistant trees, you can

grow that and 100 years from now, 200 years from now, you can still get wild type American chestnut seed from that tree. Okay, I don't think any other breeding method can actually let you do that. So, for conservation this is great because you can always get back the non-transgenic tree from these transgenic trees. Okay, I think that's an important point. As far as restoration itself, this is going to take a long time. We really need to have people involved. We want to plant in areas that are basically neutered, or places where there aren't trees; that's the best place for restoration, such as these mine lands and all. But it's really going to take people planting. These trees do not spread quickly on their own, only a couple miles every 100 years, so, it really is going to take people planting these trees to get it then restored. We think of this as a century project. Okay, so I want to stop there with this thought.

CURRENT SCIENTIFIC PROGRESS AND BARRIERS TO USE OF BIOTECHNOLOGY: TECHNOLOGY

Somatic Embryogenesis: A Multifunctional Tool for Conservation and Restoration

Scott A. Merkle

Professor of Horticulture
Warnell School of Forestry and Natural Resources
University of Georgia

I just want to introduce that what we do in my lab is in vitro propagation, and then we collaborate with other groups to do things like transformation and things like that. Although we also do transformation in the lab, but we're not a molecular lab at all—but there are different types of *in vitro* propagation. The most commonly used one is actually a commercially used one, mainly in horticulture, micropropagation. The photo on the right shows some micropropagated Eastern cottonwood trees, and this is just getting shoots to make axillary shoots in culture; then you cut off those shoots and root them *in vitro*. Then you get them out and harden them off, and you've cloned that tree. Organogenesis is another approach, and that uses adventitious shoots; that is, you can take a piece of a tree that would not normally have buds on it and get it to make new buds where they aren't supposed to be. For example, in Eastern cottonwood you can take a piece of a leaf and put it on a medium with some plant growth regulators, some cytokinins, and it'll make buds right on that leaf. Then you can elongate those into shoots and root them to make trees. The tool that I use is somatic embryogenesis, and the examples I'm going to show you today are all somatic embryogenesis. I also kind of make the case that for a lot of the applications for restoration, this is a very powerful tool to use for this. If you haven't seen what somatic embryogenesis looks like in person, or don't know what the definition is, you can read the definition on top but essentially, it's getting your in vitro cultures from different tissues to make structures that look like seed embryos—they're called somatic embryos. They have a root pole and a shoot pole and they're produced asexually, so that's why they're called somatic embryos. These can be germinated to make seedling-like plants we call somatic seedlings, at least that's what I call them. I think Bill Libby wanted to call them emblings for a while; I thought that was way too cute. These are beautiful yellow poplar somatic embryos, and the background is black filter paper on which they were collected. They all are clonal copies of an original seed embryo that was used to start the cultures, and to be perfectly clear here, all the examples I'm going to show you we had to rely on zygotic embryos as starting material. So essentially, we're not taking a piece of a mature tree and cloning the tree this way, although in some cases that can be done, but none of the systems I work with, except for sweet gum which no one wants, can you do that. So, what we're actually doing here is embryo cloning. I'll show you the whole system in just a minute, but these are the embryos. These are little germinating somatic embryos; essentially, these are induced on a strong auxin 2,4-D and then it makes embryos. To get them to develop and germinate we remove the 2,4-D and that releases them to develop into mature embryos and germinate. Then you can [make them] even a little bit bigger than this. You can pop yellow poplar embryos off of the medium pot, mix them up in potting mix and put them in a hardening-off chamber, and they will grow into somatic seedlings. I wish all of our systems worked like this.

What are the applications of using somatic energetic systems for threatened forest species? The core message is right here at the top. Mainly where I want to go with this kind of research is to combine it with selection and breeding programs. Essentially, it's a mass propagation tool

that we can use to propagate promising insect- or disease-related resistant genotypes that come out of selection and breeding programs. It has two uses: one is it's a very powerful tool for confirming that some genetic resistance you might see in the parents is genetic resistance, and it's not just by chance because you can do clonal testing. You put out 30 copies of a tree and if they all end up being resistant with that one genotype, then that's good evidence that the resistance is genetically based. But the other part is, eventually, when you can pick the very best ones, you can scale up these cultures and deploy these trees as what ArborGen used to call "varietals," but essentially cultivars that can be used for restoration purposes. The other two parts of this I'm not going to spend quite as much time on, but I do have examples to show you. Embryogenic cultures are excellent material for cryopreservation. That means that if we can start an embryogenic culture from a tree, we can essentially conserve that germplasm forever in liquid nitrogen, as long as we don't run out of liquid nitrogen. I will show you one example of that but essentially, we have thousands of copies of embryogenic cultures in cryo from many species, and we are not only conserving that genetic diversity but it's also a useful tool for this part up here while you're working with these. The progeny that come out of these cultures, you can hold them in cryo forever, or for as long as you want, until you have the results of your breeding program or of your testing program. Then go clone up the ones that are the best: Pull them out of cryo and clone them up.

Finally, I'll just briefly mention that some of the very best target material for gene transfer is embryogenic cultures, and I'll show you an example of that. Steve and Bill know that we did a lot of candidate gene testing in the FHI project using embryogenic culture–derived chestnuts, and of course from Bill's [William Powell] talk you know that there may be some deployment of genetically engineered trees soon—again, derived from embryogenic cultures.

Now one thing that you know everyone says, "oh, yeah, that wiped out American chestnut," which is true. If you go up in the mountains even in Georgia, you can find thousands of chestnuts up there. Most of them are no bigger than about, you know, waist high because the tree re-sprouts from the stumps and eventually the stems get re-infected and die back to the ground, but the root systems are resistant to blight fungus. What you might not know is that there's actually a lot of trees scattered throughout the range that they call these large surviving Americans, that Dana [Nelson] mentioned, and I think Bill may have mentioned. These two are some of the famous ones, like the Amherst tree in Virginia, the Adair tree in Adair County, Kentucky, and then these are actually a couple of grafted large surviving Americans that Gary Griffin here in the American Chestnut Cooperatives Foundation grafted called Thompson and Ragged Mountain. These trees, in many cases, when you find these large surviving Americans they're just blight-escaping for one reason or another; they haven't been killed, they haven't been infected in some cases, or maybe the strain of the fungus that they got they were able to hold off for a while. Most of them have cankers on them, but some of these, there's evidence from the American Chestnut Cooperatives Foundation, who breed these trees together to try and combine the different sources of resistance, that have some low levels of resistance. Gary, and this is Lucille Griffin, his wife, they both work in the TACF. They have some progeny where it looks like this has worked, but in order to get them to keep growing they need to be planted on very stress-free sites, and they also inoculate with hyper-virulent strains of the fungus, and they're using what the area calls "three legs." They can grow chestnut trees for quite a long time and keep them from getting killed by blight.

Now you're probably more familiar with the hybrid backcross breeding program. At The American Chestnut Foundation this is virtually

the same breeding scheme that Bill showed earlier, and as he said, this was going to be the final product, these BC3 F3 trees. But it turns out that the three gene hypothesis of resistance didn't hold up, and so the Chestnut Foundation is revamping their program both with the OxO and with looking at some of these earlier generations and picking trees that still have quite a lot of American characteristics but have high levels of blight resistance. So, this is going through a reworking right now by the Chestnut Foundation. Why am I showing you this? Because I work with both of these groups to clonally propagate material that's produced by their programs. So, how do I do this with somatic embryogenesis? We started our first cultures back in 1989 and we got cultures going. "Hey, they're making somatic embryos. We're gonna make some plants!" Look how long it took us to get to our first somatic seedlings! It took us 8 years just to get a somatic seedling germinated that had a root and a shoot, and then we couldn't get past the hardening-off phase. They kept dying on us, and it wasn't until 2001 that we got a few somatic seedlings first planted out in the field. It's been really difficult to get to work. Gradually we've improved the system to the point where we can almost have a propagation system, and part of this involved us actually scaling things up using suspension cultures.

Let me show you how the system works. Here's an immature chestnut bur; here's an immature chestnut bur with all the spines taken off. It's actually quite small, and that makes it easier for us to cut this open and get out these immature chestnuts. These were collected in August when the tree burs are still on the trees. So, we have to go out and collect these, or get someone to collect them for us, out of the trees. We then clean these off with Clorox, cut them open, cut the seeds out, and put them out on a tissue culture medium with 2,4-D in it. This [seed] is only about a millimeter and a half long, and somewhere in here is a very early stage developing zygotic embryo, but by putting it on the 2,4-D we induce it to essentially clone itself. Here it is by October; we have a little cluster of very early-stage somatic embryos, and by November it's growing more, transferring to fresh medium every 3 to 4 weeks, and by January we have the captured culture if we can sustain it, keep it growing, and it grows quite fast. When we want to make a whole lot of somatic embryos to germinate, we use suspension cultures. To get a nice synchronous population of somatic embryos coming up to germinate, we actually dump the suspension cultures through some screens, and we determine the screen sizes by, essentially empirically, by trial and error. We found out that the fraction that collects on this bottom 38-micron screen, if we take those out, plate them on filter paper or nylon mesh, we get a pretty good synchronous population of embryos that we can then pick off and germinate eventually into the greenhouse. So, what does this really look like? Here's the shake cultures and liquid medium; and this is before dumping through the screens; after dumping through the screens; looking up through the bottom of this flask; all these little light bulbs you see here, those are all individual somatic embryos. We dump those out, and up to this point here they've been in 2,4-D; when we get them off the 2,4-D, and you can barely see the nylon mesh that we collected them on, then they start developing cotyledons. We pick those off, put them in the refrigerator for a 15-week, cold pre-germination treatment because you know chestnut nuts benefit from a cold pre-germination treatment. So do somatic embryos. We get those out and let them finish germinating in this Magenta box. The Magenta Corporation makes them, and the black medium you see there has activated charcoal in it to keep light from hitting the roots and turning them dark. Eventually we can get them out into pots and harden them off and take them to the greenhouse. This is a long, a little more than nine-month process.

Now I want to show you one more slide about the scalability of this work, which was done by Dr. Li Xing Kong who was a postdoc in the lab several years ago. These are bioreactors that take the place of the shake flask. It's run by vacuum pumps that are pushing air through them. We can grow these like three times as fast as the shake cultures. Right

now, we don't have any of these running because we don't need tons of chestnut embryos. Now here's the cryopreservation part; this is the germplasm conservation. We can take those in vitro cultures, put them in little cryovials with some cryoprotectant and some sorbitol. These are Mr. Frosties: We lower the temperature in a minus 80 freezer, one degree at a time. Then when they're at minus 80, we can pop these out and into these boxes—these are all filled with these vials—and stick them in liquid nitrogen. As long as we don't run out of liquid nitrogen, we can thaw them out 48 hours later or 48 years later and about 80 or 85 percent of them will recover and regrow. Of course, when they come out they look like hell because they've been at –196 [C], right, but even within a week we start seeing regrowth, and 2 weeks they're growing again, and eventually they get back to a nice embryogenic culture. We've actually started using an alternative method with ash, because this method doesn't work as great with ash, called "vitrification," where you make kind of a glass out of things, that actually keeps freezing from happening to the material at all. Ash seems to come back from that very quickly and we've got higher survival.

So, here's how we put together the breeding programs. The Chestnut Foundation sends us immature seeds, and we start in vitro cultures. Here's that captured in vitro culture; we split that culture, put copies in cryo, and the other ones we go ahead and make plants. We can then go into testing. Once the testing is done, we can go back, pick the very best clones, pull them out of cryo, and clone them up. That's pretty much what we're set up to do, and this is actually the model for the other species. A lot of the other species we're working with are from the American Chestnut Cooperators Foundation's breeding program; they sent us somatic seedlings of crosses between Thompson and Ragged Mountain. They arranged for us to send trees or transfer trees to Carol Croy of the U.S. Forest Service; she planted them out in the Jefferson National Forest. I got one report from her in 2017 that said they were all still alive, but I have not gotten another report from her since then but hopefully they're still out there and growing.

Here's some material from the American Chestnut Foundation. Some of their BC3 F3 material, the cultures don't grow exactly the same as pure American chestnut cultures, but we can start grow cultures to make somatic embryos and make plantlets. These are some photos supplied from Sarah Cloth at Virginia Tech where they planted some of these somatic seedlings out on a mountaintop removal restoration site in southwest Virginia called Powell River Site. Just a reminder that the transgenic chestnuts are derived from somatic embryo cultures that were targeted for gene transfer, and this [photo] was our system using suspension culture and antibiotic selection to get that transgenic material.

So, I got two more species but not as many slides for each of them. I thought someone else would be talking about hemlock wooly adelgid by now, so I just put this one slide in here that shows the devastation caused by this exotic pest from Japan. It was accidentally introduced from Japan, and I'm sure the map has expanded by now into the entire range of Eastern and Carolina hemlocks. Just like the large surviving Americans, there are large surviving hemlock trees out there. The most famous ones are the Bulletproof Stand in New Jersey. We got Mark Mayer from the New Jersey Department of Agriculture to send us immature seeds. We started cultures from them and generated some somatic embryos that germinated. We haven't gone any further with this, unfortunately, because we're actually not actively involved in the hemlock research anymore. We've also worked with people breeding for resistance with resistant Asian hemlocks. This is Ben Smith with the Forest Restoration Alliance who breeds between Asian hemlocks that are resistant to the hemlock wooly adelgid and Eastern and Carolina hemlocks. It's a lot easier to make the crosses with Carolina

hemlock; for some reason, Eastern hemlock is very hard to hybridize. Chang Ho Ahn, who was a PhD student in the lab, started cultures from some of these hybrid zygotic embryos, here's what the embryogenic material looks like.

Okay, the last thing is, we started working on ash back in 2013. A visiting scientist, Dr. Jen Feng Zhang, was here and we started our cultures from immature maple samaras. Here's a newly established embryogenic culture of green ash; when we move it off of 2,4-D, it's exactly the same medium as we use for chestnut. It makes thousands and thousands of somatic embryos. These are tiny though; we have to let them get bigger, so we pick them off onto these plates and let them enlarge. We do give them an eight-week cold treatment, but they will germinate without it. When they come out, they go on this medium that contains gibberellic acid to germinate. How about the genetic material? Many of you know Jennifer Koch of the USDA Forest Service in Delaware, Ohio; she has been breeding between what are called lingering green ash. Trees that survive in stands that have been blasted away by emerald ash borer, but these trees are still growing just fine and don't appear to have been attacked or affected. So, she's been breeding, and she and Dave Carey have been sharing some of their seeds with us. When they do crosses, they'll send some of their seeds to us. In addition, from Dan Herms we got open pollinated lingering white ash seeds. Here is a culture of lingering white ash, and a culture of lingering green ash from Jennifer Koch's lab trees.

So, what's the take-home message? I told you I'd tell you twice that we can greatly leverage genetic resistance programs for these forest species for restoration by combining that with somatic embryogenesis and other mass clonal propagation technologies. This is the real core message: I'm looking for people doing selection and breeding of these trees to collaborate with me. I want to culture your stuff; okay, I don't want to keep it. I'll send it right back to you after we propagate it to test, but I hope you'll keep me in mind when you're working with these things.

Developing Durable Resistance in Populations of Forest Trees

Richard A. Sniezko

Forest Geneticist
Pacific Northwest Region
U.S. Department of Agriculture, Forest Service

I'm going to discuss developing durable resistance and populations of forest trees. This is kind of a practical tree breeder's perspective on what works, what doesn't work, and what's out there now. In the photo here, you'll see some dead trees on the left, which are a significant gene resistance in Western white pine. The green ones have quantitative resistance of partial resistance. If you get to Crater Lake National Park, you'll see white pine restoration planting at Rim Village, but this will be a resistance test of white pine and blister rust. Okay, as we've been talking about, there's a lot of invasive pathogens and pests that have come in North America and all across the world, and this is simply a list of some of that. What we find out, and I think David [Showalter] might talk about this a little bit too, is that sometimes breeding and resistance people come to it at the end, and they keep trying to save the big trees, but I think we have to get smarter to realize when diseases and pests are going to knock out the big trees. At that point, we have to say, is there genetic resistance? How much is there? What technologies can we use to get that back out in a restoration mode or a reforestation mode for our economic species? The tools for genetic resistance: You can use traditional selective breeding and new biotechnologies, and we've already heard some talks on that today. Silviculture can help, in some cases, biocontrol methods as well too. I'm mostly going to talk about resistance, so durable resistance: Many years ago, Johnson coined "durable resistance" as the disease is resistant that remains effective during its prolonged and widespread use and an environment favorable to the disease, and really, to show it's durable you have to test it over a very wide area. In this area, you can't just put one test in. It lasts for 20 years, and you say it's durable. It has to be used over a long period, and you cannot make it more durable by growing it less. It's a descriptive term that could have various underlying causes, and you know it may be stable for a period of time, but then suddenly things may change, and it may not be too durable.

There are also a couple of components. There's a virulence, in terms of the genetic change in the pathogen or maybe insect, then there's also the spread of that virulence. It also has to spread back through populations where the resistance is being deployed. The trees we modify might change the virulence definition because in crops you talk about cultivars using a single genotype, usually a hundred percent of your crop. Essentially you want resistant trees, but really for most tree species that's not the way it's going to work. You're not going to get 100 of the trees with immunity or resistance. We don't know why, and there are some exceptions to that. There are certain things that have been durable over many years. I would advocate in forest trees you have a different scenario. Certainly, if trees are killed, that's not good, but we do have where you can get trees infected but they're not really impacted. So, in a sense you could say, "well, the resistance is durable but it's at a lower level, but it is effective." So, the whole definition of resistance in trees might need to be altered a little bit for something like partial resistance. We're not really going for the same uniformity as they are in crops; we're willing to accept some impact, such as spark reactions that you'll see later, and most of the tree species aren't planted in uniform extensive plantations. We also have to deal with another thing, and I'm not going

to go into that much today, but I want you to know that without the stability of resistance, is it effective at all environments and is it effective under climate change? This has been found that in some crop species a change of just a couple degrees temperature can negate a resistance. So, we'll want to know over a range of environments. We're now testing some of our material over a range of temperatures. And then usable resistance; you know, some people when they think resistance, 100 percent of the trees have to survive. Again, probably not gonna happen unless you put a major gene out there, and that type of major gene is probably going to be overcome and not be durable. So, as you'll see in a lot of our Western white pine, we won't get 100 percent survival, but we will get high enough survival that many managers will find it to be usable for their objective. We also need to maintain genetic diversity and genetic adaptability. Many years ago, I found Port Orford cedar with homozygous dominant major genes, and one of my pathology colleagues said, "Great, you're done, you just take that one parent and cross it with everything else." Some of you will know, I'm not gonna go into the details of why that might not be a good strategy, but that strategy would have limitations, so again, there's definitions: durable resistance, stability of resistance, and usability resistance. This is often mostly focused on the durable resistance. You need to realize for trees, because they are going to be out there for decades or hundreds of years, or they're going to be progenitors of future generations, so, we have to consider the whole parcel.

Complete resistance, a lot of times whether it's in crops and some of our tree species, major gene resistances are gene single dominant. Often times, a hypersensitive type of response—in this case, in Western white pine, sugar pine, Southwestern white plant, and limber pine, four of our white pine species—seems to have major gene resistance and essentially when inoculated with wild type rust they will get no stem symptoms. They'll get needle infections maybe, but that is it. Durable Western white pine and sugar pine, if you plant Western white pine with major gene resistance, that opening slide I showed some dead red trees, well that was a major gene resistant homozygous dominant family offspring, and they were all 100 percent infected, and most of them are currently dead. So, in western Oregon from about Drena to the Northwest coast, we think the virulent strain is kind of very common and dominant there. So, if you plant only major gene resistant Western white pine in that area, you know it's not going to do very well. On the other hand, they planted up in Canada and in Washington and so far those things are holding up okay.

Partial resistance or slow rust stain is thought to be quantitatively under control, probably because of several to many genes. Is it durable? Certainly, theory says that it has much more likelihood of being durable, but there could be erosion of that going on over time with selective pressure, but we do have tests out that are 20, 30, 35 years old and there's some encouraging results. Just diagrammatically, this happens to be for Port Orford cedar but the same thing would apply to white pine blister rust. The two types of resistance, if you were to plant seedling families out, in the top one with major gene resistance, the yellow lines are homozygous dominant for major gene resistance. None of the seedlings died in our seedling tests that we have done in a root test at Oregon State University. Our susceptible line at the top in red, 100 percent of the seedlings die, and they die very quickly. Then there's a heterozygote that's kind of in the middle there, too, so that's what each of the dots on there—the 42 seedlings planted per family—this is all they do. In contrast, the quantitative resistance of partial resistance before you have a series of families down below, you see the susceptible at the top, all 42 of the seedlings died very quick, probably by day 150. But you can see in the other families there, they have different levels of mortality, at different rates of mortality, and in most of those families they never went to 100 percent mortality, so they don't show major gene resistance. But they show some sort of quantitative spread of resistance within the family, and there

was a recent paper that came out last year, that we kind of put this out in and a lot of data, a lot of information, you know we're probably working with well over a thousand parent trees and Port Orford cedar, and there is some major gene resistance but most of what we're planting is quantitative resistance. Just a warning as you go out there and look, the first 15 or 20 years at Oregon State University, and this is actually in the literature, the conclusion was there is no resistance to *Phytophthora lateralis* in Port Orford cedar or else we've got to modify our screening technologies. They were about to give up, they had two rooted cuttings surviving in a raised bed or something, cold frame one and cold frame two, and they said, "We'll do the test one more time," and that's where Everett Hansen's 1989 paper came out. And they said, "Oh, hey, there is some resistance." They mischaracterized the resistance somewhat there, but that's okay, that's when really things started going forth. So, back in 1985 if you asked, do we need genetic engineering, corporate people and the pathologist at least would have said, "There's no resistance, so yeah, we do need it." Now I would say, "hey, there's a lot of resistance, two different types of resistance, if we can breed it at age one or two if we wanted." So, Port Orford cedar, we can probably move things forward unless virulence comes into play or lack of stability. We're gonna set up some trials and tests, and David's [Showalter] going to be involved in some of those. David Showalter as well, too, in terms of testing durability and stability of resistance.

