

Yorchidding!

Morel Dilemma Episode 10 Script. Written and copyright Elizabeth S Gall 2017.

[Voicemail beep]

Leorah: Hi, Izzie, it's Leorah. So when I was an intern at the Smithsonian Environmental Research Center last fall, I discovered the North American Orchid Conservation Center. And I think that it would be really cool if you did a podcast about orchids and fungi. They have symbiotic fungi in their roots, [...] and orchids are pretty awesome! So if you think they are awesome too, I think you should do a podcast on it. Okay, bye!

[Music begins]

Izzie: Welcome to Morel Dilemma, an exploration of why some fungi are so highly sought, some are so heavily cultivated, and some are so very dangerous. I'm your host, Izzie Gall. Valentine's Day has come and gone once more, and whether or not your local florist has a sweetheart, they're probably smiling. According to the National Retail Federation, thirty-five percent of Americans were expected to buy flowers for Valentine's Day in 2017, spending two billion dollars. In 2015, 63% of flower buyers on V-Day purchased cut red roses... but only 23% purchased living plants. I was a bit surprised to learn that, because I have always preferred a little plant I can keep alive for longer, that puts out flowers regularly. I know not everybody has room in their apartment for a plant, though. They can require large pots and lots of soil. But what about plants that don't need any soil at all?

[Music ends]

Izzie: Every Valentine's Day, my mom and I get an orchid plant for my grandma. They are her favorite flowers: so bright and cheerful, with petals unlike anything else on Earth. Most of the orchids in florist shops are tropical orchids, and they are epiphytic, which means they don't need to grow in soil. In the wild, these plants cling to tree branches, and their roots and leaves give them everything they need. Even out of soil, though, flowers - whether epiphytic orchids or roses destined for a bouquet - rely on fungi.

[Music begins]

Izzie: Ninety percent of all the plants on Earth engage in partnerships with fungi called mycorrhizae, and the partnership has been around literally since the dawn of time. Both plants and fungi evolved in the ocean, then made the jump to land. On dry ground, plants were apparently sort of dismayed that nutrients couldn't just float towards them in a never-ending diffusion. They needed some help accessing minerals from hard surfaces, especially rock. Since fungi put out enzymes into the environment to digest food before they eat it, they were much better able to get nutrients out of

soil and rock. That's why, 425 million years ago, plants and fungi struck one of the most important deals of all time: mycorrhizal fungi bring the plants minerals and some nutrients, and in return the plants supply them with sugar made from photosynthesis. This partnership basically allowed the greening of Earth, making it habitable to animals and, eventually, us. Thanks, mycorrhizae!

[Music ends]

Izzie: There are two major types of mycorrhizae found in plant partnerships. The most widespread are also the original plant partners, known as arbuscular mycorrhizae, or AM fungi. These live on many crops, as well as trees and other plants. AM fungi are obligate symbionts, which means they are completely dependent on their host plant, and they can't be cultured alone in the lab. The fungal hyphae actually squeeze between the plant's root cells, working their way right into the inner root, where they finally pop through the wall and into a plant cell. Inside the plant cell, the fungus trades essential nutrients for sweet, sweet sugar.

The devoted relationship between AM fungi and their plant hosts can benefit human romance, too; arbuscular mycorrhizae have been found to have desirable benefits on a lot of flowering plants. In 2010, a study found that inoculating greenhouse-grown lisianthus roots with AM fungi significantly increases the number of lisianthus flowers produced per square meter. A study from 2000 showed that China aster plants with roots in an arbuscular mycorrhizal relationship produced almost twice as many flowers as asters without AM fungi; in petunias, the number of flowers from inoculated plants was *triple* that of plants without the fungi. AM fungi can also make more flowers appear, faster, in Mexican marigold, zinnia, and even the all-important rose. The effects are sometimes so pronounced that AM fungi can replace chemical fertilizers!

The second-most major mycorrhizal fungi are the ectomycorrhizal, or ECM, fungi. "Ecto" means "outside"; where AM fungi nestle themselves right into the plant's cells, ECM fungi form a coat or sheath around the outside of the root tips. Ectomycorrhizal fungi mostly grow with trees, including pine, spruce, fir, oak, beech, and birch. The ECM fungi include basidiomycetes and ascomycetes - that's right, this is where many mushrooms come from!

