

They're All Part Fungus

Grass blades, coffee or cacao leaves ... probably all plants

Susan Milius

"You've mistaken a fungus for a pine tree" can be a ticklish thing for one botanist to say to another. Yet, in the 1990s, one respected university researcher made that very accusation to another. Stories such as this have spiced botanist gossip for years, but in this case, the two scientists resolved their differences and published a paper telling the whole story.



In the mid-1990s, Aaron Liston of Oregon State University in Corvallis was studying the evolutionary history of pine trees and managed to sequence a long stretch of DNA from pine needles. "It was still a big deal in those days," he says. He searched databases for genetic sequences from similar pine trees and found some that didn't match his results at all.

After more work, he became confident that his lab had the wrong pine-DNA sequence. He contacted Anita Klein of the University of New Hampshire in Durham, whose graduate students had contributed the other sequences to the database and used them in a journal paper.

"I broke the news to her slowly," he says. In a series of e-mails over about 2 weeks, he persuaded her that what her students had described as pine and fir genetic material wasn't actually from a plant. Nor was it from surface contamination or DNA wafting around the lab. It came from fungi living inside

WHAT LIES WITHIN. Plant species of forests, deserts, and farms all seem to have fungi living deep within their tissues. Most of these hidden houseguests don't cause disease but can still have big effects on their hosts.

PhotoDisc

the needles.

In figuring this out, Liston says, he had the advantage of his colleague Jeffrey Stone, who was "one of the few people who knew and cared about fungal endophytes." These fungi grow intermingled with cells inside plants but don't cause any apparent disease. Thus, from the outside, a leaf may look like solid plant tissue, but deep inside, spaghetti-like strands of fungal cells twist among the plant cells. The fungi are ensconced far more intimately than are the microbes that thrive on the vast plains of human skin or in the wet caverns of animal guts.

Klein says that she now realizes that her lab's primers for the procedure preferentially amplified fungal, rather than plant, DNA. The fungi probably coevolved with their plant hosts, she says, so fungal DNA taken from seven-or-so spruce and pine species showed plausible relationships when regarded as a plant family tree. "I can look back on it now and chuckle," she says. "But I was devastated then."

Plant-entrenched fungi have been challenging to study, but modern molecular technology is finally revealing their world. Now, they're turning up all over, and their influence can be big, even though they are not.

Fungus among them

Fungi can put the greediest human land developers to shame when it comes to turning open real estate into homes. Given just a few lucky breaks, some fungi exploit the vast acreage of leaf surfaces. Other fungal species target plant roots and show up routinely on some 85 percent of plant species. These mycorrhizal fungi can boost the root system's efficiency.

Fungal endophytes slip into plant leaves and stems to set up housekeeping between, or even inside, plant cells. Some fungal endophytes, such as those in tall fescue or other grasses, also infiltrate the seeds that their host plants are forming, thereby stowing away for the ride to the next generation.

More commonly, endophyte spores waft through the air in search of a new home.

The spores are impressive at breaking and entering, says Elizabeth Arnold of the University of Arizona in Tucson. They usually don't take the easy way in, through a leaf's breathing holes. Instead, a spore typically lands on a leaf, germinates, and drills a strand of tissue right through the plant's fortified coatings.

A colony founded by one of these intruders typically grabs only a few cubic millimeters of internal leaf space, favoring locations near the plant's internal plumbing. The fungus lives off carbon and other nutrients from leaky

pipes. Under a microscope, strands of the fungal lurker can be seen curving through the brickwork of plant cellular tissue. The fungus grows, often extremely slowly, by sprouting off more strands of cells.

For many of these fungi, scientists don't know how the life cycle wraps up, says Arnold. Somewhere, somehow, most of the species must make spores. Yet the fungal lurkers have only rarely been caught sporulating in living plant tissue.

One solution to the mystery might be that the endophytes, other than those in some grasses, wait until the plant dies to make their spores, says Arnold. A big benefit of invading living tissue might be preparation for fast postmortem access. "Maybe they're like little vultures," she says.

Whatever drives the plant-endophyte fungus relationship, it's showing up all over. Every one of the several hundred plant species tested so far has yielded lurking fungi, says Arnold. They've turned up in the little dryas wildflower of the tundra and in leaves of tropical trees. In 2005, an international research team even collected an abundance of endophytes from the innards of cacti.

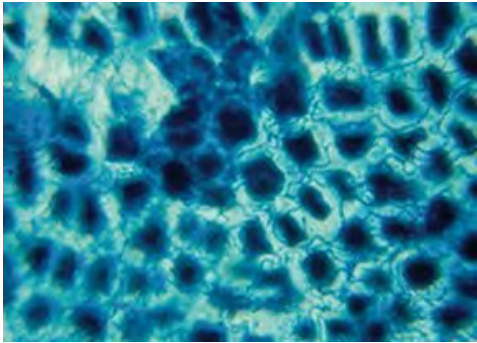
A single plant species can have a large assortment of the lurkers. Arnold says that she was first jolted into an appreciation of the variety of fungi inside plants when she worked as a research assistant in Panama. To learn how the age of leaves influences their disease resistance, she placed strips of tropical-tree leaves on a standard lab-fungus food. The dishes turned into fantastical fungus gardens with spots of white fuzz, dark slime, and colored fur. One of her prize specimens grew out in a cluster of little rounded arcs "like a green rose," she says.