There was a nice paper that came out from McDonald back in 2008 talking about the five evolutionary forces and the risk of pathogen evolution, and essentially mutation plays out real big. Population size and random genetic growth within these pathogens, gene and genotypic flow, the reproductive immediate system of the pathogen or insect, and then selection imposed by major gene resistance quantitative resistance. So, those are all factors that interplay into whether something is durable, and here's some things that they put in their highest risk of evolution: If you have a high mutation rate versus low mutation rate, things like a large effect of population sizes versus small, high gene or genotype flow, mixed reproductive system and efficiency, and efficient directorial selection. So, you know, if you wanted some of the crop species they've almost done the perfect experiment that, "hey, how can we overcome resistance?" Well, what you do, you put one culture out with a major gene that has a hypersensitive reaction, you put over many years, high risk environments, many environments, and then by golly, it's not surprising that many of those genes are overcome. Fortunately, in crops, some of those crops have a number of major genes, but they can substitute something further; and trees, we may not be as lucky to have that many major genes. So, you know we better use them a little bit more carefully. As far as we know, effective major genes in Western white pine and sugar pine, so far we've only identified one that's a different gene in each of the species. Agricultural crops versus forest crops: In agriculture crops you can plant every year, and trees, hey, the trees have to be out there for oftentimes hundreds of years or as generators of future populations. So, we not only want durable resistance, we need durable resistance. In crops you're talking about cultivars; we're talking about seed orchard mix, where every single seedling planted out there is a different genotype. You know agriculture uniformity, reinforced trees often want genetic diversity and crops you can often use major gene resistance. We're looking at using all types of resistance in forest trees and locating that type of resistance early on and not cutting off the screening cycle. So, durability resistance can be an issue in agriculture but they're working through it, you know, because they can replant and put in new varieties and whatever, but durability is essential in forestry. Then maybe CRISPRS certainly can work in trees, probably more amenable in the case where you're working with cultivars.

This is Dorena Genetic Resource Center, just to show you; we can run an operational program if you want to do things on a big scale, and we probably have the world's biggest fog chamber

at least for this type of thing. We can screen tens of thousands or hundreds of thousands of trees a year. As a lot of you know, resistance is a numbers game, and finding all types of resistance you have to screen over a period of time. So, for durable resistance to develop an assay to screen for all resistant phenotypes at once, look beyond major gene resistance. And then Western white pine, or sugar pine, or our five needle pines, what we do is we grow seedlings for 2 years, we infect them or inoculate them, and then we follow them for five years. We can pick out the early resistance early on, major gene resistance, maybe something else, but we see other things happen over time. And then we put those into different categories, and then we hopefully want to combine, for instance, major gene resistance and quantitative resistance. There is a concern in some systems. I was reading a paper that on a crop system not long ago, where they were talking about breeding in the future and quantitative resistance and with marker aid or selection, they were losing a lot of their QTLs along the way. Maybe in their system that is okay, but I think we want some input on how do we monitor over time as we're breeding so that we don't lose. If quantitative resistance is made up of 50 or 100 genes, how do we make sure we're carrying forth most of those and not just picking up three or four with a major effect? So, that in the long term, we hopefully are keeping most of it in there. Economically, somebody could set up your system, breed a few trees, and say, "hey, I'm done," and I've kept, you know, three or four of the genes in there that may not be durable in the full in the long term.

I've already talked about white pine blister rust. Our screening trials take about five years after an oscillation, although most of the results are known within a couple of years. Port Orford cedar, the early screening trials were, I think there was 120 days, and then one year, and then I started seeing some complete results. So, I asked our pathology friends to extend that to a second year. In the third year when they did that, I was able to uncover the quantitative resistance and separate that from the major gene resistance. So, you know, you got to have a good screen trial and too often I see people who say, "hey, throw together a screening trial," and they call it done. But you know for procedure, we have actually four different types of screening files. I'm not going to go into detail on that, but you know having a good trial to pick out all types of resistance is a good thing. This is whitebark pine just showing some resistance versus not resistant. Major gene resistance, I talked a little bit about that before, here's a range of reactions that we see in quantitative resistance and in five needle pines; okay, so just a range of things that seem to be slowing the pathogen.

This is how a field study would work. You could see the green lines, at the top a major gene resistance, high infection, high mortality in the field; the quantitative resistance, lower infection, lower mortality. This is how it looks in the field: the dead trees are major gene resistance, the green trees are quantitative resistance. You also have to monitor low-moderate hazard sites. This is a sugar pine over 35 years, and early on the low hazard sites had less infection, but by age 15 and 25 they had as much, almost as much infection as the high hazard sites, so you got to monitor these over time. Dead trees are when resistant genotypes, conducive environment, and virulent environment come together, and this isn't going to happen to all three of the sites, so you know, just because of virulent strain in all. Big questions: will it spread, will it spread everywhere? Can non-durable resistance be useful? Sugar pine is still in Oregon, we haven't found the virulence vein here. You know, there may be deployment strategies that one can use and that can be looked at a little bit further. Summary examples from the U.S. southern pines and Fusiform rust, probably durable, it's been out there for a long time, planted a lot of material, for the most part holding up although some of the major genes do have a problem. Western white pine and sugar pine, the quantitative resistance is probably durable, the MGR not durable in a strict sense but also hasn't spread throughout everywhere where major gene resistance is planted. Then chestnut,

we heard talk this morning, and it sounds like the gene from wheat could be one that's a real candidate for durability. Then koa over in Hawaii, I'm working with that program early in the phase that you know, we're often as a mystic, that it would be durable. So, again good screening program will help you identify the different types of resistance, you can contemplate the deployment strategy, you can avoid extreme rough hazard flights if you need to, you can plant species and mixtures, and that's that. Other than the fact that there's ways to properly do one of these programs and make sure it's kind of holistically based from the beginning. I remember 10 years ago those pathologists had a meeting down in California and said a colleague over in Italy had planted a particular species and it failed in its resistance. I said, "Well, did he just select for major gene resistance?" and the person said, "Well, yeah." I said, "Well, okay, you should have known better," so, you know, maybe involve a tree breeder or geneticist and not, you know, go beyond a single discipline.

Spectral-Based Tools for Screening Trees for Resistance/Disease

Anna O. Conrad

Research Plant Pathologist
Northern Research Station
U.S. Department of Agriculture, Forest Service

Pierluigi Bonello

Professor
Department of Plant Pathology
The Ohio State University

I'm going to be talking about the use of spectral-based tools for screening trees. I'll provide an example of its application for screening for resistance but then also how it can be used to screen for disease, which may be useful for various phenotyping efforts. So, I think this, you know, we don't really need to talk about this too much, but obviously trees are under constant threat, and these threats are changing with the emergence of new pests and pathogens and also change as a result of climate change. Because of that, we need new innovative solutions to deal with these threats. One potential solution or form of management is to use resistant trees, and Richard [Sniezko] talked really nicely about the use of resistance and breeding for resistance. Unfortunately, there are some challenges to identifying resistant trees, and this includes long generation times, which can impact seed production and how long you need to wait to see the phenotype that you're interested in, such as maybe disease susceptibility or resistance. There may be inconsistent seed production, which can make breeding challenging. The environment may impact the expression of the trait, which can further complicate your ability to screen or phenotype for resistance. The trait may be controlled by many genes. Richard talked a lot about quantitative resistance and of course you want to be able to maintain genetic diversity within your population, so the solution I'll be talking about today is the use of a rapid phenotyping tool, both in the laboratory and field, that can be used to identify these naturally resistant trees. This phenotyping tool is based on looking at the chemical properties of the trees. We're searching for biomarkers in a way that will allow us to predict which trees may be resistant. However, as I mentioned, we'll also be talking about using this approach to screen for disease. Luckily, we can also utilize changes in chemical traits of the trees to identify which trees may be diseased or not.

The approach specifically that I'm talking about is called vibrational spectroscopy. Vibrational spectroscopy: One form is called infrared spectroscopy, which many of you may be familiar with, particularly with the advent of all these different, especially remote sensing, platforms that are being used today in agriculture and more so now in forestry as well. We can use infrared spectroscopy for rapid phenotyping of a variety of different types of samples. They can be liquid. They can be solid, and they can even be gaseous samples. What it allows us to do in essence is to generate a chemical fingerprint, or you can think of it more so as a snapshot of the chemical composition of a given tissue at a given time. This is somewhat analogous to a human fingerprint, where maybe one small feature of that snapshot is not very informative, but if we view it all together, we can gather some really useful information and relate that to our trait of interest. In my case, that's typically disease or disease resistance.

There are different types of infrared spectroscopy and types that we use. One which I'll be talking about today is called Fourier-transform infrared spectroscopy, or FT-IR. What FT-IR does is that it measures how a sample absorbs light over a specific region of the infrared spectrum. Oftentimes this is the mid-infrared spectrum, which runs from around 400 to 4000 nanometers, and what this allows us to do is pick up on differences in the chemical composition—differences in what specific chemicals are present within that tissue or extract and also on their concentrations. Oftentimes, at least in my experience, we don't necessarily see different chemicals, but we see different levels of chemicals present in trees that vary in disease susceptibility. For instance, those differences will impact the FT-IR spectrum. At the bottom is just a schematic showing how this works. You have your sample, for instance a liquid extract from a tree; you load it onto a crystal, which is denoted by this blue box; and then you hit that with an infrared beam of light. That infrared beam bounces off of the sample, and then you have a detector that captures that.

Another form of infrared spectroscopy, which many of you may be familiar with, is called near-infrared spectroscopy. Instead of measuring how the light is absorbed, it measures how light is reflected or even transmitted through an object. In contrast to the mid-infrared spectral region, we're looking at around 2500 to 750 nanometers, and similar to mid-infrared or FT-IR spectroscopy, we're able to look at chemical differences in samples, but to some extent too, we can look at differences in physical properties that are reflected in differences in the reflectance. The schematic here in the bottom shows how this works. You have your sample, in this case a leaf. You have an infrared light source, this can be like a halogen light bulb, and you hit that sample with that light source. Then you have a detector looking at the reflectance off that object of that light.

FT-IR spectrometers and near-infrared spectrometers come in a range of sizes, shapes, and degrees of portability. I talked about how we're interested in developing this phenotyping tool that can be used both in the lab and in the field. There's a variety of different instruments that are available for doing that. We have our benchtop spectrometers, our more portable spectrometers, which can be moved relatively easily; we have handheld spectrometers like here on the right-hand side; and on the bottom, here are some examples of handheld near-infrared spectrometers, which are kind of on the forefront of portability and also on economic value. They're much more inexpensive compared to some of these top choices. So here on the left-hand side, we have a small sensor here that's operated off of a Raspberry Pi board, and the sensor on the right is kind of its successor, which has a bit more user-friendly interface. So, with these instruments, what do these chemical fingerprints or chemical phenotypes look like? With a FT-IR spectrometer, we can expect to see something like this from a liquid extract collected from a tree. This is over the mid-infrared spectrum, and like I said, you know each tree is going to have its unique chemical fingerprint or chemical finger phenotype that's reflective of the composition and the concentration of chemicals present within the sample; whereas with a near-infrared spectrometer, we can expect to see something like this, which is a reflectance measurement based off of the intact plant tissue. So, what can we do with these tools?

One application is to be able to predict disease resistance. Today I'll share with you an example from a project that we performed for a disease called *Diplodia* tip blight. If you're from the Midwestern United States, you may be very familiar with this disease. It's very common in the urban landscape, and while there's many different hosts of the pathogen that causes the disease, Austrian pine and two- and three-needle pines are those that are infected. It's caused by a fungus, *Diplodia*

sapinea. This disease has some characteristic symptoms, such as observed here in these two photos. So, in order to be able to relate our chemical fingerprints to disease susceptibility in the case of Austrian pine, we needed to collect phenotypes based off the traditional inoculation-based assay. To do that, we inoculated Austrian pine shoots, and then we collected the lesion lengths as a result of those inoculations' 7 days spawning inoculation. This trait is quantitative, so we see a lot of variation in susceptibility within Austrian pine. In order to develop these predictive models based off the chemical fingerprint, we needed to kind of classify samples, so we considered trees to be resistant as those with the smallest canker length in the 21st quartile. We called susceptible trees those with lesion links in the fourth quartile, and we rank based on lesion length.

What we found is that we can relate chemical fingerprints to disease susceptibility in the case of *Diplodia* tip blight. Using a partial least-squares regression analysis, we were able to predict lesion length based on the chemical fingerprint data. We're looking here at the relationship between measured lesion length and predicted lesion length, and we have a correlation coefficient of around 0.6. Similarly, we can use machine learning to classify trees based on susceptibility, so we used an approach called support vector machine, which is a supervised classification approach, and using cross validation we are able to accurately predict trees as belonging either to the resistant or susceptible group with about 67 percent accuracy. This study here was a really relatively small study, so typically we like to see hundreds of samples, but it's even possible to do some smaller-scale studies with less than that.

The next example I'm going to talk about today is an example of using this technology, not to screen for resistance per se, but to screen for the presence of disease. When you're phenotyping a tree and trying to determine which may be resistant, good candidates for breeding, or other management objectives, you need to be able to distinguish between those trees that not only show variation in susceptibility, but perhaps two trees that show symptoms versus those that that don't. Or if you want to be able to identify diseased trees in the landscape for management purposes, having a nice rapid phenotyping tool for disease presence can be very useful. This was a study that we performed in 2018, which actually has since been followed up by a graduate student at Ohio State [University] named Kerry Fuhrer. So, if any of you attended a beech leaf disease meeting last week, you probably heard about some of her more recent results on the project.

Beech leaf disease, for those who aren't familiar, is an emerging disease here in the Midwest and Eastern United States. As of 2020, the disease has been found in multiple states, as you can see here in this map on the right-hand side. The major host is American beech here in the U.S., although there are several other beech species that have been found to be susceptible. There's a nematode, *Litylenchus crenatae mccannii*, which is a subspecies of the species, which was originally identified in Asia, that has been found to be associated with the disease. However, it's possible that other microbes may also be associated. So here are some characteristic symptoms of the disease: You have this banding, which you can observe very well if you were to stand in a forest and look up; the interveinal darkening of the leaves as observed here on the left; and another characteristic symptom is this, what we call crinkling, so these leaves become kind of very leathery and tough and they have this crinkled or wrinkled appearance.

We wanted to see if we could use near-infrared spectroscopy in this case to distinguish between symptomatic and asymptomatic leaves. The instrument that we used was an economical, handheld, near-infrared spectrometer, which is pictured here on the right-hand side of the screen, and we collected this spectra from two different groups, the asymptomatic and the banding, and this is a representative kind of average spectra from those two groups. We just focused on the subset of the

spectral region for our analysis. We are able to use a machine learning approach called "random forest" to identify specific wavelengths that were associated with the group, so asymptomatic and banding. Those are pictured here in the blue. Using those three groups or three wavelengths, we built a machine learning algorithm based on support vector machine, which allowed us to distinguish between the asymptomatic and symptomatic or banded leaves with over 90 percent accuracy and our testing set, which is essentially a subset of our near-infrared spectra, which are used just to validate the model and to assess how well it can accurately predict trees. So, we can use machine learning to identify symptomatic leaves, and not only to discriminate between trees that differ in disease susceptibility as in the case of Austrian pine and *Diplodia* tip blight.

I just kind of want to briefly summarize what spectral-based tools can be used for and maybe some challenges and opportunities we have in regard to the adoption of this technology on a broader scale. We can use spectroscopy, combined with approaches like machine learning or multivariate statistical analysis to build models or predictive models that allow us to screen for resistance or disease. I talked about here where we used FT-IR spectroscopy to screen for resistance in the case of Austrian pine and *Diplodia* tip blight, and we can also use complementary approaches like near-infrared spectroscopy to classify symptomatic and asymptomatic beech leaves in in the case of beech leaf disease. These are just two examples that tested this. These approaches and many others have tested them. Particularly for disease detection, near infrared spectroscopy has been used very widely, not only in forest-related fields but in agriculture as well. I guess one of the opportunities or benefits of this approach is that you do have the potential for high throughput screening, not only in the laboratory but also in the field. In particular with the near-infrared handheld sensor, we're able to collect these chemical fingerprints in as little as two seconds, which means that we can screen hundreds of trees in a matter of hours or a day, depending on how the trees are distributed in the landscape. If you think about traditional inoculation-based approaches for screening or manually measuring things like canker length in the field, it can take a very long time. You may need to wait months or weeks or even years in some cases for phenotypes to develop. So not only can this shorten the time it may take to screen, but it allows you perhaps more flexibility or the ability to screen larger numbers of trees than you may be able to do with traditional screening approaches.

Of course, this is one benefit and it's not without its challenges. Things like making more user-friendly interfaces for how we work with the data, and also interpret it, I think is going to be really important. Ultimately, I would like to see these spectral-based screening tools put in the hands of those that are actively breeding for disease resistance or forest managers that are looking for diseased trees on the landscape. So how we go from testing out these approaches and doing proof of concept experiments to actually implementing them for disease management, for breeding, I think is going to be potentially one challenge but also, I think, an area of great opportunity as well. With that, I think I will just go to my last slide to acknowledge the collaborators and funding sources for the work that I talked about today, and hopefully there's time for questions.

CURRENT SCIENTIFIC PROGRESS AND BARRIERS TO USE OF BIOTECHNOLOGY: IMPLEMENTATION

Framework for Establishing a Rapid ʻŌhiʻa Death Resistance Program

Christian Giardina

Research Ecologist
Pacific Southwest Research Station
U.S. Department of Agriculture, Forest Service

Blaine Luiz

Rapid ʻŌhiʻa Death Resistance Program Coordinator
Akaka Foundation for Tropical Forests

Kainana Francisco

Student Trainee
Pacific Southwest Research Station
U.S. Department of Agriculture, Forest Service

Just to get folks oriented to ʻōhiʻa, it's *Metrosideros polymorpha*, it's part of the Myrtle Family, and it's our most common tree in Hawaii. It covers some 800,000 acres, which is actually a pretty large area for Hawaii. It's about a quarter of our land mass, and it amounts to about 350 million trees spanning all of our islands. ʻŌhiʻa occurs on fresh lava, like in the picture to the right, and it occurs on some of our oldest soils on Kauai. All the way to the north, for example, the tree on the left. *Metrosideros polymorpha* in particular is important because it is so common, and it supports an enormous amount of biodiversity from canopy birds, insects, and other plants. It's an anchor for our native forest making up often 80 percent of the basal area. It's hydrologically important; it's a slow-growing, large, complex tree; it traps water; it protects watersheds from invaders; it's a dense wood; and the trees are very large and so it's an important carbon stock for Hawaii. It's been described as a biocultural keystone species because it's foundational for Hawaiian culture. I'm not going to go into a lot of details about Hawaii but if you YouTube either one of these titles, there are some great stories. The first one is a short piece on ʻōhiʻa, and the second one is a longer piece, both of them touch on Rapid ʻŌhiʻa Death, which is a disease that was identified about 12 years ago. Private landowners were seeing their ʻōhiʻa trees, often precious ʻōhiʻa trees, blinking out, turning brown and dying.

The first 4 or 5 years of work starting in 2012 was directed at identifying the causal agent, and it was quickly identified as *Ceratocystis* as a fungal agent for Rapid ʻŌhiʻa Death, but it took a bit more effort to realize that there were two new species of *Ceratocystis* that were causing this disease. *Ceratocystis huliohia* with the canker pathology originating in Asia and then *Ceratocystis lukuohia* with a wilt pathology originating from the Atlantic. How they got to Hawaii or if they evolved in Hawaii is not really understood yet. Rapid ʻŌhiʻa Death has some pretty dramatic symptoms of rapid browning leaves that remain attached after they die, complete foliar death, and basically 2 weeks to a dead tree from when you first start seeing the symptoms. It's hard to know how long the trees are asymptomatic before they start turning brown, but it can be as long as a year. Signs are discoloration of the wood, and you can see the black streaking and the sapwood of the trees, and then a mild banana odor when you open up a wound. The work to isolate and identify the trees was carried out by Lisa Keith and a small team of geneticists, pathologists, doing genetics work. This work led

to the discovery of these two species *C. lukuohia* and *C. huliohia*, and in the past 10 years there's been a really dramatic spread, especially of *C. lukuohia* across Hawaii Island, that's the southernmost island in our island chain. Strangely and disconcertingly, the island with the second largest spread is Kauai all the way to the north, with just one occurrence that's been eradicated on Maui and four *C. huliohia* trees on Oahu. For more information about disease progression over the years, you can go to rapidohiadeath.org. The key takeaway though from this pathology overview is that the large-scale loss of ʻōhiʻa from Hawaiian forests would be a catastrophe for biodiversity, for carbon, for watersheds, and for the many cultural attributes that it brings to Hawaii.