[Musical tone]

Izzie: There are a few other types of mycorrhizal fungi, which mostly occur on only one family of plants. As you might have suspected from Leorah's message at the top of the episode, and my subtle, subtle hints about epiphytes, one of the plant families with specific mycorrhizal patterns is the Orchidaceae, the orchid family.

[Music begins]

Izzie: Now, for the most part, mycorrhizae are beneficial. They take their jobs very seriously, and help plants survive stressful situations like drought, nutrient deficiency, and even diseases caused by other fungi. But all the same, some plants are - shall we say - ungrateful. These plants take not just the nutrients offered by the fungi, but their sugar too, and supply nothing in return. These plants don't photosynthesize at all, but depend on mycorrhizal fungi for everything they need. Because they essentially eat fungi, these plants are called mycoheterotrophs. Surprisingly, this parasitic lifestyle has evolved numerous times and in very different plants, so that in 1994, mycoheterotrophism was known in 400 species of vascular plants. Victims may be AM fungi or ECM fungi, but our focus for today is on the orchid mycorrhizae.

[Music ends]

Izzie: A word about orchids: the kind you find in flower shops, and the kind most people think of, are tropical orchids. They're generally very colorful, and they're epiphytes, with roots that can hang out in the air instead of reaching into soil. If you live in a temperate region, you know that there aren't a lot of flowers that dangle their roots from apple trees or pine branches. That's because epiphytes need high humidity to ensure that the airy roots get enough water. This doesn't mean that orchids only live in tropical areas, though. There are between twenty-five and thirty thousand species of orchids - fully ten percent of the plants on Earth belong to the Orchidaceae, and they are unbelievably diverse. They live on every continent except Antarctica, edging right up into the Arctic Circle. You can bet *those* orchids aren't epiphytic.

Terrestrial orchids, which grow with their roots in the soil, live all over the world, including in every region where you find epiphytic orchids. There are more than 200 orchid species in North America alone. If you live in the Northeastern United States, you probably know about Lady's Slipper orchids, the Three Birds orchid, and the Cranefly orchid, as well as some others. All the same, there are a few reasons you may not have seen a terrestrial orchid in your neck of the woods. For one thing, more than half of North American orchids are endangered somewhere in their native range. On top of that, the native ranges can be very small - for example, Bentley's coralroot orchid, *Corallorhiza bentleyi*, grows in only fifteen populations, spread over a whopping five counties of Virginia and West Virginia. Lastly, a lot of terrestrial orchids stay underground, producing no leaves or flowers for years at a time. You could walk right next to a sleeping orchid, and never know.

Well, unless you have been monitoring it for nine years, waiting for it to appear.

[Music begins]

Voicemail Recording: Hello! You have reached the voicemail for the podcast Morel Dilemma, hosted by me, Izzie Gall. I can't talk fungi right now, but if you leave me your name, number, and a brief message, I will get back to you as soon as possible. Thank you!

Sabrina: Hi, my name is Sabrina and I'm calling in from Washington, DC. I'm here to talk about some "fun guys"... or I guess you would say fungi. I saw your topics that you're thinking about next season, and I have to say, flowers, flowers are the most romantic of the things. Fungi could be pretty romantic. Mushrooms, like, edible mushrooms, they make for a romantic meal. Sauté just makes me think of romance. Like, I just want to get really romantic when I think of sautéed mushrooms. This is all to say that, may 2017 be filled with many romantic flowers and fungi.

[Music ends]

Izzie: Because orchids are so visually captivating, and because so many of them are endangered, it's no wonder that a lot of botanical conservation efforts are focused on them. At the top of the episode, Leorah mentioned working at the Smithsonian Environmental Research Center, also known as SERC. That's one of the Smithsonian's nine research facilities, and it is indeed related to the world-famous Smithsonian museums. The Environmental Research Center recently developed a group devoted to orchid conservation, called the North American Orchid Conservation Center, or NAOCC. I was lucky enough to get to talk with Dennis Whigham, the founding director of the NAOCC, and Melissa McCormick, another cofounder and orchid scientist.

The formation of the NAOCC is by no means the Smithsonian's first foray into orchid research; SERC holds the world's largest culture collection of orchid mycorrhizal fungi, which includes more than 450 fungi isolated from more than 40 orchids native to North America. As impressive as that may sound, Melissa says they've only barely started.

Melissa: We've been trying to identify the fungi needed by all the orchids in North America.

