Honeybee *Apis mellifera*
Photo ©2020 Daniel Prudek

Can Fungi Ease Disease in Bees?

Fungi Bees?

Reishi *Ganoderma resinaceum*
Photo ©2020 Paul Stamets

By Connor Yearsley

Many people grew up with Winnie-the-Pooh, the good-natured bear who was often looking for "hunny" in rotting tree hollows. Renowned mycologist (fungi expert) Paul Stamets, however, may be the first person to wonder if some bees are drawn to rotting wood partly because of the health benefits that the fungal mycelia can provide as they break down the wood. Mycelia are the vegetative part of a fungus' life cycle and consist of threadlike tubes called hyphae that spread in every direction.

With researchers at Washington State University (WSU) and the United States Department of Agriculture (USDA), Stamets' hypothesis that fungi can help bees is being validated with extracts of the mycelia of two fungal species: amadou (*Fomes fomentarius*, Polyporaceae) and reishi (*Ganoderma resinaceum*, Ganodermataceae). In laboratory and field trials on the Western or European honeybee (*Apis mellifera*), these extracts significantly reduced levels of viruses that are devastating bee colonies (see sidebar). Stamets and his team hope that these and other fungal extracts can become long-term and widespread solutions to improve the health, immunity, and longevity of bees, which are critical for healthy ecosystems and human food security.[1-4]

"I believe this is a paradigm-shifting discovery that can reverse loss of biodiversity, which is a significant and immediate threat to global food biosecurity," Stamets

said (oral communication, July 3, 2019). "Many people use the analogy of the canary in the coal mine, but the canary in the coal mine probably just affects coal miners, while the loss of bees will affect not only people, but has effects that echo throughout the fabric of nature."

Stamets emphasizes that mycelia have "hidden secrets" and generally contain many more compounds and proteins than mushrooms do. "Mycelia are a lot more bioactive because they are in direct competition with microbes that are trying to consume them," he told *HerbalGram* in 2016.[5] "The fleshier mushrooms rot in a few days, so, in fact, they don't have a very good immune system, compared to the immunologically active mycelia. There are millions of microbes per gram of healthy soil, and for the mycelia to navigate through that potentially hostile environment speaks of a treasure trove of new active constituents that can help human and environmental health."

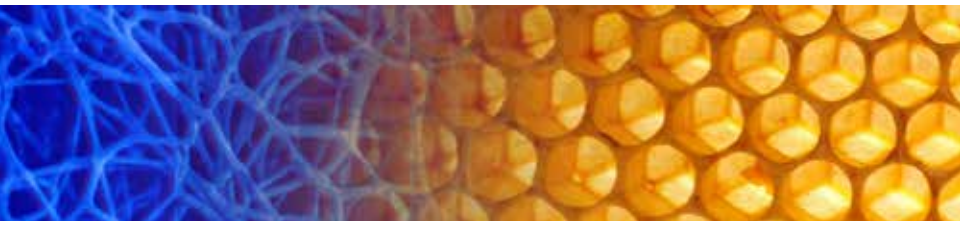
Paul Stamets with a "bee beard." The honeybees are attracted to Stamets because their queen is contained in a box around his neck.
Photo ©2020 Paul Stamets

Fungi and Bees: What's the Connection?

Because of their effectiveness in laboratory tests, the amadou and reishi mycelial extracts were validated in a field trial. In this trial, honeybee colonies treated with the amadou extract showed a significant 79.7-fold reduction in Deformed Wing Virus (DWV) levels 12 days after treatment, while colonies treated with the reishi extract showed 79.6-fold greater DWV reductions than control colonies. Amadou administration also decreased Lake Sinai Virus (LSV) levels 87.9 times more than the control colonies, while reishi decreased LSV levels 45,000 times more than control colonies. Treatment colonies were fed the mycelial extracts in a sucrose solution, while control colonies were fed the sucrose syrup only.[1*]

Amadou *Fomes fomentarius*
Photo ©2020 LianeM

* It is not clear yet how the extracts work (e.g., their mechanisms of action) or which chemical constituents are involved, but Stamets and colleagues are conducting studies to determine which of the bees' immune-regulating genes are activated by the extracts.



Over the past 50 years, the combination of *Varroa destructor* mites and Deformed Wing Virus has killed billions of bees.

This study, published in *Scientific Reports* (a *Nature* publication), remains in the 99th percentile of all tracked articles of a similar age in all *Nature* publications.[1] Stamets thinks that may be because it is one of the first reports of "a natural product giving a broader 'bioshield' of protection than a pure pharmaceutical, underscoring the complexity of animals' immune systems," he wrote (email, January 28, 2020).

Walter S. Sheppard, PhD, chair of the department of entomology at WSU who is working with Stamets, was quoted as saying: "As an entomologist with 40+ years of experience studying bees, I am unaware of any reports of materials that extend the life of worker bees more than [these extracts]."[4]

Jay Evans, PhD, a virologist and research leader of the USDA's Bee Research Laboratory who also is working with Stamets, thinks it will not be difficult to convince beekeepers to use the extracts once they see firsthand that they are safe and effective for everyday use. "Beekeepers are really interested in using natural medicines," Evans said (oral communication, November 21, 2019). "They don't like putting synthetic chemicals in their hives. Europe and South America have thousands of beekeepers, because they tend to have smaller scale beekeepers, and I think they would be eager to adopt something like this."

Why Bee Concerned?