In response to Rapid ʻŌhiʻa Death, we developed a strategic response plan. This was developed and built on an earlier strategic response plan that identified some key actions, the most important one is preventing the spread especially to other islands, but even within Hawaii island where there won't be any opportunities for eradicating the fungus, how do we at least slow it down? A second key aspect of the strategic response plan was early detection rapid response either from islands where its occurrence is either absent or very low or in new important conservation areas on Hawaii Island. The talk I'm giving is based on a major recommendation that we needed to start an ʻōhiʻa disease resistance program to perpetuate ʻōhiʻa in our landscapes. I want to emphasize that our disease resistance program is very much stone soup at this point. We have tremendous support from the USDA Forest Service but so much of our pathology work was led by the USDA Agricultural Research Service. Lisa Keith, in particular, with colleagues at universities and also nonprofits and Trop HTIRC [Purdue University Tropical Hardwood Tree Improvement and Regeneration Center]. So, basically in a nutshell, the need identified in our strategic response plan is that a disease resistance program for perpetuating *Metrosideros* as a canopy dynamic, dominant native forest is needed. We're at the very beginning stages of that, and some preliminary research led by Blaine Luis involved basically gathering, growing, and screening ʻōhiʻa from local populations in Hilo, developing basically the methods and the best practices for doing this, and trying to identify preliminary consistency efficiency of production and screening improvements to the methods. This preliminary work looked at the more virulent and problematic *C. lukuohia* through preliminary resistance screening trials that took place between 2017 and 2018. Basically, cuttings from 128 individuals of *Metrosideros polymorpha* from Hawaii Island outside of Hilo, representing four varieties, and *incana*, *glaberrima*, *polymorpha*, and *newellii* were inoculated with the *C. lukuohia*. You can see in the bottom right picture the wound that was created, a slit in the bark where a small pad of inoculated material was inserted into the wound, and then sealed. Basically, the process is after wounding, putting the plants into a controlled environment growth chamber, monitoring each of the plants, and when the plant dies—and you'll see the results here in a second, most do—exploring the wound and understanding basically what happened in the plant as the cause of the death. So, in this preliminary trial over 120 days, 82 of the ʻōhiʻa individuals died. Susceptibility was high and the most susceptible died quickly within a month and a half. Some individuals showed reduced susceptibility, they persisted for a good while longer, and the variety *incana* showed the highest survivorship rate after 120 days along with *newellii*. You know, continuing modern monitoring of the surviving individuals, mortality persisted so that by the time we got to 2021 we have five *incana* left and one *newellii* out of the original 128 individuals that were propagated from cuttings.

Survivors are still being monitored, and some of them are being propagated by either seeds or even by cuttings or even seeds. From the strategic response plan, we recognized that we needed to do a much broader sampling and a deeper screening, and so we developed an approach based on seed zones for ʻōhiʻa across the islands based on where they occur, and then also threat level

information about each of the stands. From that we prioritize the sessions. Based on these two categories, we also needed to expand our sampling beyond *Metrosideros polymorpha*, so we expanded screening to include Oahu attacks that don't occur on Hawaii Island. So, *M. rugosa*, *M. tremuloides*, *M. macropus*, and then the *M. polymorpha* varieties *incana*, *glaberrima*, and *polymorpha* but also Kauai taxa, a different variety of *M. polymorpha dieteri* and also *M. waialealae* var. *waialealae*, a different species that occurs only on Kauai. So, that screening project is married to an ʻōhiʻa survivor project, and the idea is very similar to what you've heard in earlier talks—that you have stands with 90 percent mortality and you have trees that we're calling survivors. To be optimistic, they could just be escapes but we're speculating that these survivors are potentially resistant individuals. So, we're sampling these remaining live ʻōhiʻas in rod-impacted stands; we're also sampling adjacent stands of healthy ʻōhiʻa where the entire stand may be relatively unaffected by rod. And so, we're asking the question if genotypes within the stand are overall more resistant. We've got five sites with paired stands across Hilo and Puna districts, and that's the image on the right and that's Hawaii Island that you're seeing.

We've generated some 10,000 cuttings from 200 mother trees; as you'll readily see we're not anywhere near the kind of program that Rich [Sniezko] has been talking about. This is about a year of effort to get the cuttings from the mother trees basically sampled and into an old enough stage where they can be screened. We're integrating some remote sensing into our work. This is work that's being led by the Hilo Spatial Data Analysis Visualization Lab. It's drone-based characterization of rod-impacted stands and it's helping us basically design our sampling around trees that are individuals within heavily impacted stands but also adjacent non-impacted stands, and we can use the drone data to develop spatial analyses for our different sample points. The other benefit is that the drone surveys can be conducted annually to look at mother trees and see how long they persist into the future.

We're also integrating plane-based hyperspectral imagery. We're looking at the spectrochemical signatures of what we're hoping are rod-resistant trees, using basically hyperspectral cameras mounted on the underbelly of a plane. In this image here you can see the flight lines on the left, and we're keying in on water, nitrogen, and non-structural carbohydrates. So, on the upper left is water, on the upper right is the true color imagery, on the bottom left is nitrogen, and on the right non-structural carbohydrates. Green are green trees that persist through a time series of flights from 2016 annually through 2019, and TBB are trees that become brown at some point in the time series. For example, in the bottom left you can see that the TBBs have a different color than the greens to some extent, you also see that with nitrogen, both non-structural carbohydrates. We're just at the very beginning stages of this and we'll see where this goes, but this was preliminary data that was used to secure funding from the U.S. Forest Service for basically investing in a landscape-scale approach to looking at survivor trees. One of the hopes for benefits is that it will streamline our sampling of survivor trees. If we can distinguish survivors from escapes, we can accelerate our time to finding that next generation of surviving or disease resistant ʻōhiʻa. So progress to date, we've collected cuttings or generated seedlings from 300 separate ʻōhiʻa individuals resulting in 10,000 cuttings and literally tens of thousands of germinants. A thousand plants are ready for testing, basically right away, and thousands more are coming online in 2021 and they'll be tested through 2022. Our plant inventory includes ʻōhiʻa from five *Metrosideros* species and five varieties of *M. polymorpha*. I'll end here with this slide just showing that we're a low-budget operation with a lot of support from AmeriCorps students and one full-time employee there, Blaine, on the right and a part-time Forest Service person, and with that I'll end my talk. Thank you very much.

Unintended Consequences of Biotechnology to Restore Threatened European Elms

Juan A. Martín

Associate Professor
Technological University of Madrid, Spain

Thank you very much, sir. Well, first of all, I want to thank Kas [Dumroese] and Douglass [Jacobs] for inviting me to this very interesting conference, and my talk will be focused on the answer to this main question: Why an elm restoration with resistant cultivars can fail from our experience in Spain, in the Spanish breeding program, which is focused mainly in *Ulmus minor* trees? To answer this question, I mainly will talk about plasticity of the chemistry resistance, changes in the pathogen population, failures that can be produced during the breeding process of resistant cultivars, unintended integration in the field population that are planted in the field, and changes in the host microbiome.

Probably most of you already know that Dutch elm disease pandemics started at the beginning of the past century. The first one was caused by *Ophiostoma ulmi* [a fungus], which was moderately aggressive towards European elms but very aggressive to American elms. This first pandemic declined unexpectedly around the mid-century in Europe but not in North America. The second pandemic was caused by *Ophiostoma novo-ulmi.* It was highly aggressive to both European and American elms and has now replaced *Ophiostoma ulmi* in most locations. The current situation in Europe is a post-epidemic phase with recurrent cycles of high disease incidence in recruitment trees. *Ulmus minor*, for instance, has a great ability to resprout from stumps or from roots, so the situation is like we can see in these photos.

Restoration of founder populations of elms should rely on two basic principles: availability of genetically diverse material resistant to the Dutch elm disease and deployment of appropriate planting strategies. However, both aspects are nowadays far from optimal. Regarding the availability of resistant material, it is still scarce. We only have seven native clone resistors in Spain, but I am optimistic for the future. In the next decades, this number is expected to increase. For instance, here we can see an experimental plot with crossings between resistant trees, and we are also performing new surveys looking for new resistant individuals. They are all obtained by traditional breeding. Concerning the deployment of planting strategies, we are currently making a pilot plantation of these cultivars, which are serving as an experience and knowledge to establish these strategies for the future.

Dutch elm disease resistance is controlled by several genes. There is a lot of literature about how resistance mechanisms and traits like a vessel diameter, vessel occlusion, or barrier zone formation were related with resistance or sustainability to the pathogen. In our experience, we observed that resistant trees, each resistant cultivar, has its particular set of resistant mechanisms. However, we are trying to identify common responses between resistant trees using transcriptomic analysis, which is in progress, but preliminary data suggests that some genes are overexpressed only in resistance but not in susceptible trees. But independently of tree-resistant genetics, we also find a strong genotype by environmental interaction in some cultivars. Here we have an example of seven cultivars planted in Madrid and in Valencia, two localities in Spain with quite contrasting climatic conditions, and we see how there is a strong interaction, in particular subgenotypes, that show moderate resistance level in Madrid but a high sustainability in Valencia.

Several factors can be behind this plasticity of resistance, but one of the most evident seems to be the phenology of wood formation. Dutch elm disease is a vascular disease, and the pathogen develops inside white early wood vessels, and there is a period, a window, of susceptibility that starts after these early wood vessels became fully functional and ends after a certain proportion of late wood is formed. So, in this figure, we can see the optimal inoculation date for four cultivars A to D, which is represented within these two vertical dotted lines. If we see here, the wood formation by this time in these cultivars, we see that they have already formed early wood vessels, but not late wood. But we can have late phenology trees like the cultivar E in this figure, that by the time of inoculation their vessels are still non-functional, or early phenology trees like the F cultivar, which has already formed late wood vessels, making the xylem more safe against hydraulic failure and increasing resistance to that same disease. Therefore, if phenology of different cultivars of the local condition induces a variation of phenology among cultivars, inoculation in breeding process should be carried out on different days for each cultivar or locality.

The environment of each location not only affects phenology of wood formation but also can affect other physiological traits and also can affect pathogen growth directly. This highlights the importance of conducting resistant trials over several seasons and at different locations. Attention during the breeding process should be also given to inoculum absorption during screening. Inoculation should be carried out under conditions that favor an early conductance, for instance during hot and sunny days. It should be also highlighted that any factor reducing tree growth will normally increase resistance level to Dutch elm disease. Here in this figure, we have an example of an experimental plot in Madrid, an experimental plot with three blocks. Block one showed a reduced level of symptoms to Dutch elm disease and also a reduced tree development. This was because block one was subjected to a more intensive cultivation practice in the previous years, probably leading to nutrient deficiency in this block. This could lead to unintended consequences in elm restoration.

On the pathogen side, it should be noted that the pathogens *Ophiostoma ulmi* and *Ophiostoma novo-ulmi* have been in an unusual state of evolutionary flux because of extinction and hybridization events during these overlapping periods. During these overlapping periods, for instance, *Ophiostoma novo-ulmi* acquired some useful genes from *Ophiostoma ulmi,* but *Ophiostoma novo-ulmi* itself is not a single entity but has spread as two super species: subspecies *americana* in North America and subspecies *novo-ulmi* in Europe. However, subspecies *americana* was later introduced into Europe, and nowadays hybrids between both subspecies are emerging in Western Europe, giving rise to novel pathogen phenotypes. All these events emphasize the need for regular monitoring of changes in the pathogen population, especially regarding possible changes in virulence.

Another important aspect is how trees should be challenged with the disease pathogen to avoid a false selection of resistant individuals. In some programs or in some research, mixtures of spores have been used for challenging the trees, but mixtures of species or genotypes of the pathogen are likely to compete inside vessels like we see in this photo, either as hyphae or as these cells. Isolate mixtures are probably best avoided for elm screening. Important also is the handling of pathogen isolates. *Ophiostoma ulmi* easily degenerates in artificial culture, unless it is stored under certain conditions at minus 80 degrees, and otherwise, as we can see in this photo, wild type patterns of the colony degenerate and not in non-wild patterns, which are usually characterized by a dense mycelium or pigmented areas, rendering these isolates unsuitable for resistance screening.

Concerning inoculation, there are many different ways of inoculating the pathogen, but mainly we can inoculate in small twigs or maybe in the

base of the main stem. The first method mimics a natural infection of that same disease by the vector that are elm bark beetles, while inoculation in the base of the trunk relates more with a root graft transmission between an infected and a healthy tree. This last method induced a more rapid and extensive collapse of the crown, so I recommend this method if our aim is to select resistant genotypes. Alternatively, we have developed an *in vitro* propagation system of elms that we use as an early screen method of resistance. We tested this method, and for instance, susceptible genotypes show a lower apical growth and a higher oxidative stress than resistant genotypes, so this type of method can be implemented to shorten the long previous cycles needed using traditional techniques.

If we now focus on restoration activities in the field, there is a risk to susceptible trees surviving in the landscape, either because they have escaped from the disease or because they are recruitment trees. There is a risk of outcrossing between our planted cultivars and these susceptible trees in the field. We tested this behavior of resistance by susceptible offspring, and we found that after a first inoculation year, most of the trees show a quite high resistance, but after a second inoculation year, the susceptibility increases a lot, so this restoration can fail in the long term. Also, a problem can emerge with exotics like Siberian elms, *Ulmus pumila*. If we plant our resistant trees close to these trees, there can be different outcomes, since *Ulmus pumila* is less tolerant of flooding than *Ulmus minor.* Probably the next generation will be less adapted to riparian habitats. On the other hand, resistance of the progeny might be increased, and they can be also more tolerant to drought, as *Ulmus pumila* is both more resistant to Dutch elm disease and a better adapter to drought conditions.

To finish my presentation, I just wanted to mention briefly how changes in the host microbiome can affect tree performance. It's increasingly clear that tree health and resilience is related to microbial composition. So, we are now studying microbiome composition from different populations of elms in central Spain, and we found a strong effect of the local environment on endophyte diversity. This is a potential factor of influence of plasticity on resistance, but we have also found that a small proportion of the fungal taxa are ubiquitous and can be considered part of the core microbiome. We have found within this core microbiome, two families of yeast related to the resistance level of the host plant. They are also extensive colonizers of the aerial tissues. We are now investigating the effect of artificial inoculation of these endophytes, either on field assays or *in vitro* propagation plants, and we found that, for instance, in *in vitro* plants, they increased survival against stress and improved some physiological parameters. That's all; I want to thank my team, my colleagues from the elm breeding program in Spain, and also some colleagues from other centers, which have contributed to this work. And I want to show you this nice photo of an elm tree mutilated due to the use of foliage for the cattle in Spain and the same tree several years later now. We hope to recover in the future these magnificent trees. Thank you for your attention.

Tree Breeding as a Strategy for Responding to Ash Dieback

Paul Woodcock

Joint Nature Conservation Committee
United Kingdom

Today I'm going to talk about the response to ash dieback and particularly where tree breeding potentially fits into that as a strategy within the UK. Ash dieback is caused by a fungal pathogen that was first detected in 2012, but it was probably present several years before that, and it causes very high levels of mortality in European ash. European ash is one of the most common broadleaf species in the UK; the expectation is that it will have a major impact particularly through the loss of ecosystem services and also because there are quite a lot of ash trees in urban areas along roadsides, along railways, and that may well need to be removed for safety reasons. The response to ash dieback initially focused on trying to control and contain the disease, and although that is still part of the strategy, over the past few years there has been increasing emphasis on thinking about how to adapt to the longer-term presence of ash dieback throughout the country. There's quite a few different options being discussed in that respect and it's starting to be implemented. The two that I'm going to talk most about are the tree breeding options, but I think it's important to consider these in the context of the other options that are being suggested as we evaluate them relative to these other options. I think there's a few different ways you can go about doing that evaluation, and the areas that we've probably done the most on is thinking about the biological feasibility and also the views of different stakeholders.

First of all, I want to talk a bit about the biodiversity impacts of ash dieback and what the implications of that might be as well for the options that we choose. There were a couple of really nice papers by Ruth Mitchell and others at the James Hutton Institute in Scotland that looked at which species were associated with ash and how strongly they associated with ash. What they found, or what they suggested, was quite a large number of species were either obligately dependent on ash or highly associated with ash. So, if you also add in all the species that use ash some of the time and are at conservation risk for some other reasons, you end up with what I think is quite a long list of species for the UK at least potentially at a much higher risk as a result of ash dieback. What that work also showed is that there are potentially other tree species that you can use that will support some of the ash-associated biodiversity, and planting those other species in the right combinations in the right places can help to mitigate some of the impacts. At the same time, I suppose, you would expect that really the majority of these obligates or highly associated species in the UK would be expected to either decline severely or to go extinct over the next 50 to 100 years if the levels of ash mortality are as people are suggesting.

The reason why I think that's relevant for tree breeding is because it shows that, yes, alternative species can and are an important part of the response, but to me it seems like the only way that we're going to be able to conserve this set of ash-associated biodiversity is by finding a way of keeping ash in the landscape. I think the feasibility of different options for doing that depends on a few things, partly the frequency of resistance or tolerance to ash dieback within the population and also the heritability. I've tried to show in this diagram how those two axes sort of influence which option might be most suitable. So, for example, if resistance is widespread but not very heritable then that might favor silviculture from site management. If resistance is rare but it has a high level of heritability, then that might favor within-species tree breeding. Now, I think this is an

oversimplification of things. I think these options in practice probably overlap with each other a lot more, and I think there's probably also another axis that's more to do with where the resistance is complete or partial or whether it's resistance or tolerance. I found it sort of helpful initially to just think about which option might be most effective and why. For that first question, the frequency of resistance to disease work by Tim Coker and others at Kew Gardens looked at surveys of ash mortality from across Europe and what they found was very high levels of mortality in places that had been exposed to the disease for 10 to 15 years or more. Still, there was a proportion of trees surviving, and that proportion might still go down over time I think but it at least suggests that there are so many trees that are likely to be less susceptible or to have low susceptibility to ash dieback.

The next question would then be how heritable is that low susceptibility, and the evidence from planting trials from about 11 plantings suggests that about 40 to 50 percent of the variation in susceptibility could be due to genetic factors. So, you've got that, and then you've probably also got environmental influence, which is what I think we need to better understand. I think that puts it sort of as a lowish frequency of resistance and a low to moderate heritability of resistance but with still quite a bit of uncertainty around both of those. That probably means that most of the options that are being discussed and being thought about are feasible, but there's no one really obvious one to go for, with the exception that we probably rule out to do nothing and rely entirely on natural processes, because the impacts would just be too great.

The final strand then is the stakeholder views on the acceptability of the different approaches, and there's been a few studies on this. The one I'm going to talk most about is work that was led by Mariella Marzano at Forest Research and that involved carrying out interviews with forest advisors and managers, people working the public sector, commercial sector, and NGOs to understand views on the importance of ash dieback and what people thought about some of the different options that are being suggested. There are also a couple of studies that capture the wider general public on attitudes to these different options as well, but in the responses in the interviews, people definitely valued ash both for its contribution to forestry, its associated biodiversity, and also it has a kind of a cultural value as a native species irrespective of any of these other benefits. As a result, I think you've got a generally high level of concern amongst most people that were responding. That's not universal, there were a few people saying that they want to still wait and see what happens a bit more. One or two people saying it's just one of those things, trees get diseases, we just have to live with it, and saying it was a concern but it's not the end of the world. I think the most useful part of the paper for this is thinking about stakeholder views on the different approaches to tree breeding, and conventional tree breeding particularly was one of the favored options from several of the people who were interviewed, particularly those working for conservation NGOs because of the perceived naturalness or more naturalness of that approach. The main concern tended to come from the private sector particularly because of the time that might be involved. I think the general summary for the use of hybrids, which would be cross-breeding European ash with Manchurian ash or something similar, is that it would be acceptable I think to about 50 [percent] of the people who were interviewed but not ideal and not top of the list. Then for genetic modification there would be concerns about unintended ecological consequences, concerns about the public reaction, and that was sort of the general response but that wasn't universal. There were certainly some people responding that had more of an open mind about it. So, the general benefits that people felt about developing resistant ash were in terms of we keep a native species, we maintain woodland character, we maintain the associated biodiversity, and as a result of these benefits there was a willingness to pay a bit extra for resistant ash—provided there was a guarantee that it would be resistant and some sort

of compensation if it then failed. Then there is also this set of concerns about will it take too long, how will it perform in the field, will it support the same levels of biodiversity, and particularly this question about how durable will resistance be and what will the impact of other threats be? Emerald ash borer in particular being the one that gets highlighted, and then also a final point about whether tree breeding might have consequences for genetic variation within species.

Some of the other options include silviculture management, as well as planting alternative tree species. Both of these were things that people had support for them doing and are starting to do but equally recognizing the limitations in them and that these were not perfect solutions either. So, I think my overall conclusion is sitting on the fence a little bit in that there's no perfect option for this, but tree breeding potentially could and should be part of the approach, but we need to consider all the different options in complement with each other. Some will work best in some situations and some in others. I think the potential to work with citizen scientists and volunteers to help identify resistant ash in the field and track performance is a really interesting area.

Then there are challenges that need to be addressed about the durability of resistance, the particularly potential risk from other threats like emerald ash borer, and this last point about the costs and the coordination. How do we fund and support a tree breeding program using whatever method for the necessary amount of time? I think that may well be one of the biggest challenges for the broader implications of it. I think, first of all, we found it really helpful to try and synthesize these different strands of evidence—the biological evidence, the stakeholder views—and use those to try and work through which options would be suitable and why. I think communicating with stakeholders is obviously a really important aspect. One thing that we found during the interviews is people had different understandings of what resistance meant, so some people thought resistance meant completely resistant, some people thought it meant partially resistant, and some people equated it with tolerance. So, that sort of leads into a broader point really that we need to be clear with the people who are using this material about what it's going to do, otherwise you risk losing confidence. Not just in what gets produced during that breeding program but potentially you lead to sort of skepticism about other things that happen in future. I think, again, communicating, keeping people up to date with progress and plans, is something that can be really helpful.

The last point I think with ash is more a case of mitigating an ongoing impact, at the moment at least, rather than trying to restore a species that has been lost from the landscape. I think that may lead to sort of a slightly different set of priorities and different acceptability of some of the different approaches. One thing that I'm not quite sure on, but there might be a bit of a trade-off between this need for an urgent or a rapid response versus the time it takes you to be confident in the performance of resistant material. I'm not quite sure how big of an issue that one is in practice or how you get around it, so that's, well, that's where I'm going to finish.