A sample of 83 healthy leaves from just two species of tropical tree yielded more than 400 kinds of fungus living inside, Arnold and her colleagues reported in 2000. They called the fungi not diverse but "hyperdiverse."

Endophyte variety seems to far outstrip that of the plants they inhabit. In the tropics, there may be dozens of fungal endophyte species per plant. Researchers have estimated that Earth's endophyte species outnumber its plant species by a factor of four. More than a million kinds of endophytes might be lurking around us.

Beware of grass

In the 1970s, biologists discovered that invisible endophytes can have visible effects. The first discoveries of endophyte power came from grasses on farms. One story begins in the 1930s, when University of Kentucky agronomists got seeds from a farmer with a hillside of remarkable grass.



HITCHHIKERS GUILF. Twisting strands of a *Neotyphodium* fungus (colored dark blue in image) have grown among the boxy cells within this grass-seed tissue. Some fungi ride along in seeds to new homes, but many depend on air currents to transport them to welcoming plants.

Faeth

The grass flourished, but sometimes the cattle grazing on it did not. Farmers reported that when the cattle ate primarily the fescue called Kentucky 31, they went lame more often than usual, and their tails sometimes sloughed off. While investigating the livestock troubles, researchers eventually realized that a fungus in the genus *Neotyphodium* secreted compounds that constricted blood flow in cattle extremities.

Another species introduced for grazing, perennial ryegrass, turned out to carry these fungi too. A Southwestern native species called sleepygrass hosted a fungus of the same genus. That plant earned its name from wooziness that struck animals grazing on it.

The *Neotyphodium* fungi exude toxins related to the hallucinogenic drug LSD. The fungal taints also bring ill effects to minigrazers, such as insects and nematodes, scientists discovered.

These effects vexed farmers but fascinated ecologists. By the 1960s and 1970s, those hidden fungi, once dismissed as

curiosities, seemed to be sophisticated, mutualistic partners of grasses. A plant sheltered and fed them, and, in turn, they defended it against grazers. Ecologists also reported other effects, such as resistance to drought.

Recent years have seen challenges to the idea of plant-endophyte mutualism. Stan Faeth of Arizona State University in Tempe, for example, reports that one of tall fescue's native relatives, *Festuca arizonica*, doesn't grow as well when it houses one of the supposedly protective fungal partners. He's found that the endophyte is usually a parasite rather than a pal.

Even for some of the textbook mutualism cases, such as sleepygrass, Faeth and his colleagues are raising questions, which he notes are controversial. "The hallmark of native endophytes and grasses is remarkable variability," he says. The agronomic grasses are poor models and fail to capture the wide range of endophyte interactions in nature."

In a recent example of his lab's work, the researchers sampled 17 grass patches at distances up to about 600

kilometers from a patch of highly toxic grass in Cloudcroft, N.M. How widespread the endophyte was—and its effects—varied considerably, the researchers report in an upcoming *Journal of Chemical Ecology*. They found that some patches of grass in Colorado were thoroughly infected with the toxic grasses' fungal species but had no antigrazing toxins. So, Faeth asks, is the supposed mutualist earning its keep?

Kari Saikkonen of MTT Agrifood Research in Turku, Finland, suggests that by looking only at the grass and the fungus, researchers have oversimplified the interactions. To better represent the complications of the real world, he and his colleagues set up a four-way laboratory interaction to see whether other players could alter a grass-fungus interaction.

In the lab, the researchers let a plant in the snapdragon family, the greater yellow-rattle, attack a fungus-bearing meadow rye grass. Yellow-rattle sneaks suckers over to the roots of other plants and steals sap.

When yellow-rattles parasitized grass that carried an antigrazing fungus, the researchers found that the parasite took up the fungal defensive toxins along with other goodies from the plant. In the presence of both yellow-rattle and aphids, the ryegrass with the toxic fungus didn't do as well as ryegrass without the fungus did. In this setup, fungus looked like a burden to the ryegrass, the researchers reported in the Dec. 2005 *Ecology Letters*.

If a fungus can tweak predators of neighboring plants, can its effects ripple up a food chain? When researchers at the University of Zurich herded cereal aphids onto fungus-bearing ryegrass, the ladybird beetles that fed on the aphids failed to thrive and didn't reproduce well. Jochen Krauss and his colleagues describe that experiment in an upcoming *Proceedings of the Royal Society B*.

Beyond grass

Scientists have in recent years turned to the fungi in plants other than grasses. In 2003, Arnold and her colleagues presented the first strong evidence of fungal-pest fighting by natural endophytes in a nongrass plant, wild cacao ([SN: 12/13/03, p. 374](#)).

The myriad fungi in tree leaves might create a defense system, suggests Edward Allen Herre of the Smithsonian Tropical Research Institute in Balboa, Panama. The abundance of fungi with their differing powers of interaction similarly increase the odds that for any no-good intruder, there's already an antagonist on hand.

Herre is working to manipulate such fungi to fight diseases of cacao. He says that he's getting promising preliminary results from a field test applying extra endophytic fungi.

The approach is attracting interest. The American Phytopathological Society annual meeting devoted a session to the concept last August in Austin, Texas, and the U.S. Department of Agriculture is funding research. Fernando Vega of the USDA's Beltsville facility has found some endophytes that deter insects in lab tests of coffee leaves.

Making the world safer for chocolate and coffee, among other crops, is a big job for organisms that nobody sees. That's not a problem, according to endophyte enthusiasts. As Krauss says, "It's the little things that rule the world."

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