Izzie: Nonetheless, some orchids do get higher priority. The SERC website emphasizes conservation efforts for *Isotria medeoloides*, the small-whorled pogonia - that's "whorl" like a spiral, not "world" like Earth. It's one of the most endangered plants in North America, and it's also a great example of the mycoheterotrophic orchid lifestyle.

[Music begins]

Izzie: The small-whorled pogonia is a terrestrial orchid that's even shyer than most, spending up to nine years dormant underground before sending up a flower. That's not just the wait for the first flower, either - a terrestrial orchid that has sent up a flower before can become dormant again. Orchid dormancy might be a response to stressful situations, and gives the plant an advantage against damage like getting eaten or trampled - if you don't show up above ground, nothing above ground can get you. On the other hand, it's a big gamble for the plant. Staying dormant means it can't sexually reproduce and make seeds, which is important to the survival of the species. And as long as they are underground, photosynthetic orchids like the small-whorled pogonia can't make any sugars; they rely entirely on their fungi. Even when they emerge to flower, the orchids may not be able to photosynthesize. Loss of chlorophyll has

occurred at least five separate times in the orchid family tree; it seems pure mycoheterotrophy is a very successful lifestyle.

[Music ends]

Izzie: "Wait," I hear you cry. "If an orchid isn't photosynthetic, or it is underground, it's not producing any sugar for itself; and since fungi can't photosynthesize, they sure aren't making any sugar themselves. Where is the carbon in this relationship coming from?"

Good questions. Orchids are not the only plants connected to their mycorrhizal fungi. The fungi that snake through the soil connect a lot of plants from numerous species. Some orchids rely on fungi that are connected to other, actively photosynthesizing plants. The orchid is parasitizing some other plant's sugars via the fungus, even as the orchid steals the nutrients the fungus pulls from rocks and soil. Hence, orchids are sometimes called secondarily parasitic - basically, they parasitize the fungus that has to parasitize a tree. Even if the fungus isn't usually parasitic, the energy drain from the orchid is enough to force it to parasitism. This is a pretty raw deal for both the other plant and the fungus, but surprisingly, this isn't a captive situation. Mycorrhizae come to the orchid voluntarily.

[Music begins]

Izzie: Mycorrhizal fungi are used to finding plants and giving them assistance. They've been doing it for 425 million years, after all. So when a mycorrhizal fungus encounters an orchid seed, it extends a helping hand to the orchid's embryo. When orchids grow big enough to sprout roots, instead of forming a partnership of equal exchange in orchid root cells, the fungus is induced to form balls of hyphae called pelotons that are permitted to grow, then digested by the orchid, in turns. If there are any Star Wars fans listening, you can imagine that the orchid is a sarlacc, and the fungi are poor Han Solo, getting slowly digested into starch over a period of a thousand years. Well, a few decades, in this case.

[Music ends]

Izzie: Unlike the sarlacc, though, orchids have many points of contact with their victims and undergo periods of dormancy, which means that the fungi might be more heavily relied upon at different times in the orchid's life. In the lab, it is possible to remove and isolate the pelotons from roots. If they are still viable - meaning, intact and alive - then they can also be grown in the lab. Dennis says,

Dennis: We know that if you get a root from an orchid and try to isolate pelotons from it you're not always successful. Sometimes [...] you put them on agar, they don't grow, and other times, they do.

Melissa: You take a root, and you try to get the fungi out of it, and most of what's in there is partially digested, or compacted, [...] or it's mostly been converted into starch.

Dennis: We know from sampling the orchids here at SERC that there's a seasonal component to when there are fungi in the orchid roots and when those pelotons are active. But as far as I know we're the only people that have looked at that, and we've looked at six or seven species out of 35,000. There's still a lot to be learned.

Izzie: Melissa suggested that in addition to this apparent seasonal fluctuation, it's possible that even within a single orchid's root system, mycorrhizae might be getting used faster in some areas than others. Basically, even if an orchid is photosynthetic, aboveground, and apparently doing great,

Melissa: I think the fungi are never totally absent from the orchid roots. I think it always has some fungus in there.

Izzie: From first contact with a fungus until the orchid dies, it's maintaining that mycorrhizal relationship. In fact, orchids cannot grow from seeds without fungal contact. Most flowering plants produce a few, large seeds that contain a nutritious package, called endosperm, to help the seed germinate if it lands in a favorable location. Orchids don't do endosperm. Instead they produce tiny "dust seeds" which only contain tiny, undeveloped embryos. An advantage to such tiny seeds is that orchids can produce literally millions of them, and they can travel farther on the wind than larger seeds could. The disadvantage is that those dust seeds are totally useless until they come in contact with mycorrhizal fungi, and even then, it can take them a long time to get up the gumption to emerge.