Avoiding the Knee-Jerk Reaction: A Conceptual Framework for Long-Term Response to Forest Health Threats

David N. Showalter

Postdoctoral Scholar
Department of Botany and Plant Pathology
Oregon State University

I'm going to talk first about biological invasions as one type of a long-term forest health threat. A lot of what we've been talking about are biological invasions, but there are other things to respond to as well. Then I'll lay out kind of a general response framework and that really revolves around short-term and long-term inputs and outputs, and that this is fundamentally two different approaches, not mutually exclusive, but fundamentally different approaches to managing these things. Then finally, what's the role in the truth of true breeding in all of this. So, sometimes long-term forest health threats are single actors. If you think about a chestnut blight in the dashboard, you know, a single agent that is introduced into a native ecosystem and explodes. Sometimes they're a little bit more thorny and complicated, like with high elevation pines that are kind of suffering from direct and indirect effects of climate change that are impacting their growth, the growth of mountain pine beetle and its reproduction, as well as the introduction of non-native fungal pathogens that are all really working together to just hammer some of these high elevation pines. So, how do we respond to these things, be they, you know, somewhat simple or somewhat more complicated? To kind of get us all in the same place here, I'll briefly, you know, kind of talk about classic biological invasion theory and where management fits in.

So, in these circles here, we have different stages that are separated by red boxes that I'm going to call barriers, and then over top are sort of the management interventions. So, you have a source, in this case I'm calling it a PIP, a phytophagus insect or a phytopathogen. It comes from a source introduced into a naive ecosystem, and there's a barrier to establishment that is survival and reproduction. Not all introductions result in establishment. You can intervene here and try to eradicate before a local population becomes widely established, and there's been a lot of studies that show putting your money into prevention and eradication is money well spent. It's much easier to take care of these things early but invariably some of these organisms make it through. They're able to disperse into this naive environment, and they spread, and then really the only barriers left are those between an orgasm being present and an organism outbreaking and then that outbreak. In our case, the things we're talking about result in tree death, and so there are a number of factors at play here. Host tree defenses, so bottom-up pressures; natural enemies, top-down pressures; and there's some abiotic environment as well. So, here we're kind of focused on these established pests. I mentioned prevention and eradication are hugely valuable and important, but they kind of come off the table once you get a widely established threat like a biological invasion.

So, this is a simplified version of the framework we proposed, and you can check out this 2018 paper that I led and actually involved several other people here. First step is to identify and then you can facilitate a three-step, three-part research response. As Paul [Woodcock] was saying, you need to assess the different values of the host trees across these settings, be those cultural, economic, ecological, and then how those things might vary in different settings. You know, taking care of urban ash you want a very different outcome than taking care of naturally generating forest ash.

There's different inputs, different outputs, then you need to characterize the hosts for your pest, both co-evolved hosts and the naive introduced hosts. Then you also need to understand that the pest ecology, as well as the factors that contribute to overcoming resistance, what they have to do with how the pest reproduces and things like that. Then you have to kind of check your toolbox to see what approaches are available to respond to this pest, and as I mentioned, I'm going to kind of classify them into short-term ecosystem maintenance approaches or long-term ecosystem transition approaches. Finally, you take all of these inputs together, you look at things like effectiveness, feasibility, acceptability, things that we've talked about here, and then you set goals within your response capacity. Really importantly, this is an iterative process. Our knowledge is imperfect, particularly at the beginning of these invasions. We really just know very little, and so it's important to review these as the invasion progresses. Also, it's important once you've identified what tools might be helpful to try and develop some of those tools and strategies, and perhaps that will allow you to revise your goals down the road. That gets to what I think Richard [Sniezko] was kind of talking about as well—if upon first assessment you really don't have any tools in your toolbox you know the acceptability of some of those might not be very high, and yet if you develop some tools and show their usefulness you may need to reassess acceptability and viability of some approaches.

When making distinctions between long-term and short-term responses, I take from this paper from Science a couple years back, where short-term ecosystem maintenance is about protecting the trees that exist now, protecting the forest as it is now. So, prolonging the health and productivity of the ecosystem services of the existing trees, which eventually then fall off and need to be replaced by an alternative ecosystem versus an approach that's a more long-term ecosystem transition, which may work to establish the long-term ecosystem. Sometimes this is at the expense of the existing but with the long term in mind, and these are not mutually exclusive, but they are fundamentally different. When we talk about suppressing a pest or enhancing tree health with silviculture, limiting the spread, this is all to protect existing trees, and it's valuable. It has usefulness as well, but when we talk about long-term solutions we're talking about self-sustaining biological control, resistant tree restoration or as Paul [Woodcock] talked about, using alternative tree species.

What I'd like to introduce is sort of some theoretical ecology that might help us pick our targets. I think we're going to have to be selective about when we try and throw sophisticated resistance breeding programs at threats, and I think a useful way to think about it is tree resistance fills defense free space. There are two theories of why biological invasions happen. Among others, one is this idea of enemy free space where you get a pest that comes in and there are no predators or parasites and allows it to, you know, explode due to the lack of top-down pressures of enemies versus the other saying. It's something to do with the bottom-up pressures, the defenses of the host plants, that are inadequate, and so these invading organisms can explode. And again, these are not mutually exclusive, they're sort of two ends of a spectrum, which is how I'm gonna want you to think about it from now on. So, if the mechanism of invasion is enemy free space, the primary influence on the pest population is top down from natural enemies, and you would imagine that a plant that has co-evolved with this type of pest would tend to favor tolerance. So, survive long enough to allow some of those top-down factors to come in, knock down an outbreak, and outlive the problem versus the other end of the spectrum. Here you have an invasion of defense free space that is determined by the host defense quality from bottom-up pressure and a co-evolved system. You would expect a plant to utilize active resistance to fight off any attack or feeding. Then alongside that we came up with two factors that we thought might help predict some of these traits. The first being the cost of tissue damage and the second being the intimacy of association. I'm gonna unpack these two axes in just

a minute, so the idea of the potential for resistance as a fitness cost. There are a number of factors that contribute to how damaging you know a pest can be. It's the amount of damage: the pest or feeding group size, when does the damage happen, is it on a short window, is it on newly emerging tissue, which tissues are involved? So, meristems and vasculature are very hard for the plant to replace, or very costly tissues to be damaged.

Then finally you can think about the role of vectors, be they beetles that make a fungus have more access to a tree. This isn't the case with the beech scale and *Neonectria* species in the beech bark disease. Then the second factor to think about is the intimacy of association, so think about the proportion of the life cycle and the proportion of the organism that is in contact with the host. If you think about it like a foliar-feeding insect, you might have just a stylet or a mouth part in contact with the host, and it may feed on multiple hosts and move around to different trees. Or something like a powdery mildew; it's only a haustoria or a very small part of the fungal tissue in contact with the host versus a vascular wilt or a phloem borer where you have almost the entire organism surrounded by host tissue under its entire life cycle, because this can facilitate the exchange of signals and sort of the building blocks for the co-evolution of active resistance. This also may have an indirect effect in that it may limit the apparency or availability to natural enemies thereby limiting top-down forces. So, you tie these all back together and you think about tree-folivores' relatively low-cost tissue that's being removed. Trees have adapted to replace lost foliage and it's not a very intimate association. There's not a lot of signals to go on up the scale here; you have, you know, gall formers that cause a bit more deformation particularly if they're meristematic or things like that. When you have phloem and wood borers, cankers and vascular wilts, occupying this upper right corner and the shading here corresponds. We expect these to have the highest active resistance, and that invasions by these organisms are probably occurring in defense free space.

I think the response frameworks with biological invasions may inform other responses as well be they climactic, and it's important I think that tree breeding can be a primary tool back to what's been said. It's sort of thought of as a last resort, but it really is a very strong long-term reaction. It will not always address the short-term impacts but really these are different costs and different inputs, it really should be used to address invasions of defense free space. We have a couple of candidates for determining when that's happening, and it also benefits from starting early though—you have a higher population size for selection, greater genetic diversity, so you can leverage some of that urgency that stakeholders feel.

Causes and Consequences of Forest Pest Invasion

Andrew M. Liebhold

Research Entomologist
Northern Research Station
U.S. Department of Agriculture, Forest Service

Randall Morin

Research Entomologist
Northern Research Station
U.S. Department of Agriculture, Forest Service

Songlin Fei

Professor
Department of Forestry and Natural Resources
Purdue University

I'm [Andrew Liebhold] going to be talking about both the causes and consequences of forest pasture invasions. In terms of the causes, it really goes back to continental drift and the fact that as the continents drifted apart, we separated the land masses into these separate regions so they were largely isolated. So, they evolved, and communities organized separately, but now in modern times we're basically putting these species and communities back together. And because they have not evolved together, you very often get sort of weird and sometimes bad things. In terms of forest insects and diseases, the main mechanism by which we've accidentally been moving these species around, probably the most important one, is the movement of live plant material. We've now learned that's the most dangerous method for moving species around. The second, especially in modern times, there's been increasing use of solid wood packaging material, and we've also learned that's a very effective way of moving certain species around, especially wood-boring insects. There is actually a third category, sort of less common, but what we call "hitchhiking," where life stages move on sort of inanimate objects.

In terms of the big picture of things here in the U.S., when we've put the data together we have somewhere around 450 non-native forest insects established in the United States, and we've had sort of this constant rate of accumulation going on since somewhere around 1860. So, we get about two-and-a-half species establishing per year but of those only about a fifth are species that we actually notice that cause any damage, so the rest are just things that they're out there in the forest and we don't really see them. Most of the time this problem is something that's not going on just in the U.S.; obviously it's happening throughout the world. Then within the United States, historically this is a map where a few years ago my colleagues and I put together county-level data on the distribution of non-native forest insects and diseases, and there are about 90 species that are damaging. You see there's kind of a remarkable concentration of species in the Northeastern United States. I'm not going to get into the reasons for that, but I think it has partly to do with the historical pathways of people being concentrated there but also forests in the Eastern United States are much more diverse, and so there are essentially more targets, but it's definitely a very strong pattern that we see.

I'm going to switch now to talking about the consequences of forest pest invasions, and I think from a sort of food web perspective, of course, you can consider insects and diseases are sort of herbivores. They're basically feeding on producers,

which are mostly trees, and so the reasons why we sometimes get explosive growth of some of these introduced species is when, as I mentioned before, they have no prior evolutionary contact with their hosts. So, you get this lack of host resistance, that Dave [Showalter] was mentioning, but you also have this phenomenon of enemy release, they arrive in their non-native habitats without any of their natural enemies that may normally regulate their populations in their native range. So, we get this very explosive growth, and there can be varying types of ecological damages, but certainly one of the damages that is more impactful in terms of impacts on ecosystem services is tree mortality. Not all forest insects cause tree mortality, but some do and sometimes for some of these species it's been quite spectacular. As I mentioned before, we have a total of somewhere around, it again changes by years, 450 and 500 species of non-native insects established in the U.S. A few years ago, we went through and did a study to try to analyze the overall impact of all these species, and around 90 of these species were reported not to cause any damage at all. When we studied the literature, we decided there were about 15 of these species that really caused widespread tree mortality. A lot of these don't really cause tree mortality at all. Then we determined, based on analysis of data from [U.S.] Forest Service Forest Inventory Analysis [FIA], that only about nine of these species are causing any regional impact on tree mortality. So essentially, I'm not going to go to the methods but, we basically use FIA data to compare the tree mortality that we can attribute to these pests with sort of background levels of tree mortality. We found at the time of the study, which is based on inventory data from 2005 to 2015, that Dutch elm disease was causing the largest amount of extra mortality followed not too far behind by emerald ash borer. I wouldn't be surprised that now it was more modern, with the expansion of emerald ash borer this is sort of switched, but Dutch elm disease is right up there too. You see beech bark disease is up there as is hemlock wooly adelgid.

One thing I want to sort of clarify here though is that very often I've heard people today talking about we're losing species. I think in most cases that's not the case; having a lot of tree mortality does not necessarily translate into those species declining in their overall abundance. In fact, most of the hosts I think are not at all threatened. I think the only examples, the two pests that maybe come closest to having impacts on the viability of host species, are probably butternut canker and chestnut blight. For most of these species, we're not losing, I mean we're not losing elm, there's a ton of elm in North America. There's American elm, there's slippery elm, there's a lot of beech, there's a lot of hemlock, and we're not losing these species. The other thing is, very often these impacts take very long to express themselves. There can be quite a long lag between when these pests establish and when they cause impacts. I think a good example is provided by hemlock wooly adelgid, which we don't even notice on a landscape scale. On a regional scale there's an increase in tree mortality for maybe 30 or 40 years after the pest establishes. The other thing is, for example in hemlock wooly adelgid, we have this because of successional processes. Without hemlock wooly adelgid, we would have this huge increase in the biomass of hemlock going on. So, the combination of successional changes and tree mortality caused by the adelgid sort of cancels each other out, and there's about an equal steady state for hemlock abundance. The other thing which I think is very important to keep in mind is in terms of when you look at the overall societal impacts of these forest pests. The biggest impacts by far are in urban settings, even though these species are all having ecosystem impacts, by far the biggest impacts, I think if you quantify things economically, are in urban settings because trees are just much more valuable there.

I'm finally going to switch gears and talk about management, and again this maybe overlaps a bit with what Dave [Showalter] was mentioning. Again, in invasion biology we talk about the sort

of stages every invasion goes through. One is the arrival, then establishment, spread, and then finally the permanent range of the species. According to those same categories, we have specific types of activities we use to manage invasions. So, things like quarantine inspection are practices that we implement in order to prevent species from arriving. Also, we do surveillance and eradication to try to prevent this permanent establishment of species, and then we use barrier zones in domestic quantities to slow or prevent spread. As David mentioned, when you do the economics, by far the most effective way of dealing with these invasions is early on, investing in quarantine and inspection. But, as David also mentioned, many of these slip through all these nets and we get establishment. Then the question is, how do we manage species once they've established widely?

Essentially these methods would include human adaptation, which in a lot of ways it's simply a matter of society getting used to and adapting to changes in ecosystem processes and properties. Silviculture can also be effective, very often it's a question of applying silvicultural methods to change species mixes; biological control, which we tend to mostly think of classical biological control where we're importing non-native natural enemies from the native range; and finally host resistance breeding, which of course is the subject here. One of the things I wanted to emphasize is that I feel there really is not enough emphasis given to the question of deploying resistant genotypes. If we're seriously talking about using resistance breeding to make landscape-level impacts on restoring species over a whole region, and there's a very serious problem of restoration, one of the issues is tree planting that obviously, you know, you're only going to deploy resistant genotypes through deployment by planting trees. Most of the tree planting that's currently done in the U.S., I'm sure all of you know this, is either in the Southeastern United States or lesser in the Pacific states. Elsewhere in the Northeastern region and elsewhere, there's very little tree planting that goes on. Again, so plantation forestry on international scales, places like the southern hemisphere, are also places where plantation forestry is really a huge thing and there's probably good opportunities for using resistance breeding, and as there is in our urban forest since most urban trees are planted. I mentioned before that most of the greatest impacts and greatest concentrations of these non-native forest pests are in the Northeastern United States, and in the Northeastern United States they are almost entirely naturally regenerating. As many of you are familiar with the ecology of these forests, the recruitment is intense, and so this is especially the case for some of these high-impact species that we're seeing from say the impacts on elm, ash, and beech. These are species that invest a large amount of their energy in reproduction, and in most cases I don't think they're going away. To make any of these hosts go away, you actually have to work pretty hard. Many forest managers in the Northeastern United States don't like beech because it's considered to be of low timber value, and there's actually quite a bit of research that's gone on, basically on how to kill beech, and it's really difficult because it's this prolific root sprouter. When a tree dies from beech bark disease, or if it's cut down, it produces massive numbers of sprouts, and so, if we're really talking about seriously doing a tree restoration program where we're replacing these naturally occurring genotypes with resistant genotypes, you're really going to have to work hard. You're going to have to use a lot of herbicides or mechanical methods to get these things established, and once they're planted there's going to be a need to go back and invest a lot of money in these areas again, either using herbicides or some combination of mechanical methods to prevent competing vegetation over in these areas. To be honest, I actually find it hard to believe that this is a realistic thing, that it's going to be done over large areas of the Northeastern U.S.

In conclusion, I feel that from my perspective as an outsider looking at this, I feel like restoration with resistant genotypes has its greatest potential in places where people are already planting trees. That includes places like southern pines in the

Southeastern United States or, for example, the Southern Hemisphere where pines and other species are planted in plantation forestry. In the Northeast, north-central areas, where we've had the greatest concentration of many of these pests, I think resistance probably has less potential as a long-term solution.

CURRENT SCIENTIFIC PROGRESS AND BARRIERS TO USE OF BIOTECHNOLOGY: POLICY AND SOCIETY

Public Perceptions of Forest Biotechnology and the Role of Engagement

Jason A. Delborne

Professor
College of Natural Resources
North Carolina State University

I'm going to speak today partly about the chestnut project that Doug [Jacobs] referred to [when introducing me]. I, along with Richard Sniezko, who's on this conference, participated in a national academy study. Forest health and biotechnology has been referred to a couple of times, and I'm going to talk about some of the findings from that report as well as talk about one of the activities of the GE [genetic engineering] chestnut grant that I have from the National Science Foundation. So, one of the points we made in the report, and this is fairly obvious to those of you in the room or to many in the Zoom room, is that biotech trees are designed to spread and persist in unmanaged environments, and so some of the first biotechnology trees that we saw were fruit trees like the papaya and the arctic apple. Those look a lot like the first-generation GMOs [genetically modified organism] that appear in agricultural fields, for example, and this is really different in terms of how they're perceived from deploying biotech trees in unmanaged environments that look more like these kinds of forests. So, this raises different kinds of issues in terms of public perception and the kinds of values that get triggered when we consider the deployment of these technologies. One of the things our report talked about is, we used an ecosystem services framework to think about the way that forest health could be valued, and we also agreed that the values around the potential use of biotechnology and forests extend beyond ecosystem services. So, it's not just about trying to put evaluation in the way that trees serve us as human beings, in terms of managing water or offering benefits like hunting and fishing, timber, things like that, but that we need to think beyond the ecosystem services and one of those is the notion of intrinsic value. One can think about trees or forests having a kind of spiritual nature. There are some cultures where there's an understanding of kinship between trees and people and that kind of relationship creates a different kind of value than we can capture with an ecosystem services framework. We also have to think about this issue of wildness. This is not an easy term to define, it's probably even harder than defining forest health, which our committee certainly struggled with, but this notion of wildness has a huge impact on the way that we value forests and trees in those forests.

Finally, there's a set of values around social justice, which have to do with the distribution of benefits and risks, the kinds of procedures that we create to make decisions. Even if a decision isn't our favored one, there can be procedural justice, a sense that there was a fair procedure that was undertaken. Even with things like intergenerational issues that come with organisms that last longer than a human lifetime. For example, one of the conclusions, there's a really nice review in one of the chapters on attitudes about forest biotechnology, and I won't try to review those—they're in the report if you're interested.

One of the key findings of our committee was that public attitudes about forest biotechnology are mixed. So, if you take a look, this is one study that we took a look at, on the last column here with reforestation with GMOs. Perhaps not surprisingly, the public was fairly negative about this compared with something like natural regeneration, and so if we're thinking about how we might want to restore forests or deal with invasive species, invasive pests, here we have less enthusiasm for something like reforestation with GMOs. That's maybe not a surprise, and that seems to fit into the narrative of,

"well, the public is scared of GMOs, this is going to be a dead-end street"; we have a huge battle ahead of us. But look also at this category, there's almost the same level of concern about reforestation with non-native species, so there's something complicated going on here that's not explainable just by a kind of anti-GMO or pro-GMO partisan divide. Our values around forests are complex; what people see in forests, what they want in forests, is complicated and if we're going to really wrestle with attitudes about forest biotechnology, we need to wrestle with some of these complicated values around how people value forests.

Our report stated that we need more knowledge about societal responses to biotech trees for forest health. We need to understand how different social and cultural groups might respond to genetically engineered trees, for example, we need to explore the stability and consistency of attitudes towards different kinds of interventions and different contexts for those interventions. We recognize the importance of thinking about differences and attitudes towards different kinds of genetic engineering techniques, and those have been discussed today in terms of cisgenesis, transgenesis, and CRISPR-based gene editing. Some of the surveys out there do take this kind of complexity into account, but there's more work to be done in terms of understanding people's responses. There the role of deeper value orientations is something that's difficult to get at. With some of the surveys that are done either online or by phone, understanding these differences in value and orientations across cultures and geographies are really important. Then, finally, the importance of thinking about and knowing that people make trade-offs all the time. So, what are the trade-offs around values such as wilderness and species protection? How are people going to approach that and understand that, in the context of thinking about biotech trees for forest health, and we agreed that there was a need for engagements. We talked about respectful, deliberative, transparent, and inclusive engagement not only to understand attitudes towards biotechnology but to understand how people understand forest health threats themselves. We want to uncover complex responses to any of these potential interventions, and biotechnology is just one of those interventions. The graph showing a concern about the planting of non-native trees is a good example of that complexity. We recommended analytical deliberative methods that engage stakeholder communities and the public, and I'll talk a little bit more about what I mean by that.

A previous National Academy study that I served on around gene drives defined engagement as "seeking and facilitating, the sharing and exchange of knowledge, perspectives, and preferences, between or among groups who often have differences in expertise power and values." There's a lot packed into that definition but what's important here is to recognize that the way we've defined engagement here is not just a one-way communication of either educating an ignorant public or persuading them of some outcome that we're sure is correct ahead of time. This is about sharing an exchange of knowledge, perspectives, and preferences, and we acknowledge the kind of power differentials that are part of these engagement exercises. This report also further went on to distinguish among the types of engagement that we might pursue with different audiences. Ranging from communities, which are geographic in nature, to stakeholders, which has a more political orientation, all the way to public in terms of thinking about broad segments of society. One of the points I think is that whenever we do engagement, we are constructing a particular audience and making choices about who we engage, when, and how.

So, I'll talk for a couple of minutes now giving you a sense of what that engagement can look like. I had a grant from the National Science Foundation, that Doug [Jacobs] mentioned. I'm looking at the genetically engineered American chestnut tree. I started in 2016, so this was well before it was submitted for regulatory review, and one of the activities that we undertook was a diverse stakeholder workshop that we held at NC [North Carolina] State in April of 2018. We were

focused not on a narrow question of, you know, is the genetically engineered American chestnut tree a good idea or a bad idea, but instead we brought a diversity of stakeholders together to have conversations about the role of public engagement in governing this technology going forward. That workshop report is available online, the URL is down there; it's at the genetic engineering and society website if you're interested. I just want to emphasize that we did a lot of work to invite a broad, diverse set of stakeholders to this meeting. We had some of the scientists who developed, the tree people from the Forest Service, someone from ArborGen, which is the main industrial organization pursuing forest biotechnology, and The American Chestnut Foundation. Also, staunch opponents to GE trees, people from the Global Justice Ecology Project, we had some Indigenous representatives from the Center for Native Peoples in the Environment, Nature Conservancy, and Chestnut Growers of America. So, our goal was not to establish a kind of representative public sample but instead to get a real diversity of perspectives in one room, to have a discussion over a couple days about these kinds of issues.

So, what was on our agenda? One of the important things we did early on to build a sense of community and trust among the participants was to really talk about each individual's interests and values around these issues. This was an intensive listening session where there weren't interruptions and people heard from each other and got a sense of the overlaps and the conflicts that were existing in the room. Then what we did was, we focused on this question of engagement across different decision phases. What I mean by that is, as a technology is developed and potentially deployed, there are different kinds of decisions that are made along the way. The first phase is research and development, and there's lots of decisions that are made in terms of what kind of techniques are used, even defining the problem to begin with, as well as the kind of safety testing and efficacy testing that might be done as a kind of proof of concept. Then there's another set of decision phases around regulatory review. These tend to be the focus when people think about public engagement and public perceptions of this moment, when we either deregulate or approve a particular genetic engineering technology. But there's a lot of complicated decisions there, and the chestnut is a great example given that it engages three different regulatory agencies in considering different aspects of its safety and efficacy. Finally, there's a third decision phase which is if it is deregulated and approved for release, there are lots of decisions to make around how this technology is deployed, how it's managed including how we might monitor this tree and the environment over time. We talked about the ways in which each of these phases involve decisions that could be influenced by stakeholder and public engagement. We specifically had a session on Indigenous perspectives on chestnut restoration and biotechnology. That was a thrust of our research project, and I can answer questions about that if you're curious.

We then ended the workshop with talking about ideal engagement scenarios, so how might we look forward to future public engagement around this particular technology. Some of the outcomes of discussion: One was a sense by the people in the room that the deliberative public engagement has been limited. So, this has not been a private technology that's been hidden away. The folks at SUNY ESF [State University of New York, College of Environmental Science and Forestry], Bill [William] Powell and Andy [Andrew] Newhouse especially, have been very public in terms of talking about this technology, but there hasn't been much deliberative public engagement. The interests and values shared showed a mix of conflict and common ground among the stakeholders. Most people in the room agreed that the narrow definition of safety during our standard regulatory review in the U.S. discourages the use of more diverse sources of knowledge and different kinds of perspectives. The group generally agreed that strategies for what might happen after deregulation in terms of deployment, management, and monitoring are fairly unclear. There's

certainly been some work in that area, but it does raise questions about responsibility and authority about this tree, and finally, that public engagement could play a role in doing things like guiding safety studies, prioritizing conservation needs or targets, and determining priority areas for chestnut restoration if it is approved. I'll just mention that there are some other publications that my research group has gotten out in the literature. Here are a couple of them. I'll just mention two of them in particular that looked at our engagement with Haudenosaunee representatives in Upstate New York. Some interesting findings there, which I'd be happy to take up during the Q&A. And so I will end there, and I'd be happy to take any questions from the audience.

Understanding Public Perceptions of Biotechnology

David Flores

Research Social Scientist
Rocky Mountain Research Station
U.S. Department of Agriculture, Forest Service

What I'm going to present on is not necessarily a research study but rather, [I will] provide a review of public perceptions of biotechnology and specifically, genetically modified trees. I think it's important to note that this is a review of several other studies on this topic in order to give you an idea of the social science research that's been done, and it isn't actually my area of expertise. Kas Dumroese likes to push me outside of my comfort zones, and so he has helped me sort of think about this topic a little bit more. My original research is more on wildland fire and social perceptions in that area, but this was very interesting to learn more about. What I did learn, first of all, is there's relatively few research studies in the social sciences that have been done around the public perceptions of genetically modified trees as opposed to social science of public perceptions of genetically modified foods; or in other disciplines in the social sciences such as anthropology, psychology, even in economics. This topic is pretty young; especially compared to the research that's been discussed all day, it's a relatively young topic when we look at public perceptions in the social sciences around that.

Why is knowing about the public perceptions of biotechnology important? Why should we care? I think Friedman and Foster sum it up well when they state, "The challenge of managing public lands is to balance the current and future needs of people and the long-term health of ecosystems." In addition, "public land management requires being responsive to the public and their elected representatives and ensuring that the public agencies are efficient, effective, and representative of the diversity of the American public." So, I think that it's been discussed quite a bit already that as we move forward, the public perceptions of this topic are incredibly important in order to move projects forward and hopefully be successful in getting some of this work done.

When it comes to the social science of public perceptions on this topic, I found that there's four general groups that are studied in this research. There's the perceptions of forestry scientists, forestry managers, the perspectives of wood product consumers, and the perspectives of the general public. I also found that within these groups there's four dominant perspectives that social scientists identify. First are general perspectives that are shared by research participants, second are perceived benefits of biotechnology, the third are perceived concerns, and the fourth are underlying values. Now this is not how they are labeled in the literature, but this is how I label them given my reading of the research. In addition, note that while I categorize these different perspectives into four groups, they're not mutually exclusive of each other. Actually, the same person can hold a combination of these perspectives or even all of these perspectives at the same time, and that's what can make these diverse perspectives increasingly complex when they're applied to the real world. I think for analytical purposes it's helpful to dissect them down to these separate parts.

I'll begin with discussing general perspectives from forestry scientists and managers. Generally, these are two groups that view biotechnology for the benefits that they provide to restoration, and their argument is that there are regulatory requirements that impede research. Also, how products are marketed and labeled is important to both of these groups. On the other hand, when it comes to the general public and consumers, their

perspectives about biotechnology tend to fall into lack of trust in government and skepticism toward decision makers. They often hold perceived threats from technology, and they have questions about economic competition. There's also a difference among the general public when it comes to residential proximity to forests, whereas groups that live closer to forests tend to be more supportive toward the use of biotechnology. But a significant challenge for support for biotechnology is that as soon as a credible group provides a negative message or argument, support from the public tends to drop dramatically. In other words, all of the science and all of the positive messaging and support of biotechnology can quickly be torpedoed by one negative message from what the public identifies as a credible source. Moving on to perceived benefits of biotechnology, the public recognizes many of the economic benefits provided, such as reduced production costs and job flexibility. They also recognize more job opportunities and improved production of biomass; that it uses fewer chemicals to fight against pests and increases tree productivity.

When it comes to social perceived benefits, GMOs [genetically modified organism] for enhancing resistance of tree disease and less need for pesticides is highly acceptable to the public. Also, when people are informed that biotechnology will lead to other favorable outcomes such as aesthetics, pest control, disease and fire outbreaks, support for biotechnology and perceived benefits tends to increase. In terms of perceived concerns from an economic standpoint, purchasers report that health and safety issues and environmental impacts are very important, but the public and consumers note perceived risks to biodiversity, increased herbicide resistance, and vulnerability to other tree diseases. More generally, environmental NGOs and First Nations or Native Americans tend to have a less positive attitude toward marker-assisted selection. At the same time, they do see some benefits, as was mentioned earlier in the talk before me. From a social, non-market perspective, there's a high acceptance for breeding and replanting with local seeds, which has been discussed today. As well, when it comes to concerns, there's a perceived risk of loss of biodiversity, the risk of needing more herbicides, and vulnerability to viral diseases. There's also concern about negative impacts on wild and native species and on human health. Also, acceptance of biotechnology decreases when using non-local seeds and species out of their natural range. Then finally, people who oppose GMO trees tend to be relatively more in support of environmentalism than market-driven goals in forest restoration. In terms of overall underlying values, government and industry tend to have more positive attitudes toward biotechnology and supporters tend to have higher levels of trust in science, in forest industry, and in government. In regard to management, there's less support when wildlife is impacted; also, applications of biotechnology in the medical field tend to be more acceptable than non-medical applications such as modification of trees. There's also overall less acceptance for genetic modification compared to traditional forest management. So, for example, methods that are seen as quote/unquote "natural" tend to be preferred, nonetheless respondents to studies do care about this issue. People do not want no activity happening, which was discussed earlier; overall, doing nothing tends to be the least acceptable management preference.

So, just to recap, in my review I found four dominant perspectives that social scientists identify in this research. First are the general perspectives that are shared by research participants, second are perceived benefits of biotechnology, third are perceived concerns, and fourth are underlying values. So, what are the policy and management implications of this work? First residents and stakeholders really need to understand the management practices and strategies that are taking place and what's the role that technology is playing here. In other words, groups that are opposed to biotechnology really need to be invited into the conversation rather than scientists fighting with them and telling them that they're wrong. It was mentioned earlier,

and that is really important. Second, explanatory models and visualizations tend to be a valuable starting point to engage stakeholders and the public. Third, labeling of transgenetic forest crops tend to be an approach that everyone seems to agree on including scientists, managers, wood products consumers, and the general public. That concludes my review, and I look forward to any questions.

Perceptions of Land Managers to the Use of Hybrid and Genetically Modified Trees

Andrea Brennan

Program Coordinator
Biology Integration Institute
Morton Arboretum

This is part of my dissertation. I'm currently at the Morton Arboretum in Lyle, Illinois, now, but it's my research. Now this presentation is perceptions of land managers on the use of hybrid and genetically modified trees. So, it definitely pairs well with the other presentations we've just had for this session. Before I really get going, I just want to acknowledge that numerous, well hundreds, of land managers in Indiana helped make this study possible. They spent a lot of time with me, helping to make sure that this was some really well-informed data that was getting at what I was trying to answer. I also want to thank my committee including Doug [Jacobs] and Zhao Ma who will be co-authors on this study. I just submitted the paper for the New Forest Special Issue. A variety of other folks including committee members and grad students and lab mates help pilot this study, which takes a lot of time, so I really appreciate that, and the van Eck Foundation supported me financially. This is not a crazy presentation, your typical academic format, except I do combine results and discussion. I think it's a little more interesting for the narrative.

Starting with a brief intro, I just pulled some stats from the IUCN, the International Union for Conservation of Nature, and in the last 200 years, 522 plant species have been classified as extinct and over 40 percent of the roughly 40,000 plant species evaluated by the IUCN are listed as threatened. These are both considered to be severe underestimates of the true situation. We can't do much about those that are extinct, but we can do a lot to try to help the ones that are going, and so hybridization and genetic modification are two potential tools. As we talked about a lot, American chestnut is a really prime example of this, but really key to this issue is learning from past biotech rollouts in other areas, like crops, and realizing that we really need to be proactive in understanding these perceptions. Learning from those experiences and trying to engage a two-way channel of communication on these issues to help inform our science as well as our messaging. So, an important demographic here is tree land managers, and so we took this more broadly than just the field of forestry and defined it as professionals and/or volunteers involved with wide-scale tree selection, sale distribution management, and/or planning of trees in Indiana. Indiana was a good case here because it has both American chestnut and butternut, two trees in which these biotechnologies are being considered as native species in the states. The land managers would have a pre-existing knowledge of these species and the biotechnology related to them. It's a very diverse group operating across the natural to urban spectrum.

I have three main research questions: One is simply, what are the perceptions of language to using hybrid and GM [genetically modified] trees? Just that baseline would be particularly helpful. What factors and characteristics were associated with these perceptions? Lastly, what factors and characteristics determine the current use of hybrid trees? Obviously given the legal barriers to the use of GM trees, that is not what we did. My methods involved using an online survey, and it was performed by preliminary interviews with nine land managers. I can't tell you how important I found these interviews to be—making sure that I actually got quality data from the research. And these land managers were so willing to talk, so willing to help

me, because they were so excited to be listened to. The study was made of 26 multiple choice and Likert-scale questions, and it was sent to land managers in 46 different organizations across Indiana including professional societies and government folks, municipalities, even landscape architects. I tried to really hit the big movers and shakers in terms of mass, like wide-scale tree planting in the state. It was open from February to April of 2019. Everybody's favorite slide for the perceptions I just focused on, descriptive stats and frequencies because we just need the baseline to start with. For the factors and characteristics associated with perceptions, look at humidity models, Pearson's chi-square tests of independence, and Fisher's exact tests if any non-assumptions being met or not met. Then logistic regression to really get at the factors and characteristics with use of hybrids.

Ultimately, 273 responses I was able to use in the study, which was really fantastic. The ages were about 23 to 78 years old, mean of about 47. Heavily a male study with nearly 80 percent male, very well-educated population with 86 [percent] having a bachelor's degree or higher. I was really pleased that we managed to get good coverage around both the northern and southern half of the state, because there's a lot more farmland in the north and more forestland in the south of the state, but as we found out that actually ended up being a significant factor, interestingly. Then the land type managed, so it's predominantly natural lands, and we asked folks whether they manage mostly natural lands, urban lands, or roughly equal both types. It's predominantly natural, about a quarter urban, and 60 percent about equal parts full.

What were land managers most concerned about with using hybrid GM trees? Here we developed a list of about seven different concerns that were brought up during these preliminary interviews, and they were either ecological or economic concerns. The ecological concerns were far greater consistently than the economic concerns. The top concern overwhelmingly was a potential for invasiveness with nearly 70 percent of folks being very concerned about this for hybrids and GM trees. The least amount of concern is for an economic concern of low availability and 61 [percent] for hybrids and 54 [percent] of dramatically modified trees just indicated they really weren't even concerned about this issue. Then we asked about what purposes did land managers mostly agree and disagree with for using hybrid trees, and we gave them this list of a bunch of different purposes. Again, gathered from these preliminary interviews, and we allowed them to rank from strongly disagree all the way to strongly agree, and they're arranged here in decreasing agreement. You'll see that at the top: the top three agreed-upon purposes for using hybrid trees, for non-timber production, like fruit and syrup, timber production, and conservation restoration of at-risk species. At the bottom it was using these trees for aesthetic value, recreation, or promoting biodiversity.

Now the same thing again, but just for genetically modified trees, and you'll notice that it's again the same three variables but they're in a slightly different order, and conservation restoration of at-risk species was actually considered the most agreed-upon purpose for using GM trees as opposed to with hybrids, it was number three. The same purposes at the bottom again, trying to look at a little bit about the characteristics that were associated with these perceptions. What land manager characteristics were associated with them, and age was a big one for hybrids. The younger the land manager, the more negatively hybrids were perceived and the older the land manager, the more positively hybrids were perceived. This is over a range of questions. So, this is the predominant trend of responses. Interestingly though, this flipped when it came to perceptions of genetically modified trees. With younger individual land managers seeing them more positively, and older individuals perceiving them more negatively. What might be going on here based on a wide variety of literature beyond just biotechnology in general but just technology more widely, is that older individuals tend to perceive the older technologies, here hybridization, more positively likely because

it's just the technology we're more familiar with versus younger individuals might be more aware of genetic modification. It doesn't seem so strange to them because it was just there from the start of their lives. Also, we have some other perceptions that not only affected perceptions but actually translated to actions, like use of hybrids. So, type of land managed was a big one, a very strong one here with those who managed more urban lands perceived hybrids more positively; those who managed more natural lands, perceived them more negatively. This trend was not present with genetic modification perceptions, but it did translate directly to actual use of hybrids. So, the urban land managers used hybrids a lot more often, and this makes a lot of sense since urban land managers are under intense pressure to plant trees that are not only tolerant to a stressful urban landscape but also to maintain a diversity of species and families so that if a single pest event comes through, like with American elm or ash, it does not wipe off an entire urban area. Further, because of these issues, hybrids are just being developed specifically for use in urban situations, and so it makes a lot of sense that you're going to see a lot more use in urban situations of hybrids.

Another characteristic that affected both perceptions and use was concerns specifically for hybrids. The greater the ecological concern, the more negatively hybrids were perceived, but the greater the economic concern the more positively hybrids were perceived, which is interesting here. So, both concerns but depending on the type, you perceive the tree differently. Again, I did not see the differences when it came to perceptions of GM trees, and it partially translated to actual use of hybrids in terms of ecological concern. So, echo having a strong ecological concern about using hybrids negatively affected the amount of hybrids the land manager used, but economic concern did not actually ultimately affect whether a land manager, at least in our models, actually used hybrids or not in their landscapes.

Before I wrap it up, I just want to say, this is a tiny part of the study. There was a lot of other things we looked at, including nativeness perceptions, that is just really interesting, so if it goes in the special issue, I urge you to take a look at that paper, see the other things we were able to look at. Some specific takeaways: so, biggest concerns here were ecological especially for invasiveness, but ecological and economic concerns had an inverse effect on perceptions, but only ecological concerns affected predicted use. The most agreed upon purposes for using both the two types, more for using them for species restoration, conservation, and production purposes. Age, type of land manager, and concern types most strongly affected perceptions and/or use. We looked at other variables: education or gender and even the type of organization that the land manager worked for, and ultimately, they only were significant, and a small handful of scenarios are just mostly not at all. Just some broader takeaways, there is general but conditional support for using hybrid and GM trees to restore at-risk species among Indiana land managers. If you looked at those purpose percentages, you can see that the amount of agreement even at the bottom, the bottom-ranked purposes, were still greater than the amount of disagreement. Then there was a whole section that I didn't cover today about specific types of tree improvement advantages to these trees. Again, it was mostly agreement rather than disagreement, but it will be critical that ecological concerns be addressed first because even though they indicated high agreement, they indicated a lot of concern about ecological issues, particularly invasiveness. So, like we've been discussing in prior sessions, active engagement communications with land managers on these issues will be critical for maximizing effectiveness of tree restoration in general. If we would want to use these biotechnologies, that two-way dialogue is going to be really essential.

Regulatory and Social Acceptability of Transgenic Chestnut

Andrew E. Newhouse

Researcher
College of Environmental Science and Forestry
State University of New York (SUNY)

On the topic of regulatory and social acceptability, I'm not a regulator and I'm not a social scientist. I'm a biologist, and so I see my role here to describe kind of our experience as biologists, or developers of a transgenic product like the chestnut, with regards to the regulatory and social processes. Bill gave a great background of the chestnut project in general earlier. I'm not going to go into a lot of detail on that, but just in case you missed that first episode, I'll do the 30-second version here. American chestnuts were nearly wiped out by an invasive blight. We have developed a transgenic chestnut with a gene from wheat called oxalate oxidase that allows the chestnut to tolerate blight infections. We've done lots of different kinds of testing focusing mostly on environmental or ecological interactions as well as efficacy. One of our primary focuses moving forward will be enhancing or increasing genetic diversity and adaptability to hopefully make restoration a reasonable possibility. As far as history of our project, and kind of some of the different aspects of communication that we've looked at, the whole idea of a transgenic chestnut tree for restoration was initiated by the public by a group that would soon become the New York Chapter of The American Chestnut Foundation. They approached Bill [William] Powell and then colleague Chuck Maynard in 1989, and so that is how the whole thing began. It's nonprofit, no patents, we're not intending to commercialize this, and so that really makes it one of the unique uses of genetic engineering—not for agriculture but for wild release and environmental restoration as most of you are familiar with. Our conversations with both regulators and other scientists have been frequent and ongoing and evolving as we learn more about the process and the product. We also have frequent interactions with the public with largely presentations and tours and things like that. We've incorporated feedback in different ways over the years, resulting from those interactions with public and presentations, and so by far the most frequent response we get is, "how soon can I get a tree, I want to plant these trees." So, that has been encouraging.

I'm going to go through kind of a quick overview of where we are in the regulatory process. Like I said, I'm not a regulator, I'm not really here to describe the whole process, but I want to give a kind of an update on where we are in the process. Hopefully you'll be hearing more about this from the experts themselves tomorrow, but currently we have several confined release sites under USDA [United States Department of Agriculture] permits. We have trees planted in various field environments, undergoing tests, and have been for several years now. The first of the three agencies that regulate our transgenic chestnuts is the USDA. We submitted a petition for non-regulated status to the USDA. That was accepted early last year. There was the first public comment period last fall. I think Sarah is going to be talking more about that tomorrow, but the USDA's assessment review of our petition is in progress. If you want to see the petition, that is available for download. The public review from the federal register, I don't have a link handy, but I can supply that if you want to send an email or whatever. Should be easy to search just "petition transgenic chestnuts," something like that.

The next of the three agencies I'll mention is the EPA [U.S. Environmental Protection Agency], who regulates pesticides. As Bill [William Powell]

mentioned earlier, there have been some interesting conversations about whether we should even be regulated due to the unique mechanism of the oxalate oxidase that isn't a typical pesticide. Ultimately it was decided that we are regulated as a what's called a PIP or a plant-incorporated protectant under the FIFRA [Federal Insecticide, Fungicide, and Rodenticide Act] law, and so we will be registered. We will be applying for a registration and tolerance exemption, which is kind of a typical path for new microbial pesticides, but we're also going to be requesting a 25B exemption from registration, which would really make this a lot more feasible for just wild release and restoration. We expect to be submitting this paperwork hopefully soon. One more note on that exemption from registration: We just found out about the proposed rule change, which I imagine we might be hearing more about tomorrow. But there is a proposal on the federal register right now that's open for public comment. They're looking at potentially streamlining this exemption process. I'll leave it at that for now.