Melissa: There certainly is variation from one species to the next, but there are some photosynthetic orchids, like, say, *Goodyera pubescens*, which is a common orchid in this area –

Izzie: Commonly known as the downy rattlesnake plantain –

Melissa: – That I think can go from a seed to a seedling, which is to say it has a leaf, in probably about a year. And during that time, until it forms that first leaf, it is entirely reliant upon its fungi. Now that's not to say that once it becomes green and photosynthetic, it stops relying on its fungi. It still needs them.

Izzie: Adult orchids may not rely entirely on their mycorrhizae, especially if they are photosynthetic. But they don't seem to relinquish their fungi, either.

Melissa: We've done some other work that demonstrates that when orchids are stressed, so for example if they suddenly become quite shaded, or if they are experiencing drought conditions, then they shift over to relying much more heavily on their fungi again. So it's a relationship that continues throughout the orchid's life.

Izzie: The relationship is often very specific. Nobody knows what makes certain mycorrhizae suitable for partnership with orchids, but some orchids can only use a single fungal species. Or that may be a single complex of closely-related species. As Dennis told me,

sometimes, it can be hard to determine whether one fungus or multiple fungi are useful to an orchid species.

Dennis: We can get different fungi out of the roots of a given species, and they may not be exactly the same, but they're all related. And nobody knows whether it's a different species of fungus or what, but they're all in the same ballpark.

Izzie: Apparently, identifying the species an orchid uses is a whole other can of worms.

Melissa: Let's just say species delimitation among fungi is problematic.

Izzie: One reason is that soil fungi are extremely diverse. Another, closely related, reason is that among all those soil fungi, orchid mycorrhizal fungi are very hard to detect. Even the Tulasnella, one of the fungal families most commonly used by orchids, isn't found very often. SERC is one of the few research facilities in the world that's able to detect them in the wild.

Melissa: I know that if I take a soil core from out in the forest here I might get, for example, ten different species of Tulasnella in a single soil core. Yet most people don't detect any.

Izzie: Lastly, the species may be impossible to identify because they've simply never been found and named before.

Dennis: We have the largest collection of living orchid mycorrhizal fungi in the world in our lab, [...] somewhere between 600 and 700 isolates that we have. Melissa's analyzed some component of those, not all of them yet, but basically everything that gets looked at is new to science.

Izzie: In fact, on January 6 of 2017, the team published the research that first identified the nine species used by the small-whorled pogonia I mentioned earlier. DNA techniques are letting scientists find a lot more diversity than they could otherwise, largely because identifying fungi without DNA comes down to sexual morphology, and orchid mycorrhizal fungi don't fruit in the lab. They may not even fruit in the *wild*. As a result, lab cultures of these fungi - when successful - all look essentially the same.

Melissa: Yeah, originally, all the orchid fungi were described as Rhizoctonia species, (30:00) and it turns out that they clearly belong to multiple families.

Izzie: Even if scientists have trouble identifying the fungi, the orchid knows what it wants, and some are so picky that they can die when their preferred fungus disappears. The downy rattlesnake plantain, *Goodyera pubescens*, tries to keep one fungus host for its whole life. When SERC performed a decade-long survey of the fungi in the roots of these orchids,

Melissa: We had a couple of individuals who had the same fungus for ten years. We sampled one root, we came back ten years later, we sampled another root from the same plant, and it had the same individual fungus in it.

Izzie: There was a bad drought during that study, and some of these root fungi died. When the SERC team returned, some of the *G. pubescens* had managed to retain their fungi, and were doing pretty well. On the other hand,

Melissa: The other plants that had switched fungi, most of them were not doing quite as well. And there was a pretty high death toll associated with this very severe drought.

Izzie: In fact, all the orchids that had had to switch fungi died within two years of the drought. So, *Goodyera pubescens* is an orchid that requires the same fungus its whole life, or suffers. This is not always the case.

To make orchid conservation more confusing and difficult than it already is, many orchid species are not monogamous with their fungi. There are orchid species that can partner up with multiple fungi, though one individual only ever takes on one fungus at a time. Other orchids begin life with highly specific fungal partners, but are able to use more species effectively as they mature.

Melissa: *Tipularia discolor*, the crane fly orchid, needs one of two fungi in order to germinate. Those fungi are only abundant on decomposing wood. But once that orchid gets to maturity, often that decomposing wood has decomposed right out from underneath it, and it's now growing in the soil. And once *Tipularia* produces a green leaf, it becomes able to take on a much wider range of different fungi in its roots to form mycorrhizal associations with.

Izzie: This can be good news for the orchids, since after severe stress, they have more options than *G. pubescens* did. If another suitable fungus is nearby, they can grab onto it instead. But it does make cultivating those orchids in the lab even more grueling and even less effective than cultivating picky orchids. With a species like *Goodyera pubescens*, scientists can reach into the orchid's roots in a season when the pelotons are viable, culture them in the lab, and then place *G. pubescens* dust seeds nearby, with the reasonable expectation that these seeds will be able to germinate.

Dennis: What we do is we isolate the fungus, grow it in the lab, we know the orchid that it came from, and so when we want to do experiments of germination or whatever, we just put them back together.

Izzie: If the adult roots of an orchid hold a different fungus than the one that made first contact, that technique isn't viable.

[Music begins]

Izzie: This is a shame, because SERC studies have found that the orchid mycorrhizal fungi best suited to a species are particularly abundant near adult orchids of that species,

and, for at least three terrestrial orchids, the abundance of the germinating mycorrhizae is directly related to germination success. Also, for the small-whorled pogonia at least, increased abundance of desired mycorrhizae in the soil is also correlated with aboveground emergence of the plants. That means that in areas where the requisite fungi are more abundant, it's possible to have more emerging orchids producing seeds, and those seeds are more likely to germinate, supporting a higher orchid population – *if* the germination mycorrhizae are the same mycorrhizae that support adults.

[Music ends]

Izzie: To recap, we have plants that only germinate if certain, hard-to-detect fungi are present, and are more likely to flower if *other* certain, hard-to-detect fungi are present. Because of their acute sensitivity to soil fungi, orchids are considered good indicators of forest health. The thought process goes like this: when more orchids are sprouting, they need very specific fungi, so the soil mycorrhizae of the forest need to be healthy enough to provide a suitable home for that fungus. Unfortunately for orchids, when forests are disturbed - whether by human activity, invasive species, or other massive changes - the fungi they interact with are less abundant. For example, many of the fungi that orchids rely on grow with hickory, oak, and beech trees, which can only grow in forests more than 100 years old. Therefore, an undisturbed, healthy forest is expected to have more diverse tree species, more diverse soil fungi, and a more abundant population of native orchid species. By the same logic, an abundance of orchids indicates that a forest is healthy and undisturbed.

Dennis: We try to convince people of the notion that the orchids are very sensitively adapted to the environment where they occur, which is probably not the case for all of them, but if the fungus isn't there the orchid isn't going to be there. So if you have the orchids present in an ecosystem, that's at least an indicator that that ecosystem is very healthy.

Izzie: Unfortunately, orchid dormancy makes it hard to accurately estimate orchid populations. Orchids go dormant even in areas where their mycorrhizae are fairly abundant. For example, seventy-five percent of *Corallorhiza odontorhiza* orchids may stay underground during a given year. Dennis told me about one species that presents an additional puzzle. SERC has been monitoring these orchids for a while, marking off plots of forest where the emerging orchids are mapped each season.

Dennis: Over the many years that we've done this, what we find is that new plants appear regularly [...] but what's intriguing is that this particular orchid, as far as we know, has not set any seed out in the forest.

Izzie: The fruits tend to either rot or be eaten by deer before the dust seeds can be released. So where are the new plants coming from?

Dennis: The question is whether these are seeds that have been in the ground for a long period of time and then were able to germinate and then, within a year or so, appeared above ground; or whether these are plants that germinated and are living below ground for a long period of time off of fungi, without any photosynthesis, and then all of a sudden decide it's time to come above ground.

Izzie: One answer could indicate the spread of highly specific mycorrhizae, a possible sign of forest health or recovery. The other could indicate the maintenance of forest status quo, or indicate nothing, one way or the other.

Using orchids as yardsticks for forest health also assumes that the presence of their mycorrhizal fungi either derives from or contributes to a healthily functioning ecosystem. Actually,

Melissa: We really don't know how widespread these fungi are, we don't really know what they're doing in the ecosystem besides associating with orchids.