The third of the three agencies is the FDA [U.S. Food and Drug Administration] who regulates safety of food and feed. For the transgenic chestnut specifically, that will be probably the most straightforward, definitely the shortest of the petitions, paperwork, that we'll be submitting. They're requesting the biotech consultation document as well as an allergen exemption because the gene we used came from wheat. By kind of the default, it would need a label that says it contains ingredients from wheat. Which doesn't really make sense; that's not really how genes work but there is a reasonable path by this allergen exemption, so we don't need to put wheat ingredient warning labels on chestnut trees hopefully. Since everyone asks right up front how soon can I get a transgenic chestnut tree, my best guess is 2 to 3 years. That's hard to estimate because even some of the published timelines have already been longer than the published estimates. So, that's my guess.

Moving forward in this session, potential barriers, a few different kinds of categories, and these range in relevance and perspective. So, initially kind of general concerns that people express about GMOs [genetically modified organisms], kind of as a category, might be associations with pesticides or food supplies or impacts of agriculture. A lot of these get brought up with discussions about things like corn and soy and cotton. Then associations with the biotech industry; there are these criticisms that there's just a transgenic chestnut because it's opening the door for industrial use of other transgenic trees, which is not the case. Another kind of common concern about GMOs is that you'll get these unrelated traits, other aspects of wheat along with the one gene from wheat. Again, that's just not how genes work so these are not really well-grounded criticisms. I think we've been able to address some of them, some of them persist, at least in public perception, but other types of questions [persist]—one that was brought up earlier is that as soon as we release a transgenic tree into the forest, it's just gonna take over. It's gonna be everywhere, and we'll never be able to get it back and it's going to spread rapidly, but then also people are saying, and we just talked about earlier, it's actually going to be difficult to get this to spread fast enough to implement effective restoration. So those contradictory questions are tricky. There are also more kinds of philosophical questions about who initiated the work, who benefits from the work, who gets to make decisions about how it's used, about the processes, about the decision-making processes in general, and then longer-term comparisons to alternatives like to planting hybrids. We can test some things and unreasonable time scales, but over the life of a tree there are some questions that are worth studying. And this kind of category of questions I would say are important and worth talking about but not easily solved with, you know, an experiment we could complete in the lab this year kind of thing.

Then the last group are things that we have largely addressed or are still working on, but some of the early criticisms were that people just wouldn't be interested, people wouldn't want a transgenic chestnut tree, and that's very clear that

there is demand, there's definitely interest. Another early criticism was that all regulatory review for an academic group and a nonprofit thing for restoration, it'll just be impossible, too expensive and too convoluted, it'll just never happen. Earlier I think the quote was something like that it would be possible, and I guess I would change that, too; it'll be probably possible. We're in the process, we're starting the process, and I think it's convoluted but likely to be feasible. Then there are some other kinds of more direct, more tangible questions like insertion effects of where the transgene is inserted, whether there might be effects on the host plant, whether there might be changed environmental interactions, whether there might be effects on the pathogen, and those are things that we've really tried hard to address in the petition to the USDA and with several types of experiments. Those are things that we as scientists can at least supply data to address those types of questions.

Moving to some kind of broader goals and challenges. The intention with this project is safe and effective light tolerance in an American chestnut tree, that has the traits and features that people like, that people want, and that are historically relevant and ecologically relevant for American chestnut countries. As Bill mentioned earlier, we're not necessarily trying to replace native trees. One of the interesting aspects of this hemizygous transgenic tree is that it will always have non-transgenic wild type offspring, which is not the case for some other methods. Some of the challenges, again, we've kind of addressed some of the early criticisms that this just won't be possible. Well, I think we've shown that at least the early stages of this are possible, but other challenges currently involve distribution and genetic diversity; we're working on that. That's kind of in process, but the scope of restoring a tree that was prevalent across, you know, whole regions of our continent and numbering in the billions is just hard to fathom, and really, a project that goes beyond our lifetime, but we have to start somewhere. I guess acceptability as others have mentioned, in terms of kind of social and land manager acceptability, is mixed. We've seen a lot of demand for the trees, but that's not universal. One kind of interesting question is that different types of, I guess I would classify them as stakeholders, different interested groups might be interested in different types of data. Whether it's kind of silvicultural and how this will grow in forests versus the environmental interactions with other native organisms versus what will this look like in my backyard, require different types of data to answer these different types of questions, which is the challenge. Bill already talked about some of these different options for dealing with chestnut blight, so I'm not going to go into them in detail, but I'd like to bring up here which of these options are associated with risks. Bill mentioned a few of them specifically with hybrids and with hybrid breeding. We've absolutely seen risks to chestnut trees, we've seen differences with environmental interactions with insects, for example, there would be risks or at least significant differences between American chestnut and say Chinese chestnut or hybrids, but which of these options are regulated. At this point it's only genetic engineering with the possibility of some biocontrol, depending on how that's implemented.

Moving on to a little bit of what Jason [Delborne] talked about. Some of the things that I've kind of found interesting along those lines are the underlying values surrounding a restoration project that we really didn't question initially, that I think most of us haven't questioned, are that chestnuts are good and restoration is good. Most would feel comfortable saying, most of us from kind of a Western scientific background, think that addressing a human-caused environmental problem and trying to restore damaged environments is a good thing and that's a worthwhile goal. But it was interesting for me to hear with some of the recent research that that's not necessarily a universal good, that there are people or groups of people who might not value restoration as an activity as a whole. Another interesting unspoken concern is the allegation that genetic engineering must be riskier than these alternatives because it's regulated and

so therefore, it should be tested more carefully and is held to this higher standard because it's regulated and that's not necessarily based in science.

I guess thinking a little bit beyond chestnut blight, some other kind of considerations that we're thinking about for chestnut restoration, in general, there's a whole separate disease called phytophthora root rot and it was actually introduced in North America well before the blight fungus. This is probably mid-1800s if I'm remembering correctly. So, this is something that will need to be addressed before chestnut restoration can really be successful range-wide. Basically, this is kind of an active area of research for collaboration with The American Chestnut Foundation. So, we might hear a little more about this in talks tomorrow, but I think one of the things I'll highlight is that there's really a lot of potential here for combining approaches from breeding. Specifically, with backcrossing and genetic engineering, what we're seeing right now is that oxalate oxidase seems to be the most promising avenue toward light resistance, but that backcrossing seems to be very effective against phytophthora. Probably more effective than it was against blight, so there's, I think, a lot of room to combine these efforts or to kind of use different techniques or strategies or combinations of strategies for different parts of the chestnut range or for different applications. I would encourage people to be open to different strategies. No single silver bullet will be useful for all situations in all environments.

A few next steps thinking beyond the oxalate oxidase tree that we currently have, that we're starting the regulatory process with, we call it Darling 58. Some things that we'd like to work on is inducible expression of the same gene. So, currently it's expressed by what's called a constitutive promoter. This gene is kind of in all tissues all the time and that seems to impart a bit of resistance, but it could be potentially more effective or more efficient if it were targeted or induced in the stem tissues or just around cankers where the blight has infected trees. We would also like to work on incorporating genes from Chinese chestnut. Again, that could kind of be complementary approaches along with the oxalate oxidase and could make it even more effective and durable. I think we're going to hear more about increasing diversity tomorrow from [The American] Chestnut Foundation folks Jared [Westbrook] and Sarah [Fitzsimmons]. Again, I've kind of mentioned this but it would be nice, it's an active area of interest and early research, if we could identify these genes, like the lactase that Bill [William Powell] talked about earlier, that might be present in American chestnut but [could be] more effective if they were upregulated or if we could enhance expression of the genes or move parts of genes from Chinese chestnut that might not be present in American chestnut. That might work well with some of the proposed rules from both EPA and USDA that we've seen some changes to recently.

With that, I'm wrapping up a few kind of key things that this is really a different way to think about genetic engineering, for developers but especially for regulators, for the public, for communicating science from the perspective of biologists. I would argue that the regulators and other interested groups would benefit from better engagement and better communication. Also, I put myself in that group by continually trying to be better along those same lines. Community involvement and support is absolutely essential. We've seen a lot of support recently from The [American] Chestnut Foundation and I would say this wouldn't be a realistic restoration project, wouldn't be a feasible option, if it weren't for support from the Chestnut Foundation. If we didn't have this community engagement among people who are interested in chestnut restoration, then it wouldn't be relevant. I think that's, it's, a bold statement, but I think that's so critical to get the community involvement. As most of you very well know, trees are slow, and working with federal regulatory processes and laws is slow, so this project requires patience.

ISSUES FOR THE USE OF BIOTECHNOLOGY FOR THREATENED SPECIES REINTRODUCTION: POLICY AND REGULATORY

U.S. Department of Agriculture Regulatory Perspective for the Workshop on Biotechnology Risk Assessment for Restoration of Threatened Forest Tree Species

Christina M. Vieglais

Team Lead Biotechnologist
Plants Branch
Biotechnology Regulatory Service
U.S. Department of Agriculture, Animal and Plant Health Inspection Service

The three agencies have separate authorities for implementing the biotechnology regulation for the federal government, and the USDA [United States Department of Agriculture] is concerned with modified organisms safe for agriculture and environment. We look at plant pest and weedy impacts and harm to organisms beneficial to agriculture. Amanda [Pierce] will talk about what the FDA [U.S. Food and Drug Administration] protects. For specific traits and crops, you can have one or all three agencies regulating. The first example I've put up there is the fungal-resistant chestnut that's pest resistant. So, the USDA would regulate it and do a review for agricultural environmental safety, EPA [U.S. Environmental Protection Agency] would review the plant-incorporated protectant that Andy [Andrew Newhouse] talked about yesterday for environmental food safety and feed safety of the pesticide. FDA would regulate it because people eat chestnuts and so do animals, and so there's food and feed safety and there's other examples there for other types of things. You can see that not all three agencies regulate all at the same time.

The coordinated framework was modernized because of a July 2015 White House memoranda that gave us directives to modernize them, to clarify roles and responsibilities, develop a long-term strategy to ensure that the federal regulatory system is equipped to assess risks of the future due to those changing genetic technologies that we have in different ways of genetic of modifying organisms. There was also a National Academy of Sciences presentation on future biotechnology products, which was discussed yesterday. For USDA and the rest of this, I'm talking about what we're doing. We use the Plant Protection Act authority to regulate the import, interstate movement, and environmental release of plants and other organisms developed using genetic engineering, found at 7 CFR part 340. The approach represents, it's revised, for U.S. regulation of plants based on decades of experience and advances in science and technology, and we hope that you think it establishes a clear, consistent, science-based and risk-based regulatory framework for biotechnology; and to a degree provides regulatory relief and a better focus on regulatory resources on our areas of plausible risk, instead of what we were previously regulating, which was anything that may have had a plant pest sequence in it, even though it wasn't itself inherently risky.

There's three key components, I'm going to talk about all three of these regulatory processes. So, regulatory exemptions for certain plants—we'll move into that. I guess I should talk regulatory status review for plants developed using genetic engineering and then permitting when you have to stay regulated. So, there's three exemptions for plants modified in a manner that could otherwise be achieved through conventional breeding. A developer can use one of three express exemptions to make a single targeted genetic modification. So, we're not accepting multiple targets on one thing at this time, and I think that was discussed yesterday as well. A change resulting from cellular repair of a targeted DNA break in the absence of an externally provided repair template, a targeted

single base pair substitution, and introduction of the gene known to occur in the plant's gene. The organization's rationale for exempting modifications achievable through conventional breeding is because plants developed through conventional breeding have a history of safe use, and they're not regulated to the degree that genetically engineered or modified plants are historically but, this is a change like we now are not regulating them, we're trying not to regulate them differently. Exempt plants could have theoretically been developed through conventional breeding; it would have taken a long time. It's much faster using the modern technology but it's conceivable that they could be developed through conventional breeding. Also, there's no evidence that use of genetic engineering in and of itself introduces plant pest risk. In fact, there's some really good studies which say the opposite about mutation breeding, and certainly Bill's [William Powell] example of putting half of the Chinese chestnut genome in a plant or in the chestnut compared to a single targeted gene. So, when a plant meets one of these exemptions it's not expected to pose any greater plant risk, and that is our standard. Does it cause increased plant pest risk? If it does, then it's regulated.

Additional modifications are achievable through conventional breeding, such as the ability to list modifications that plants can have and be exempt. So, to cover multiple modifications that are achievable through conventional breeding in a specific plant species, APHIS [USDA Animal and Plant Health Inspection Service] can initiate or stakeholders can request—you can request any time—the addition of a modification. We've got these three set ones, but then there's this other category where you propose what you think should be exempt and this ensures that it will remain current over time, whereas our past regulation was sort of stuck in agrobacterium-achieved genetic engineering. We exempt previously reviewed plants under our petition process and under our new regulatory status review. Once we've got a plant trait mechanism of action combination that's the same as something that's previously been evaluated and determined not to be regulated, then those evaluations are exempt under our current regs as well; there's a process for confirming exemptions. You can see this on our website, so I'm going to skip through this, but basically you have to provide a lot of data in a letter; not a lot, I mean it doesn't even have to be something that you've actually done, it could be something that you're proposing, and we will give you a response in 120 days. Here's some description of the requirements for confirmation. Basic stuff about the plant, a clear statement on which regulatory exemption you're coming under, the description of the trait, and the description of the intended or actual genetic modification to the plant. We're only doing plants at this time. Some more information requirements, methodology used or intended to be used, because again I said you don't have to have this developed ahead of time, and Steve [Strauss] mentioned that yesterday as well in his talk. Requirements focus on the information that is necessary for us to confirm that the plan is or isn't regulated, and optionally you can let us know up front the function of the modified gene or genetic element, molecular characterization, data, and DNA sequence data.

Okay, so past exemptions, we have a regulatory status review [RSR] process where the plant doesn't meet a regulatory exemption. You can seek a regulatory status review to determine whether or not it's regulated. This is somewhat similar to our old petition process, which is the process that the genetically engineered chestnut is going through right now. Through this RSR we evaluate plant pest risk based on the biological properties of the plant, the trait, or the new characteristic, and the mechanism of action [MOA]. The evaluation examines whether the trait and MOA could change any of the following factors in a way that could plausibly increase plant pest risk. So, there is the charge, does it plausibly increase plant pest risk? We look at the distribution, density, or development of the plant and its sexually compatible relatives and that has to do with the exposure side of the risk equation. Risk is exposure times consequence, so the exposure has to do with,

"would this trait change the distribution, density, or development of the plant, or any of its sexually compatible relatives," because we have to assess that it's going to be able to cross-breed them.

Secondly, the production, creation, or enhancement of a plant pest or a reservoir of a plant pest. So, we look at direct and indirect effects to plant pests' harm to non-target organisms because USDA's goal is to protect agriculture natural resources. So, we're concerned about that, and then any weedy impacts of the plant and its sexually compatible relatives. As someone who spends a lot of time trying to get rid of tree of heaven and barberry in all the forest on our little farm, I feel, as USDA [does], what we don't want to do is to release something into the environment or allow you or the public to release something that could have harm for ecosystems. It's a big issue, as we heard a lot about it yesterday.

The regulatory status review can be one or two steps depending on the plant developed using genetic engineering. We'll go through those; there's some definitions here, I'll let you look at them later in the presentation, you can find them in our new rules. We have some new definitions for new terms. Step one evaluates the characteristics of the plant relative to an appropriate comparator plant. We do a full review of the wild type plant and the species and then compare the modification to that plant. If we do not identify a plausible pathway to increase plant pest risks up front, the plant is not subject to the regulations, never was, never will be. Therefore, we don't even take it under our authority, we just say it's not subject to our regulations and we take no further action. We do that in 180 days, that's our proposal. Now, if there is a plausible pathway to plant pest risk, it still doesn't mean that it's a plant pest. What we have to do then is a full risk assessment. So, we look at exposure and consequences to determine the likelihood that it could increase plant pest risk. If we determine that it's unlikely, then that's your other way out of the system. If it is likely, then you go to the permitting process, and we can pause it depending on what we receive from you. It could take longer, so it just depends on how quickly we receive from you, the public, the information that we need. There's benefits to this process, in that we can rapidly identify in stage one whether or not it's subject to the regulations. It also focuses our staff time on the true risk things that are risky and not risky.

Now permitting is the third part. This is not changed too much; we still have permits for plants that haven't been exempt and haven't gone through a regulatory status review and found to be unlikely to pose a planned pest risk. So, we had only the changes 45 days versus 60; it used to be 60 for an importation or movement. Now it's 45, and it still is 120 days for an environmental release. The timing of this new rule, the revised rule was published May 18th, 2020, and as of now these actually need to be updated, but you know the exemptions took place on August 17th, and just recently, on April 5th, our new permitting rule took place. We're only accepting RSR requests at the time for corn, soybean, cotton, potato, tomato, and alfalfa. Later in the year we'll open it up to other crops. So, summary, I think I've already touched on those. We don't have notifications anymore, and determination of regulatory status not through the petition process, but through the RSR, our process. Summary of revised biotech regulations, so what's the benefits to this? It should better focus our regulatory resources on protecting plant health, which is our main mission. It's likely to decrease unauthorized releases and reduce costs for developers and also allow innovation to take place without having to necessarily go through our permitting process every time. It will facilitate, we think, availability of a lot of new varieties of crops and trees and plants that are not currently available, and hopefully things that are good for the environment. We're doing a lot of stakeholder outreach, you can catch some of those on our website. There's webinars, and we're going around the country talking about this—we meaning biotechnology regulatory services, thank you.

Overview of U.S. EPA Regulation of Plant-Incorporated Protectants

Amanda A. Pierce

Biologist
Biopesticides and Pollution Prevention Division
U.S. Environmental Protection Agency

Today I'm just going to give a general overview of how EPA [U.S. Environmental Protection Agency]regulates plant-incorporated protectants. Christina [Vieglais] already gave a nice overview of the coordinator framework here, so I won't spend too much more time on it. Indeed, there's the three regulatory agencies, and we use our existing laws to regulate different biotechnology products. Now we're at EPA in the office of pesticide programs; where I work we regulate the use of pesticides. One thing I just wanted to point out, as Christina mentioned, different products can be regulated by one, or if you're really lucky, all three of the regulatory agencies. There's a website that was created, I think in 2019, called the Unified Website for Biotechnology Regulation, and this allows you to submit questions to the three regulatory agencies. You can choose to submit it to just one agency, or multiple, or even all three agencies. So, this is just a nice resource, if you have general questions over who's regulating you, to be able to reach out and get an answer from the different agencies.

So, getting back to what EPA was working on: In the office of pesticide programs, we have three different types of modern biotechnology products that we regulate. There is the first one, which are genetically engineered microbial pesticides and then, as was sort of mentioned in my bio, we also have the genetically engineered mosquitoes for mosquito suppression or population suppression. Then the class that's most relevant to this group is our plant-incorporated protectants. So, our role in regulating plant-incorporated protectants [PIPs], here we've got the sort of regulatory definition for PIPs. That's what we call them for short, and that just means a pesticidal substance that is produced and used in a living plant or in the produce thereof, and the genetic material necessary for production of such a pesticidal substance. The types of products that we typically see here are things like Bt [*Bacillus thuringiensis*] corn or Bt cotton. Most commonly for PIPs, what we see is a gene encoding the synthesis of an insecticidal substance, like a protein that's been engineered into a plant. It's also relevant to note that in addition to the pesticidal trait itself, PIP also includes what we call inert ingredients that are also in the plant or the produce thereof. What an inner ingredient means in "regulatory speak" here is that it's any substance that's intended to confirm or ensure the presence of the active ingredient. The definition is important because, you know, it means if you're genetically engineering a plant and you just have other traits that you're interested in that are not pesticidal and are not related to ensuring or confirming the pesticides' presence of the pesticidal trait, then that's not a trait, you know, that EPA is necessarily regulating.

For crop plants, the types of inert ingredients that we've typically seen are ones that are used for selecting a plant line to ensure that it contains the active ingredient. So, a lot of times this will be an herbicide-tolerant trait or an antibiotic-resistance trait. Just some more history about PIPs. In general, with PIPs we've seen a reduction in conventional chemical pesticide usage. So, there appears to be, you know, environmental benefit to these sort of products, and to date we registered over 100 different PIP products. Like I said, most of these have been Bt-based cry proteins for insect control and sort of the major crop species, corn, cotton, and soy. We've also started seeing more products that are looking at using an RNAi mechanism. So, different plants that are producing dsRNA, for example, to control different insects,

and then also there's the plant disease-resistant PIPs. From these we've seen plants that have incorporated viral co-proteins, or defense proteins, or r proteins for disease resistance.

With the increase in terms of the sort of democratization of biotechnology and sequencing, as well as gene editing, we expect to see sort of a change in the PIP landscape in the near future. Both in terms of greater involvement from smaller developers but also increased product diversity, like minor crops, and of course with all of you, possibly trees. So, just getting into the statutes that we're working under with our regulatory oversight of PIPs. We work under two different statutes: there's FIFRA [Federal Insecticide, Fungicide, and Rodenticide Act] and FFDCA [Federal Food, Drug, and Cosmetic Act]. Under FIFRA, this is our pesticide statute essentially, and it's how we regulate the distribution, use, and sale of pesticides. Then we also under FIFRA re-evaluate older pesticide products. Then finally we also look at field testing and distribution of experimental pesticides. This will be something like an experimental use permit, for example. And then under FFDCA, this is where we evaluate food safety that is associated with the PIP. So, under FFDCA we establish tolerances for pesticide chemical residues that are on food and feed from that specific crop application. For conventional pesticides, for example, you know perhaps there's a certain maximum amount that's allowed to be on certain produce before it would be then considered contaminated in some way. These tolerance exemptions apply to both domestic and imported foods. Important to note: All PIPs registered to date have tolerance exemptions. So, rather than a maximum residue limit, there's just a full exemption on the tolerance side of things, and our tolerance exemptions can all be found in our regulations and online. I think this also speaks to a lot of the history of safe use that we've seen with these PIP products.