Izzie: In order to support the orchids, orchid mycorrhizal fungi that aren't ECMs with trees are probably decomposers known as saprobic fungi. They help return dead material to forms that forest organisms can use. But what do they do when the orchids aren't sapping them? What do the trees or other plants get out of the fungal association? These mysteries of fungal function pose as much issue for orchid conservation as orchids that swap mycorrhizae.

Melissa: That's why most orchid conservation efforts to this point have focused on just conserving the landscape [...] not enough has been known about all of the different connections that are necessary so that those orchids can grow, to be able to successfully go out and muck with what's there without making it worse instead of better.

Izzie: If scientists find a space that seems to be supporting a good community of orchids, they try to preserve the area as quickly as possible - essentially heading off the necessity of trying to tease apart the relationships in the lab later.

[Music begins]

Izzie: Mostly, though, conservationists are working in environments where the damage has been done and orchids are in decline. In this case, it's their job to figure out what the orchid species needs, grow some individuals in the lab, and reintroduce them to the area in an effort to recover the population. Unfortunately, there is no complete protocol for propagation and conservation for any North American orchid. I don't know if complete protocols exist for orchids elsewhere in the world, though I know reintroduction efforts have definitely been launched and have met some success.

The first step to bringing back an endangered orchid is to identify the fungus that the orchid requires, which is, of course, no mean feat. If the pelotons in the adult orchid don't belong to the germination species, scientists can try leaving seed packets out in

the forest. The packets are made of a fine mesh, and the idea is that the dust seeds inside will attract the germinating mycorrhizae from the soil. After leaving the packets for four or more years – because, remember, dust seeds don't germinate right away, even if the right fungi are present – scientists can recover the packets and try identifying and growing the fungi that reached into the dust seeds. This technique only works if the right fungi are present and the seeds actually germinated, which isn't often the case.

[Music ends]

Izzie: Assuming the right fungus is identified and isolated, scientists must then grow the mycorrhizal fungus in the lab. If it's a saprobic fungus, then it can usually be cultured on a petri dish with the right nutrients, with dust seeds placed nearby for germination. Of course, what constitutes the "right nutrients" is a trial to figure out all on its own, if the fungus' function in the wild isn't known. Put a peloton in a dish with the wrong stuff, and it can't grow and support seed germination. On the other hand, giving the fungus too much of what it wants can also cause problems.

Melissa: So if the media is too rich, then we can end up with a fungus that will totally overgrow the germinating seeds [...] and we will have little balls of brown mush instead of a nice, healthy, green plant.

Izzie: Ectomycorrhizal fungi pose more of an issue, since they need to grow near trees, and that's very hard to do in a lab!

Melissa: There's not really a way to grow a tree seedling on a petri dish.

Izzie: The SERC team has tried what might be considered the next-best thing: growing trees on soil held between sheets of plexiglass. That didn't turn out very well, and it introduces a new issue of sterility: if you try growing a tree in a sterile, fungus-free environment, when and how do you introduce the orchid mycorrhizae? How do you guarantee that it will establish with the tree? On the other hand, if you don't grow the tree in a sterile environment, opting instead for natural soil with native fungi present, how do you make sure the fungus the orchid needs is in there?

When seed packets and petri dishes fail to isolate, identify, or grow the right fungus, scientists may try growing orchids on fungi that they don't associate with in the wild. This was more common in the days before DNA techniques made fungus identification even remotely possible, but it's still employed today, if rarely.

Dennis: When people didn't have the molecular tools, they would just take a whole range of fungi that they had isolated in culture and then put them in petri dishes with the seeds, and it might get germination and the seedlings would grow, and so you ended up having people matching up orchids with fungi that they probably never would encounter in nature.

Izzie: One of Melissa and Dennis' associates, Larry Zettler, had luck with this technique in Hawaii - having had no luck isolating a threatened orchid's root mycorrhizae, he placed a lot of the orchid's seeds with a lot of fungi isolated from the roots of different orchids in other areas.

Dennis: He found one that did great. The seedlings grew really well!

Izzie: But rather than fixing his troubles, this discovery only brought up new, equally difficult problems.