The protection goals that we have under these different statutes, under FIFRA, we can register a pesticide based on its label usage, when it will not cause unreasonable adverse effects on human health or the environment. The unreasonable adverse effect part is important because FIFRA is actually a risk–benefit statute. So, we take into account potential risks but can also take into account the benefits of using the pesticide product. Under FFDCA, this is a different standard; as I mentioned this is again for food safety and so we can establish a tolerance or tolerance exemption if the product is determined to be safe. And so, not, you know, unreasonable adverse effects, it actually just has to be safe. This means there's a reasonable certainty that no harm will result from, generally, the exposure of that PIP.

A bit more information on when you would need to come in for a PIP registration. If a PIP is going to be tested in the field, at greater than 10 acres, then an experimental use permit is required. Even before you potentially reach that stage, if you have a product that you think meets the definition of a PIP, then you're certainly encouraged to come in and talk to the team and the regulators to get some feedback on your product, and potentially some feedback on the types of data that you might want to be collecting for when you eventually do come in. If a PIP is distributed or sold for commercial use, including what we call a seed increase registration, which is likely more relevant for major crops like corn, then you would require a full pesticide registration. For both of these, if the PIP is going to be used in food or feed, then a tolerance or tolerance exemption from under FFDCA is also required to ensure that should the PIP enter the food supply in some way, that it would still be safe for people. Just to note: For small-scale field trials that are less than 10 acres, if these do not require the issuance of an EUP [experimental use permit] or a corresponding tolerance, it is required that all the plant material that potentially contains the residue of the PIP is destroyed or used for additional experimentation. This is to ensure that it does not enter the food supply.

I want to switch queues now and briefly discuss the types of data that we evaluate when we're registering a PIP product. So, to evaluate the safety of PIPs for human health in the environment, we

consider a number of different topics here, and we look at data but also other information like scientific rationale. The main topics that we evaluate are product characterization, human health, non-target organisms, environmental fate, gene flow, threatened and endangered species, and there can also be risk resistance management considerations. So, considerations on how to extend the longevity and efficacy of the PIP product. There's also a link down below—I hope I'll be able to share these as a slide deck—which links to a symposium that discussed the data requirements that we typically see for PIPs. Some more information on product characterization. What we mean by this is, we will receive information on the origin and nature of a PIP trait. As I said, typically for a lot of products they're cry proteins and so the origin of those would then of course be *Bacillus thuringiensis*. So, we would receive that information, and in terms of the nature of the PIP trade, that might be information on the mode of action. Additionally, information on the transformation system and sort of molecular characterization of the inserted DNA and additional molecular information as well, like information ensuring that the PIP has been stably transformed into the plant, and you can see that across perhaps some multiple generations in some way. Then additionally, expression level data of the PIP protein, and a lot of times people will also show that data across different plant tissues. Also of importance, if you require a tolerance exemption, there's also a residue analytical method. This is basically just a way that people can identify, or, you know, measure, that the PIP is present in the food or produce.

For the human health side of things, our review is primarily focused on looking at allergenicity and toxicity. So, for looking at allergenicity, we evaluate *in vitro* digestibility studies as well as heat stability studies, with the idea that if these proteins, for example, are rapidly digested or are heat labeled then they're unlikely to stick around enough to pose as much of a hazard for toxicity. There's acute oral talk studies, a lot of times this might include rodent studies. Then finally to inform both allergenicity and toxicity, there's also bioinformatics analysis, which is looking at amino acid sequence similarities and comparing these in online databases, like allergen online, looking at similarities between your protein and known allergens. It could also be run through something like the NCBI [National Center for Biotechnology Information] database, to see if there's similarity between your protein and toxins.

Then, getting to the environmental assessment side of things, we have a few different assessment categories. Here, the first of which is environmental exposure. So, this is looking at the environmental fate of the PIP. What is the environmental degradation? What is the pollen dispersal? Is the PIP expressed in the pollen? Where is it likely to end up in the environment? What is likely to end up exposed to the PIP based on that? The other side of the risk equation there, from exposure, is looking at the hazard. Is there any known hazard to non-target organisms? This can be determined based on toxicity assays, but also a lot of people submit rationale based on the mode of action as well. For example, we also look at the possibility of gene flow and development of invasiveness, and so from this perspective what we're looking at is whether there are wild relatives around that your plant might be sexually compatible with, and could the transfer of the PIP to one of these wild relatives result in increased invasiveness of that relative.

The last side of the thing that we're looking at here is threatened and endangered species. So, of course, what is the impact of this PIP on threatened and endangered species, and will it affect them in any way? This takes me to my last topic that I'm just going to briefly talk upon, and that's our update to our current PIP exemptions. So, we're in the proposal phase right now, so it's relatively brief. So, you know, as Christina [Vieglais] also mentioned, due to recent technological advances, gene editing and whatnot, EPA proposed last October to update our existing exemptions for certain PIPs. This is meant to accommodate PIPs that are formed when genetic materials transferred

using biotech between section compatible plans. We just want to point out that this proposed rule is intended to implement a number of policy goals that were supported across the years from multiple administrations. Since 2001, PIPs that are moved between sexually compatible plants through conventional breeding are exempt from regulation, except for the adverse effects reporting requirement, which is basically, if the unexpected happens, you would report that to EPA. An issue that we have is that with conventional breeding exemption that's currently defined in our regulations, its definition specifically excludes biotechnology. What this means is that if you have a PIP that is identical to what could have been made using conventional breeding techniques, but you've used bad technology that currently requires a registration, we proposed a rule to allow certain PIPs created using biotech to also be exempt under our regulations. In cases where those PIPs pose no greater risk than PIPs that EPA has already concluded meet the safety requirements and could have otherwise been created through conventional breeding.

So, our current proposal also includes a process for determining eligibility for exemption, and that would require that a developer submit a self-determination letter and/or if they could also request an EPA confirmation that their PIP meets the criteria for exemption. The goal of the proposal is to limit the exempt pesticidal substances to only those that are found in plants, that are sexually compatible with the recipient plant. To do this we created a genes and native alleles, and these are meant to limit the substances that are eligible to those that are found in plants that are sexually compatible with the recipient plant. The use of these phrases is also meant to specifically exclude the use of transgenes that could have been moved between sexually compatible plants through conventional breeding. So, for example, let's go back to Bt you genetically engineered. That plant is sexually compatible with a different plant doesn't mean that Bt suddenly counts as a native gene, because that Bt of course originated from a source that's not sexually compatible. So, by limiting the pesticide substance to only those that are found in plants sexually compatible with the recipient plant, we believe that this is how we can rely on the history of safe use associated with conventional breeding to ultimately conclude negligible risk of novel exposures or hazards. My last couple slides here are mainly just links. Here we've got some online resources for the proposed rule; there's a link to the document which contains the rule as well as you'll find the public comments that we received. There's also a YouTube link to a webinar that we presented last fall that goes over the rule in more detail than what I've done today. Like I said, we proposed a rule in October of last year, the public comment period is currently closed, and we are currently working through the comments we received in order to move towards finalization.

FDA Oversight of Food From Plants Developed Using Biotechnology

Patrick Cournoyer

Biotechnology Team Lead
Office of Food Additive Safety
U.S. Food and Drug Administration

I'll get started. I'm here to talk today about the FDA's [U.S. Food and Drug Administration] oversight of food from plants developed using biotechnology. The agency explained its approach to how it regulates foods derived from new plant varieties way back in 1992 in the advent of biotechnology, in a policy document that was broad in nature. It didn't only address genetically engineered plant varieties; it addressed the whole gamut of new plant varieties. What it did is that it identified potential safety concerns that could arise during both plant breeding and plant breeding using biotechnology, and what it found was that by and large the considerations are fairly similar with or without genetic engineering. Then what it also found was that genetic engineering is a bit unique relative to other types of plant breeding in that you have a very wide potential source of substances that you can introduce into food through recombinant DNA technology.

The agency already had a lot of experience evaluating new substances added to food, and what you would think of, let's say, if you're adding a new gene that imposes new proteins into a plant that makes its way into food through biotechnology. It would be similar to how the agency evaluates any new protein added to food, and that's actually why the biotech program at the FDA lives within the office of food additive safety. It's the same people; it's the same scientists; it's the same consideration when you're adding a new substance into food, whether it's through biotech or in a recipe. So, in addition to identifying the potential safety considerations from biotech implant breeding, it also pinpointed the applicable legal requirements, and it concluded that the existing legal requirements that are already out there that regulate the food supply as a whole, those legal requirements in that legal framework are adequate to encompass and capture all the concerns that were previously identified in that document that would apply to plant breeding biotech. Then what it finally did was that it proposed approaches to check all those boxes to make sure that all those safety considerations are indeed accounted for by developers before they bring a new product to market.

I mentioned that there were pre-existing food safety requirements out there that were applicable to planting and biotechnology, and so on this slide, I'll explain what they are. First of all, I've broken them down into three pillars on this slide. The first one is general safety, and this is really the overarching king legal requirement for food. The law says that food must be safe, and so some might criticize that they feel the agency doesn't do enough for biotechnology or what have you, pointing to the fact that there aren't specific regulations that require that companies view a, b, or c, specifically in the context of genetic engineering. To that I would say that the act is very general; if something is unsafe, it is illegal and this is how the food supply has been regulated for a long time. If there are foods that have toxic components, like let's say mercury and fish or any poisonous substance in a plant, usually it would be this general safety requirement that the agencies could use to take action. So, any time plant breeders alter the content of a food by potentially toggling a toxicant that's endogenously present, this provision would come into play and allow the agency to make sure that the food is safe. That provision has been there since really the beginning of the FDA's work.

Later in time, around the time when more processed foods were coming onto the scene in 1968, an additional legal provision was added that applied to added substances. These are basically food ingredients, and back then it became clear that there ought to be some special oversight for food additives, and that required pre-market review and approval by the agency before they are put into food. That shifted some of the burden on insurance safety from a kind of post-market surveillance and pointed action by the agency to obligations for a food manufacturer very early on. That's what the added substances provisions deal with, and if a substance is introduced into a plant through genetic engineering, let's say a new protein is added, then it really falls into this added substances paradigm. These food additives require pre-market review and approval by the agency, but there is an important exemption to this. One of them is pesticides and that has to do with the EPA's authority over pesticides, but the big one that applies to the FDA's oversight is an exemption for substances whose use is generally recognized as safe. The universe of substances added to food is large, and depending on how narrowly you define an added substance, it could be almost infinite. This exemption is really important, because it recognized that for a lot of the things that are added to food, there would be scientific consensus that it would be safe; therefore, it wouldn't be a good use of the agency's resources to have to review each and every thing that has ever been added to food. This recognized that a general safe exemption applies in a lot of cases, especially with genetic engineering, since a lot of the things that are introduced into foods through genetic engineering are things that are already infused. So, if you were to add a simple enzyme that confers herbicide tolerance, it's usually coming from biology, it's usually part of the diet, and proteins generally are fairly benign substances. They're usually just digested and used for nutrients with a few rare exceptions that I'll talk about a little bit later.

Finally, the third provision of the act that is applicable to biotech is labeling. Biotech can be used to change food, to change food ingredients in many different ways. The act says that foods must be labeled in a way that is truthful and not misleading, and they must use names that are a common or usual name for food. Sometimes biotech can be used to make foods from plants different from anything they were before, which raises questions about what you are going to call it. For instance, soybean oil has been altered so that it's high oleic soybean oil, which has different food properties in several different ways, and so just calling it soybean oil isn't really the right name. Oftentimes in biotech, we have to address these types of questions, and then there's another labeling issue about the labeling of substances from allergenic sources and those allergenic sources have to be declared. I'll get to that in a future slide. On the subject of labeling, I wanted to point out that when you talk about biotech and you talk about labeling, most people think about GMO [genetically modified organism] labeling or non-GMO labeling. There is the bio-engineered food disclosure standard of 2016 that has to do with GMO labeling, for lack of a better term, and that is the responsibility of USDA's agricultural marketing service. So, this is a labeling claim that relates to marketing and not safety, and it's not under FDA's person, so that's not what I do when I say the labeling under the Food, Drug and Cosmetic Act.

I mentioned these three pillars and that addressing these main legal issues is what the goal of the consultation with FDA is. The 1992 policy that I referred to at the beginning talks about these legal provisions and safety considerations, and what it recommends is that developers come to us and talk to us and go through a process to resolve all these questions before marketing. The goal really is a compliance audit that you can go through with the product that you intend to put out there just to make sure that indeed it is safe and that all of these legal issues are addressed before we're going to market. Companies can consult with us to ensure compliance. It is a voluntary process. This consultation program is not a market approval. It's not something that you must go through; however,

it checks for compliance with safety standards and legal requirements that are mandatory. So, it's a voluntary process, but what it does is it ensures compliance [with the] mandatory aspect of the law, and this has really become an established practice. Developers have routinely participated in this. We've now evaluated over the past 25 years well over 150 genetically engineered families, so you know this approach that we take has worked for us well. However, we'll be the first to admit that forest trees raise different issues than you would normally get when you interact with developers of agricultural crops, and so it's not typical for us to deal with developers of native trees, but some do have food uses. Of course, the American chestnut is a great example that has come up a lot during these past 2 days. Sugar maple is consumed for their sap, or another example is walnut, and there are several others, particularly various types of nuts.

One of the real, biggest concerns is introducing new things into food, like protein, and as I mentioned before, new proteins infused into food are generally benign things. However, there are a few specific potential hazards and addressing them is a prudent thing to do before putting any new variety out there. Although these may not be developed with the intent of them entering the food supply, it could be a prudent thing to ensure that these issues are addressed in order to pave the way to a successful rollout. These hazards I mentioned are allergenicity, toxicity, and then whether the biological activity of that protein raises any other question. There's a weight of the evidence approach that is taken that includes a lot of different questions that help build a case for these things not being allergenic or toxic and raising any other issues. One, is this protein already safely consumed in effect? If yes, then that's strong evidence towards species. Another element is, what is a source organism and is it known to be allergenic. If it is, can it be demonstrated that it's not the determinant of that allergenicity?

A big part of it is bioinformatic comparison of sequences to see if this protein is similar to any known allergies or toxins, and whether it's in another aspect of producing that protein, and seeing if it is readily degraded by digestive enzymes, because this has been shown to be a feature that's correlated with proteins being well tolerated and not allergenic. Then finally, what about a technological function, does that raise any safety concern? Viewed singly, any of these criteria is a real slam dunk in terms of safety, but when you put them all together, it creates the weight of the evidence that can be used to support faith. It is of a lot of importance to point out the Food Allergen Labeling Consumer Protection Act [FALCPA], and what the act does, is it requires that levels of food containing an ingredient or protein from a major allergen declare the presence of that allergy. The law defines major food allergies, and we call them "the big eight." One was recently added to this list from sesame, or at least there's a proposal to do so, and so the fact is with the transgenic chestnut that the source organism was leaked. Protein from wheat was added to it, and that does raise this output issue. However, they requested a food ingredient covered by FALCPA be exempt from false labeling requirements, and from what we heard yesterday from the transgenic chestnut developers, that's something they're planning on engaging with the FDA to do, if it can be shown that this protein is not the allergenic determinant.

Then finally another element of what we would look at in a consultation is a comparative compositional assessment to show that the new type of food is comparable to previous versions. This involves identifying what might be the key components of that food, like toxicants, fancy nutrients, or nutrients, and then grow it next to the progenitor variety and show that the compositional value, the nutritional value, is comparable. Data on other varieties can be used to put things into context and to show whether there's a lot of variability for various components.

ISSUES FOR THE USE OF BIOTECHNOLOGY FOR THREATENED SPECIES REINTRODUCTION: PUBLIC PERCEPTION AND SOCIETY

The Nature Conservancy's Perspectives on Biotechnology and Its Potential to Protect Forest Trees

Leigh Greenwood

Forest Health Program Director
The Nature Conservancy

The Nature Conservancy's Forest Health program, which I'm the director of, follows the mission of The Nature Conservancy, which is to protect the lands and waters on which all life depends. Forest Health specifically focuses on invasive pests and pathogens of trees and forests. So, forest health in this definition does not include forest fire. Sometimes people get confused, just wanted to clarify. Let's start on firewood. Today we're here to talk on the other side of the spectrum. When we talk about firewood and forest pests we're often really clear on the fact that this is a very obvious part of the management perspective, but when we get all the way to biotechnology, host-on-host trees, or on the pests themselves, people get a little bit into a different headspace. The Nature Conservancy starts thinking about things in a perspective that includes a lot more of not can we do these things technologically, but should we do them at all and do we have the license to do them at all. That's a different place, so I just wanted to kind of shout that out, that's part of what The Nature Conservancy thinks about frequently when it comes to biotechnology. Furthermore, when we talk about can and should, the real question is how do you know the difference? What is the threshold? What's the boundary there between what you can do and what you should do, and what you will put time, money, capacity, social license towards in terms of the management through biotechnology means?

I'm in a little bit of a bind with this presentation because I have good news. The Nature Conservancy recently heard of the acceptance of a major paper that was written by a multi-NGO group regarding the principles governing biotechnology use and environments. So, I can't really show you all of the major headings of the paper or exactly detail exactly what it says because obviously when you have a paper in press that's a little bit of an issue. What I decided to do instead is just do a lot of talking and a lot of really nice photos because that makes it easier. We're going to start with some really nice photos here, so generally speaking The Nature Conservancy views genetic modification and all biotechnology tools as tools themselves. They are not considered explicitly under a threat framework. They are not considered unacceptable risks, they are considered tools in the toolbox of how you might protect agriculture, human health, or ecological- or biodiversity-based goals, which is what The Nature Conservancy focuses on. When we look at them as tools, we see whether or not you should use the tool and whether or not the tool is the right tool for the job. This was really the question, and in the past many decades, The Nature Conservancy has had a bit of a reputation. Rightfully so, for having some sort of theoretically conservative viewpoints on things, like, for instance, biological control organisms in the past. As well as pesticide use in the past, but like any organization we do progress in time along with science, public opinion changes, and threats, etc. So, in the same way we are now viewing biotechnology in a viewpoint of the science is improving and changing, and these tools are becoming more important to assess on the sort of, can we, should we, what is the benefit, what is the risk, how do we mitigate, kind of systematic framework.

In order to actually come up with what the organization as a whole feels like is the right answer from the scientific perspective and the work that's currently in motion, is between two

different groups within The Nature Conservancy's departmental structure. One is the protect food and water global group, which is a name that you can kind of think about as being basically our international agricultural support group, if you want to like rephrase that into your own language. Then also our invasive species advisory council. Now those two bodies could not be more separate, because our global protect food and water group is very high up in the executive structure, and the invasive species advisory group is a community of practice. So, they basically are totally different, but the thing that unites them is that both of them have a really high interest in The Nature Conservancy's guidance and perspectives on how biotechnology can be used to achieve the overarching goals of their groups. So, in that we are working together between the two different structures in order to make sure that biotechnology, like gene editing, any type of gene modification, is researched, used, supported or not supported in a way that aligns with our values and with the tools that are available to us. The invasive species advisory committee, I serve on the leadership group of that, and it is like I said, it's a community of practice. It spans all the different parts of The Nature Conservancy's taxa base. It goes all the way from zebra mussel potential gene drives to daughterless carp to mosquito-based interventions for both human health and, in mosquito's case, bird health.

I worked with a lot of folks on chestnut blight as an issue, through my leadership of the Forest Health, so we have a really wide scope of practice, but we do touch down on forest health frequently. I picked this particular slide because I went to Hawaii a few years ago as part of looking into Rapid ʻŌhiʻa Death and while I was there, I found out that actually one of the most interesting historical uses of biotechnology was on papaya. I thought it was a really good picture for us to focus our thoughts on the many different dimensions of the use of these tools. The paper itself that I alluded to has been accepted into Nature Biotechnology Journal. Hopefully it will be published within the next few months, and the working title is "Principles for governance: gene editing applications in agriculture and the environment." It has been co-signed by various authors from multiple NGOs in both the environmental sector, like The Nature Conservancy, as well as a food and agriculture sector presence. I'm going to kind of summarize the basic principles.

The basic principles are that gene editing technology needs to be applied in a safe and ethically responsible fashion. Societal engagement is absolutely critical at every single level in order to ensure these technologies are accepted and respected. Government relation regulations are absolutely critical to those two different points, as well as because without a science-based governmental approval process, you won't be able to achieve the other two ends. At the same time, voluntary stewardship and societal integration during and after the approval process is going to be key. We see this a lot when I start to talk about chestnut, people immediately think about, like "okay, well, when it's on the landscape, then what will we do." That's one of the strengths of that sort of volunteer organization engagement that we're seeing. I think you need to see that for absolutely every single forest tree proposal that we're going to see put forth in the next few decades. Another one that I thought was really good, is that the public should have access to clear information. Now remember this paper is written partially from an agriculture food consumer basis perspective. So, that clear information is not just about the environmental realities, impacts, benefits, and potential negative impacts but also for the consumer's ability to exert their own sort of free will and preference on what they are doing, what they are eating, what they are buying, what they are supporting.