Dennis: Do you take seedlings that are inoculated with a fungus that you don't know whether it's even in Hawaii, and put those seedlings back there with that fungus? It's a tricky, tricky business, and what we hope to do in working with all our collaborators is to try to get them to work with the fungi that we know come from the orchid that we're working with.

Izzie: If the orchid scientists want to bring balance back to the ecosystem, the best way to do it is by reintroducing native orchids with the correct, native fungi in their roots. The second-best way might be reintroducing native orchids with no fungi, and hoping the right mycorrhizae come along. In south Florida, Melissa is working with a regional team of the NAOCC that grew epiphytic orchids in a sterile lab environment, hoping to bait the right fungi during the orchids' reintroduction. To increase their chances, the team gathered dust seeds from several members of the local orchid population - an attempt to maintain local diversity while keeping the traits that evolved in the endangered area.

Melissa: So basically their approach is to plant out a bunch of orchids across a bunch of different trees, and hope that some of them establish with the right fungi. [...] The chances of any one of them surviving is actually pretty low.

Izzie: If the orchids' preferred mycorrhizae are known and can be found in soil samples, scientists can look for areas of the environment where those fungi are relatively abundant and preferentially reintroduce the orchid in that area. However, Melissa and Dennis' team isn't just loyal to the orchids; they are also responsible for the fungus' wellbeing, in a way.

Melissa: Particularly under stressful conditions, orchids can be taxing to their fungi, and if you've got a whole bunch of individuals all taxing the same fungal individual, then they would risk possibly killing off the fungus.

Izzie: Certain features of orchid lifestyles can help scientists predict how orchid reintroduction will affect the mycorrhizae.

Melissa: So, you know, if you've got an orchid that can use a really wide range of fungi, chances are that if you plant out a bunch of individuals, they're going to all be using different fungi. Whereas if you have an orchid that's very specific, then chances are that orchid's going to be - they're all going to be drawing on the same fungus.

Izzie: All the lab cultivation in the world amounts to nothing if there's no native fungus left to support the endangered orchids. That means the team has to be very careful with how many orchids they attempt to reintroduce to a habitat at one time. So while at the start of the conservation pipeline, scientists have to identify just a few fungi that help the orchid grow and germinate, by the end, they really are trying to tease apart the relationships of the entire forest.

[Music begins]

Izzie: The next time you walk by a terrestrial orchid, take a moment to think about all the fragile networks that go into keeping that orchid blooming, and keeping the forest healthy - both natural networks and human ones. It's humbling, isn't it?

As a closer, I'd like to ask you to remember the small-whorled pogonia, the most endangered orchid in North America. There's some good news and bad news where the pogonia is concerned. In January of 2017, Melissa and Dennis' team published work that identifies the fungi needed by the small-whorled pogonia for the first time. Unfortunately, the fungus is ectomycorrhizal, so the NAOCC is going to have to sort out the trees-in-plexiglass process to get it to grow in the lab. Also, the seed packet method hasn't been any help for gathering germinated seeds from the wild. I asked if they might try giving some seeds to Dr. Zettler, after his partial success with the Hawaiian orchid and non-native fungi.

Melissa: He gave up on this species. [Laughs] He wished us best of luck.

[Music ends]

Izzie: Still, having the fungus identified gives the team some new avenues to explore. For example, they know that in the soil, this fungus forms rhizomorphs, which are thicker than ordinary hyphae. The thicker mycelia don't fit through the mesh of the seed packet, so if the team widens the mesh a bit, they may achieve germination.

Melissa: Of course the problem with that is orchid seeds are so small that once you get a little bit bigger mesh you risk the orchid seeds coming out as well.

Izzie: Still, it's progress! And Melissa says that as fungal DNA techniques continue to improve, the team expects orchid conservation to keep getting easier.

[Music begins]

Izzie: Not that it could be much harder, at this point.

"Morel Dilemma" is written and produced by me, Izzie Gall. Our theme song is "Fungi Among I", composed and performed by John Bradley, and background music is "Underground Happy Hour" by Mihai Sorohan. You can find more of Mihai's music at mihaisorohan.bandcamp.com. Special thanks this episode to Leorah, for inspiring it, and to Sabrina, for that very romantic intermission!

Yorchidding!: Morel Dilemma Scripts, Episode 10

I also want to extend a very special thank-you to Melissa and Dennis, for taking the time to talk to me about SERC, pogonias, pelotons, and the incredible work they're doing. Just so you know, they don't just work on orchids - between them, Melissa and Dennis also contribute to projects dealing with salmon populations in Alaska, invasive wetland grasses in Chesapeake Bay, and even tigers in Bangladesh! (Okay, tiger *habitats*.) It's all very cool work, and you should check it out at serc.si.edu.

Morel Dilemma is on Patreon, where you can receive cool rewards for donating, and donations start at just \$1 a month. You can also post a review on iTunes, or wherever you listen. Next episode's topic is mushrooms that really need gills - because they grow underwater! Call 347-416-6735 if that tickles your interest or your imagination, and leave a mushroomy message to have your voice in the next episode. However you show it, thank you for your support! Mycelia later!

[Music ends]

Resources

- Aboul-Nasr A, 1996. "Effects of vesicular-arbuscular mycorrhiza on *Tagetes erecta* and *Zinnia elegans*." *Mycorrhiza* 6: 61-64.
- Deacon, Jim. "Mycorrhizas: Study Notes." *Environmental and Community Biology* 1h. 1998. Accessed 13 Jan 2017.
- "Endangered Orchid on the Edge." *Ecosystems on the Edge*. Smithsonian Environmental Research Center. 28 June 2013. Web. Accessed 17 Jan 2017.
- Ecosystems on the Edge*. Smithsonian Environmental Research Center. Web. Accessed 17 Jan 2017.
- Garmendia, I., Mangas, V.J. "Application of arbuscular mycorrhizal fungi on the production of cut flower roses under commercial-like conditions". *Spanish Journal of Agricultural Research* 2012 10(1): 166-174. Web.
- Gaur, A., Gaur, A., Adholeya, A. "Growth and flowering in *Petunia hybrida*, *Callistephus chinensis* and *Impatiens balsamina* inoculated with mixed AM inocula or chemical fertilizers in a soil of low P fertility." *Scientia Horticulturae* 28 April 2000 84(1-2): 151-162. Abstract.
- Go Orchids. North American Orchid Conservation Center. 2017. <http://goorchids.northamericanorchidcenter.org/>
- Landers, Jackson. "A Mystery of Hiding Orchids, Solved." *Smithsonian.com*. 19 Jan 2017. Web. 20 Jan 2017.
- Leake, J.R. "The biology of myco-heterotrophic ('saprophytic') plants." *New Phytologist* June 1994. 127(2): 171-216. Abstract.
- McCormick, M.K. Personal Interview. 21 Jan 2017.
- McCormick, M.K., Taylor, D.L., Juhaszova, K., Burnett, R.K. Jr., Whigham, D.F., O'Neill, J.P. "Limitations on orchid recruitment: not a simple picture." *Molecular Ecology* 21(6): 1511-1523. 24 Jan 2012. Abstract.
- McCormick, M.K., Taylor, D.L., Whigham, D.F., Burnett, R.K. Jr. "Germination patterns in three terrestrial orchids relate to abundance of mycorrhizal fungi." *Journal of Ecology* 104(3): 744-754. DOI: 10.1111/1365-2745.12556 19 March 2016. Summary.
- Meir D, Pivonia S, Levita R, Dori I, Ganot L, 2010. "Application of mycorrhizae to ornamental horticultural crops: lisianthus (*Eustoma grandiflorum*) as a test case." *Span J Agric Res* 8(S1): 5-10. Abstract.
- Nowak J, 2004. "Effects of arbuscular mycorrhizal fungi and organic fertilization on growth, flowering, nutrient uptake, photosynthesis and transpiration of geranium (*Pelargonium hortorum* L. H. Bailey 'Tango Orange')." *Symbiosis* 37: 259-266. Abstract.
- Rock-Blake, R., McCormick, M.K., Brooks, H. E. A., Jones, C.S., Whigham, D. F. "Symbiont abundance can affect host plant population dynamics." 6 Jan 2017. *American Journal of Botany* doi: 10.3732/ajb.1600334. Web.
- Rothacker, Eric. "Orchid Fungi." *Orchid Webpage For The Non-Scientists And Scientists Alike*. 2016. Accessed 13 Jan 2017.
- Rothacker, Eric. "Achlorophyllous orchids." *Orchid Webpage For The Non-Scientists And Scientists Alike*. 2016. Accessed 16 Jan 2017.
- "Valentine's Day Floral Statistics." *AboutFlowers.com*, The Society of American Florists.
- Whigham, Dennis. Personal Interview. 21 Jan 2017.