Then, last but not least, the inclusive access to gene editing technology for groups. So, for instance, to not have the governmental regulatory burden be so tremendous that it excludes smaller groups or smaller-scale problems from participating in this system. I think that's a really interesting one to consider because it also touches into the

aspect of patents. Whether or not these things should be patented is an interesting ethical question when they look at environmental impacts, and I think that's one that I will leave to the philosophy majors. I'm much more of a scientist, but I think it's a really important element. So, those are the basic principles that are going to be represented in this paper that should be published within the next few months. The Nature Conservancy led but was not by any means the leader of the effort to publish this paper. It was the result of the effort of many different folks interested in the sector across both environmental and agriculture consumer-based products.

One of the things that I wanted to impress upon the group is that we've been looking a lot at what we can do to address threats to trees now, and issues that we know about now, and how we can improve regulations coming up soon or improve societal acceptance coming up soon. One of the things we need to look at is the future. We know that international trade is what almost always brings in invasive species threats to forest trees, specifically solid wood packaging in the nursery industry as primary accidental vectors of these threats. When we look into the future, we're going to be tackling this problem of how to protect host trees—whether the trees themselves, whether by altering the trees themselves, or whether by altering the pests in some way through biotechnology at threats that we haven't even imagined yet, attacks that we didn't even realize are going to be threatened, yet these things will happen in the future. The work that we're doing right now needs to prepare for this theoretical future where we're not entirely certain what it is we will be facing. I want to really kind of highlight that we're all looking at this now but it's going to affect our environment, our personal, and our professional lives for decades to come in ways that we may not be able to predict, and that's an important concept to think about. The legacy of this work is going to be important past our own professional lives. Just wanted to say thank you to all the partners that I work with and special thanks to USDA APHIS [United States Department of Agriculture Animal and Plant Health Inspection Service] and the U.S. Forest Service. They are the primary funders of this work in this space.

Resolving Uncertainties About the Use of Biotech for American Chestnut Restoration

Jared Westbrook

Director of Science
The American Chestnut Foundation

Sara Fitzsimmons

Director of Restoration
The American Chestnut Foundation

[Jared begins] I'm going to talk today, just five slides, just mainly about what I'm interested in. For this transgenic American chestnut, it's efficacy. I think the safety has been demonstrated, and for us, we're thinking about this in terms of probably not hundreds of years but maybe thousands of years, if you talk about the whole hundreds of millions of acres of the range. We have to be thinking about this in terms of almost evolutionary time, and so what is the efficacy of this resistance over a long period of time.

Criteria for success—not even thinking about what the methods are, but what we're actually looking for in restoring the American chestnut—is that the trees have to survive indefinitely with chestnut blight. They have to grow as tall as the American chestnut. The Chinese chestnuts tend to grow shorter than American chestnuts, so one of the trade-offs with the breeding is that we're making the trees maybe grow less tall, so they may not be competitive in the Eastern forests. The trees have to function ecologically like American chestnut. They have to have similar kinds of insect pollinators, mycorrhizal fungi associations, things like that. We have to adequately represent the diversity remaining in the species, so that it can continue to evolve on its own and adapt to climate change. We also have to worry about the second pathogen, **Cryphonectria parasitica**, besides chestnut blight, *Phytophthora cinnamomi*, which infects the roots of the trees. Those are the main things we're kind of focusing on in our breeding program with TACF [The American Chestnut Foundation], and particularly for the transgenic trees. I think dozens of trees thus far have been evaluated for resistance over a relatively short time frame. Going forward, having the trees deregulated will actually help us to look at these trees' long-term resistance in natural settings. There's been a number of trials done so far, planting the trees with drier sites, intermediate sites, mesic sites, and looking at the resistance and competitive ability of our backcrossed trees. I'd really love to see some of these forest trials established with transgenic trees with our partners with the U.S. Forest Service, for example. We want to be able to look at whether under the stress of flowering and energy resources, does that affect the resistance of the trees. We see this often with the backcrossed trees, that when they're really stressed out, they'll flower to make a bunch of seeds to reproduce before they die. Or when they flower, they tend to get more blight, so there's a huge environmental component to this, and we need to measure this out in a lot of sites when we breed this gene into different trees' backgrounds. The genetic background can have an effect on the expression of that gene and then, therefore, the resistance potentially. We want to capture a diverse population of trees in our breeding program. How much is that expression going to vary, and are we able to systematically capture a lot of that diversity or is it going to vary so much that we will see some trees that we breed with that will not be able to be represented? I don't know. These are the things that I love. We're

establishing some common-garden experiments, we're looking at wild type trees that we bred from Virginia, Maine, New York, and we're planting these in common gardens in those locations and seeing how local adaptation and the trees' genetic background interact with resistance. I've done a lot of work with looking at the backcross program and looking at the genetics of resistance. It looks like it's complex, meaning there's genes on all chromosomes that are affecting the resistance.

One of the mechanisms is, does the tree form a barrier to infection that's grown when they get the fungus, there's this formation of a lignified barrier to that infection. So, do we need to be incorporating these other mechanisms of resistance into the oxalate oxidase? In other words, should we be breeding the Darling 58 trees with our backcross trees to potentially pair these types of resistance mechanisms, or should we be considering other types of genes to be putting in alongside with oxalate oxidase? Other kinds of gene editing to stack different kinds of resistance mechanisms? It takes a while to evaluate the resistance of the tree, so we're doing some of these crosses now, but we probably won't have the answer to these questions satisfactorily for like a decade. So, it takes a long time to really answer these questions, but we are breeding the backcross trees and Darling 58 trees to answer that question. Should we worry about bringing [other genes] in if we're doing breeding combined with crossing with Darling 58? Bringing in some of these Chinese chestnut genes along with the oxalate oxidase gene, what is the potential trade-off of bringing in these additional genes that maybe have nothing to do with resistance? So, things like climate adaptability when the trees flower, ecological associations, all of that is influenced by these other genes that have non-target effects. We might get more resistance, but we might also have a trade-off. If we cross a Darling 58 tree with a backcross tree that inherited 80 percent or 90 of its genome, those progeny from those trees basically function similarly enough to American chestnut to have, like, an ecological parody. We're going to compare the growth rates and ecological associations of these trees in the field, and then what will it take to generate trees that have resistance to both chestnut blight and phytophthora root rot? I think it's going to take a couple generations of breeding. I mean, when we have identified some phytophthora-resistant backcross trees in our program but when we breed them with Darling 58 they'll be heterozygous for the phytophthora resistance genes so they'll be intermediate in resistance. Then we'll have to breed inner cross and select individuals that are homozygous for potentially both oxalate oxidase and phytophthora. So, we're talking a long-term investment. All of this takes a while to assess and to do the breeding so there will be time. If there's any unintended consequences, the benefit of tree breeding is it's slow and we can learn through a decade or more of effort. I'm not worried about these trees escaping immediately after they're regulated and, you know, becoming like super prominent in the landscape. I think it's gonna be a slow process, so with that, I'll leave it to Sara [Fitzsimmons].

[Sarah Fitzsimmons begins] Thanks, Jared [Westbrook]. I'm going to switch gears a little bit and focus more on the combined efforts between TACF and ESF [State University of New York (SUNY) College of Environmental Science and Forestry] toward the public comment period. TACF stands behind the three-bur method that Bill [William Powell] talked about yesterday. Where we want to evaluate the application of pretty much any means, safe means, that we can to restore the species. One of which, of course, is the OxO and Darling 58. In August, TACF paired with ESF and several other people within a committee to help promote the public comment period that the USDA ran on the petition to deregulate blight-tolerant American chestnut, Darling 58. Many of you on this meeting made a comment; thank you for doing that. So, the process was, we did a lot of preparation ahead of time. The petition was submitted by ESF by Bill and Andy [Andrew Newhouse] and a lot of other players in January of 2020. At that time, we put together a committee of several representatives of both of those organizations.

We hired a public relations firm to help us with crafting some language that we could use for our outreach. We had some legal representation, just to make sure we were staying on the straight and narrow. We created a database of personal contacts so that we would be prepared once the petition went to public comment; so that we were prepared to reach out to folks individually, and also prepared messages to all of our contacts between ESF and TACF. That public comment period opened on August 20th and concluded on October 19th. It was a 60-day public comment period.

Our strategy was, again, we had the lists of all of the supporters of ESF and TACF. I can't speak a lot about ESF's list, I don't know exactly how many people were on ESF. As far as TACF goes we have a constant contact database of a little over 12,000 people. You can see in the lower right-hand corner, these are the blasts that we sent of various public comment period related email messages that were sent out. We have a great open rate on those, you can see upwards of 40 percent of the people, of our contacts, actually open the emails that we sent through constant contact, and the click rate was pretty good, too. We saw upwards of 25 percent even of people clicking on the links for the public comment period. We can't do one-to-one of who clicked on those and who commented, but we think we had really great outreach just through mass outreach to TACF people. Even though this wasn't a popularity contest, I think you know, many of you guys are aware of that from the regulatory standpoint, it's not how many people are against. Really the USDA is trying to evaluate substantive comments, but from a TACF and engagement strategy, this was phenomenal because this is something where you can say "you can help restore the American chestnut, all you have to do is click on this link," and you know, "here's some strategies that you can follow in order to make either a supportive or substantive comment during this period." We got amazing feedback from people who participated in this project. So, apart from actually participating in the public comment period, this was a great engagement tool that both ESF and TACF were able to utilize. Besides the public outreach, just as many contacts as we could. Like I said, we had individualized messages to organizations for whom we had direct contacts, and especially in the academic community, that was where we were hoping to gain, again what those are called, "substantive comments." People who would give primarily supportive scientific arguments toward this, the safety of Darling 58.

Here are the overall results, don't pay attention to the specific numbers. A few comments are not included in this table, but we had over 4000 comments. Coming to the public comment, 63 percent were positive. Really interesting how this broke out. Obvious TACF and ESF organizational contacts were overwhelmingly positive. The public was really split 50/50. We can't say who were TACF supporters and maybe ESF supporters, we weren't able to pinpoint where those public folks came from, but it was pretty much 50/50 on that public outreach. From that public, those critical comments that were on the negative side, 50 [percent], over 50 [percent], of those were form letters. So, they were handed a form letter by an organization to which they belong; they cut, copied, and pasted it into the public comment period. From the positive, supportive side of the comments, less than one percent were form letters. Part of our strategy was to inform our stakeholders that we wanted them to give very unique substantive comments as much as possible. Academic response was excellent, we had 90, over 90, individuals identifying from over 70 universities and over a hundred organizational comments. The Nature Conservancy, even the Sierra Club submitted a positive comment, SAF, The Society of American Foresters, we had lots of really great organizational support. You can see that direct outreach to people who were directly affiliated and had personal connections with TACF, with ESF, were overwhelmingly supportive and that makes sense.

What can we learn from these comments? We didn't really see any sort of temporal trend. A lot of people submitted right at the end, especially

from the negative side of things, but we weren't really able to match up any sort of bump in terms of weekends or days of the week, or any of the outreach that we did specifically, but still interesting to look at. For that last-minute bump, there at the end geographical trend, that's probably one of the more interesting places. The critical comments were largely outside the TACF or ESF network and came largely outside the natural range of American chestnut. Over 33 percent of the negative comments were from five states alone, all of which are outside of the native range: California, Washington, Oregon, Texas, Florida. That's where a great majority of the critical comments came from. Comments from Canada were overwhelmingly negative, which was a little surprising. Then at, I think, 11:59 there was one comment that came in right at the last minute from a German organization called Rainforest Rescue. They submitted one comment with about 120,000 signatures critical of the regulation. To the positive comments, 94 of direct affiliates with the organizations were overwhelmingly positive and 72 percent of people commenting from states within the range submitted positive and supportive comments. So, obviously there's a direct connection of people who have interest or a stake in the American chestnut, and it's restoration versus those who are a little bit more separated from it both geographically and culturally.

Just to kind of dive into analogous public comment periods, so, I love the APHIS [United States Department of Agriculture Animal and Plant Health Inspection Service] website in that you can really dive in and see these are public comments. You can really see what the breadth of things that have been submitted from 1992 until present. So, I just categorized all of the different crops that have been regulated or submitted petitions. The overwhelming majority of them are soybean and corn, which makes sense. You have five different tree species that have been looked at; the only tree species that has not been deregulated is that freeze-tolerant eucalyptus [and another tree species that was deregulated,] apple. It's interesting if you go in and you click on these public comments and just randomly select apple. You see in the general public, "I want to eat this apple," "can't wait to taste it," "fantastic, I want to eat it." Eucalyptus, you randomly click, you see many of them, "this is going to destroy the environment," "please don't do this," "it's terrible." For both of them generally the public's criticism was higher than the public support, but you see way more support for apple, something people can eat and utilize versus something like eucalyptus where they have, again, that disconnect with a species. They don't feel as though they can really have any connection to, and again you see these comments, these last-minute submissions of small comments with lots and lots of signatures. Apple was deregulated, eucalyptus is still stalled in an EIS.

As Doug [Douglass Jacobs] mentioned, I've been working with the foundation since 2003 and not as long as some people. The organization's been around since 1980, but I will say that biotech has absolutely gained support over time. I've been giving talks to the public, I probably give a talk a week, at least two talks a month, and when I first started people would always say, "why don't you just use genetic engineering and solve this problem, why are you wasting your time with breeding," and I say, "well, we're working on it." Then you would inevitably have someone in the crowd, "ah, that's gonna destroy the environment," and now I don't hear that. I don't hear the criticism against biotech that I used to when I first started. I see a general shift toward acceptance of biotech as a strategy, particularly for forest health, and I think you see that too with something like the Sierra Club. As an organization, they had vehement opposition in a policy statement where they will never support a GMO [genetically modified organism] tree, to now with chestnut, I'll call it lukewarm non-opposition. In fact, the article that came out earlier this year, where, you know, they say, "we're not gonna support it but we're also not gonna not support it." So, I think that you can sort of see this trend. I see that in the public, too, when I talk to folks. They really want this for forest

health, they absolutely still are absolutely against commercial applications, so if they see corporate greed in this, whatsoever they want no part of it, but forest health, okay maybe. "Are you gonna make any money out of it? Well, then, I don't want any part of it, but if it's for forest health, okay I'm onboard," is the general trend that I see.

Jared [Westbrook] put this out there, we have a lot of questions about the efficacy of OxO. It looks good, it looks promising, we still need to test it. There's no doubt about that, this is a long-term, long road to go, and OxO is just the beginning. One part of the strategy, there are other events out there, other techniques. You know, CRISPR is sometime in the future that we can implement. Regardless of that, we need to be able to have people embrace these technologies for chestnut, for other species, there are lots and lots of other threats that are out there. Lee made that point really well, so we need to be able to apply these other biotech strategies to chestnut, to the pests themselves. We've got biotech strategies applied to the chestnut, maybe we can apply them to other pests and diseases. So, hopefully something like the outreach that TACF can do and these other organizations, like TNC [The Nature Conservancy], can again continue to increase public acceptance of this technology over time.

Biotechnology Is Not a Shortcut—It Must Be Part of a Comprehensive Program

Faith T. Campbell

President
Center for Invasive Species Prevention

In the view of the Center for Invasive Species Prevention, we think there's a real crisis of non-native insects and pathogens attacking our trees. At least 200 tree species in the lower 48 have been found to be under attack by one or more non-native pests, and one study said at least 40 are under immediate threat. There are additional tree species in Hawaii and Guam that are also under threat and probably in the Caribbean. One estimate found that 41 percent of the forest biomass in the lower 48 is threatened by just the top 15 of these pests. The U.S. lacks a comprehensive program to deal with this, we lack a well-funded, long-term foundation for acquiring the knowledge we need in a range of fields. We lack a system to evaluate the most promising strategies for each species, and the current system for testing the safety of biologically based organisms has many holes. I'm going to look at each of these.

We need a well-funded, long-term foundation for breeding programs that would promote understanding of complex triangles to host, the pest, and the environment. Each of these areas needs time and a variety of academic disciplines. To make progress, we need to assure continuity among the institutions and the personnel and training of the next generation of practitioners. We need to develop, test, and apply tools, and we have to have a geographically dispersed land base within the historic ranges of the various tree species on which to plant and maintain them. Sandy told us yesterday how hard that's going to be. So, these shortcomings are well known but they haven't been resolved. There's some additional factors that I've learned from Jeanne Romero-Severson, and I wish she had been on yesterday, but anyway. The environmental factors in the triangle cannot be learned through genetic studies, and the forestry-related programs in academia generally are too focused on quick answers, tools technology, and techniques, rather than understanding whole organisms and ecosystem relationships. We don't have a system to evaluate the most promising strategies for each species, which should be started early in the invasion process, because it takes time. We need to look at the probability of breeding success and the probability of pests overcoming the breed resistance. In my mind, there are a bunch of questions, some of which we've been discussing these last 2 days. Is genetic engineering more or less likely to speed resistance breeding? Will genetic engineering develop resistance? Will that be more persistent unwanted impacts more or less likely using traditional GE [genetic engineering] breeding, and if there are multiple threats to the tree host, such as six threats to chestnut and five threats to beech, will GE facilitate addressing these threats or at least the most important?

In my view, genetic engineering might speed up some steps in the breeding process, but it doesn't affect other steps. Even under the current inadequate procedures, the lengthy approval process is very long; public concerns might hamper application planting out of the trees. On the other hand, GE is considered to be more exciting and new, so it might be more successful raising funds. Let's not forget the values of traditional breeding, utilizing the resistance that most tree species apparently have to some degree. We have new techniques to develop interesting chemistries in the tree that Anna [Conrad] spoke about yesterday. We had the challenge of reaching flowering time, but Scott [Merkle] talked about how that can be compressed. In all cases I think we have to allow the seedlings to mature to ensure that resistance is lasting, and breeding alone cannot substitute for simultaneous

application of appropriate pest mitigation strategies. I think breeding makes little sense in the absence of a strategy for planting out and maintaining the resistant trees. Looking at the current system for testing safety, Jason [Westbrook] mentioned the National Academy of Sciences study in 2016. I came away from that process focused on two conclusions: proof of concept lab studies are not sufficient to support a decision to release gene-derived, modified organisms, and the existing risk analysis protocols including NEPA [National Environmental Policy Act] are not adequate. We have a new player on the block, the Revive and Restore [organization] developed an intended consequences statement. This is the consensus of 57 conservationists and scientists. They agreed that *inter alia*: We need new risk assessment tools; we need to consider the risks of an action as well as a proposed action; we need transparency about the social and cultural values that we're seeking to attain and engaging stakeholders; and we need monitoring of results to design successful interventions, manage uncertainty, and codify the lessons learned. I'm going to focus on the monitoring program. I find the most troubling aspect of this is the absence of required monitoring of the organism once it's been introduced into the environment. That applies to the traditionally bred ones as well as the genetically engineered ones. It's going to take decades, as we've just been discussing, to determine how these different trees will interact with the environment. We need someone watching that process, but who's going to pay for that, how will we address possible conflicts of interest? Who will review any kind of assessments of how well these trees are doing in any incidents? Something I left off the slide but should not have, is who is responsible for responding if an undesired effect is detected? We don't have an answer to any of these questions.

We've discussed the chestnut innumerable times, and the Center for Invasive Species Prevention is one of those 4200 groups that endorsed it, but I am concerned about the voluntary nature of any monitoring that will occur over the next decades. We don't know whether there might be some wrinkles that haven't been anticipated. Other than the monitoring aspects, what I'm most concerned about is the totally inadequate funding and infrastructure for breeding of resistant trees; it's getting worse and worse. The Forest Service's Forest Health Protection Program and the Invasive Species funding under the research program have been cut more than 50 percent over the last 10 years. I keep hoping to turn it around, but I haven't yet, and the results of all this are that all these activities are sadly missing and difficult to implement. How are we going to solve this problem? We need funding for biocontrol and genetic manipulation of the pests for enhancement of host resistance mechanisms in the trees, other strategies, dissemination of tools, and information from research. There are a lot of entities that might participate if they could find funding, but finding funding is extremely difficult. Let's not forget that planting of trees is going to be difficult, it's going to be expensive. Again, many of the similar institutions would be involved but it's probably different people with different skills that will be engaged. The Center is on record in saying that projects need to integrate the components that you see listed here on the screen.

There is a partial effort underway, a bill in Congress, H.R. 1389, introduced by Peter Welch, a representative from Vermont. It would fund research into various strategies for restoration and application and planting of trees in the forest. Unfortunately, this bill would do so through grants, which is not the long-term funding that I'm seeking but it's at least a step forward in the right direction. The bill would also mandate a study to identify actions to overcome the current low priority that I at least perceive in the USDA agencies that are responsible for addressing pest cause mortality; identify those agencies' expertise and resources, improve coordination among them and with partners, and develop national strategies for saving tree species. So, I hope you'll take a look at H.R.1389, consider supporting it, consider asking the scientific and other organizations that you're part of to support it, and contact your representative and senators in support of this bill.

Now with more than 200 species under attack, we need to set priorities. I'm suggesting here a couple of criteria that might be used. One would be the threat level to the individual species, and is it from one pest or more. Should there be a higher priority for monotypic genera? Should there be a higher priority for species that are keystones and unique ecosystems? Then there's some practical criteria. What projects appear to be most promising and might provide prompt successes that we can then point to? Which projects are partners willing to fund? I think genetic engineering might have an advantage there. This is one effort to set priorities, the CAPTURE [Conservation Assessment and Prioritization of Forest Trees Under Risk of Extirpation] project. It was carried out by several Forest Service scientists. They have a couple of publications out, both in 2019, I think. They concluded after a pretty thorough review in 48 states that these 15 species are at greatest risk and should have the highest priority. Most of those are probably pretty familiar to most of us. However, I see no evidence that any priority setting system is driving funding, and from what Sandy [Andrew Liebhold] said or some people said yesterday, I think the U.S. Forest Service might have decided at the chiefs' level to kind of abandon breeding 10 years ago. Certainly, there are individual scientists, most of whom are in on this call, who are taking action, doing really good work advocating, forming consortia, to try to build support for this approach. I consider you all heroes, but this is far from having a national strategy on the topic.