An estimated 87.5% of all flowering plants are adapted to animal pollination.[6] Pollinators strongly impact ecological relationships, ecosystem stability, plant genetic variation, evolution, and more.[7] Bees, which include about 20,000 known species worldwide, are the main pollinators in most ecosystems and have been found in all habitats with flowering plants.[6]

Apis mellifera is the most widely managed pollinator. It is not native to North America. Colonists brought the species from Europe in the 1500s and 1600s. Many crops also were brought to North America from elsewhere and evolved in the same places with honeybees. Now, *A. mellifera* pollinates more than 100 food and other commercial crops in North America. In the United States alone, *A. mellifera* colonies add at least $15 billion in agricultural value per year. Also, pollinators, including honeybees, are responsible for an estimated one of every three bites of food that humans eat.[8,9]

Almonds (*Prunus dulcis*, Rosaceae), for example, depend almost completely on honeybee pollination, which is significant with the popularity of almond milk and almond butter.[8] Dairy aisles would be much less diverse without pollinators, including bees. Most fruit-flavored yogurts would disappear. Pollinators also are responsible for the chocolate (derived from *Theobroma cacao*, Malvaceae) in chocolate milk. Milk products could decrease up to 50% without bee-pollinated clover (*Trifolium* spp., Fabaceae) and alfalfa (*Medicago sativa*, Fabaceae), which are fodder for cattle.[10]

One honeybee can visit between 50 and 1,000 flowers per trip and can make seven to 14 trips per day. So, for example, a colony with 25,000 forager bees, each making 10 trips per day, can pollinate 250 million flowers per day![7] According to an expert peer reviewer of this article, bees prefer to stay within two miles of their hive, but can travel up to five miles, so one colony can cover an area ranging from 12 square miles to 78 square miles. This creates challenges for "organic" beekeepers, because they need to center their hives where the bees will not come in contact with insecticides, fungicides, or herbicides, all of which affect bee health.

During winter 2006-2007, beekeepers in the United States lost many bees to a set of symptoms later called Colony Collapse Disorder (CCD). Specifically, CCD occurs when most worker bees in the colony disappear and leave behind a live queen, food stores (honey and pollen), capped brood (bees usually do not abandon a hive until all capped brood hatch), and a few nurse bees to care for the remaining immature bees.[8,9]

CCD does not describe all colony losses, and no single factor is known to cause CCD. Instead, multiple factors likely are involved and possibly not always the same factors in the same order. Since 2010, CCD cases have decreased, but beekeepers continue to lose bees for other reasons.[8,9] In fact, colony losses in the United States have averaged about 30% per year in recent years.[11]

Threats to honeybees include *Nosema*, a parasitic fungus that infects bees' midguts and causes symptoms like dysentery.[8,9] Also, neonicotinoids, a class of insecticides, are absorbed by plants and can be present in pollen and nectar, making them potentially toxic to bees.[8,12] Poor nutrition from a loss of diverse foraging plants also weakens bees' immunity, making them more vulnerable in general. However, beekeepers have identified *Varroa destructor* mites as the most serious problem.[8] These parasitic arachnids, native to Asia, have spread to almost every beekeeping region in the world. They latch onto bees and suck their hemolymph (a blood-like fluid in most invertebrates), which weakens the bees and shortens their lifespan.[11]

Varroa mites and other parasites can spread among bee colonies through bee drift. That is, while bees have amazing navigational abilities, a foraging bee sometimes returns to the wrong colony. Typically, the colony accepts her because of the pollen or nectar she brings. This can infect the colony if the drifter carries *Varroa*.[13]

Still, even larger field trials are needed to confirm if and how the amadou and reishi mycelial extracts might work in the field, according to Evans. Field trials, in general, are challenging partly because it is difficult to control where the bees forage and what beekeepers do to them. An entire field trial can be compromised if the bees bring in a pesticide from outside. "We think the effects are additive, so if you treat the viruses, the bees might do better against other stresses, but there is a lot more noise at the colony level," Evans said.

Two bumblebee species, *Bombus mixtus* and *B. melanopygus*, at a bee feeder containing the reishi extract in sugar water.
Photo ©2020 Paul Stamets

Sometimes, even if a treatment is effective, uncontrollable variables in the field may prevent a measurable effect from being seen in a month, which is the length of some field trials. The treatment needs to work in this un-ideal, real-life situation, though. "A lot of treatments don't survive the field trial stage, which means you need to have more candidates or be willing to keep refining it," Evans said. "And I think Paul is doing that."

Evans and his colleagues have tested dozens of candidates for antiviral properties in bees, including thymol, which is derived from the essential oil of thyme (*Thymus vulgaris*, Lamiaceae), and some compounds from nectar. So far, Stamets' extracts are working better than all of those, Evans said. Still, he "would love to see more" fungal candidates tested. "In science, you are really lucky if you find the winner on the first attempt, so I think it is worthwhile to keep looking."

Stamets is screening other fungal species for antiviral activity in bees, including agarikon (*Laricifomes officinalis*, Fomitopsidaceae), a rare fungus from old-growth forests. "Keep in mind that these tests are complicated," he wrote

The mites transmit viruses, most notably the Deformed Wing Virus (DWV), which they inject directly into the bees' hemolymph. DWV causes shriveled wings and shortens the lifespan of bees. Colonies without *Varroa* maintain low and often asymptomatic levels of DWV. With *Varroa*, however, the percentage of infected bees in a colony can increase from 10% to 100%. Over the past 50 years, the combination of *Varroa* mites and DWV has killed billions of bees.[11]

A 2016 study found that male drone honeybees with DWV can infect queens during mating.[14] Also, flowers can be hotspots for viral transmission, a 2019 study found. Honeybees were shown to deposit both DWV and Black Queen Cell Virus on flowers, but viruses were not evenly deposited on all analyzed flowers. Floral traits, virus ecology, and/or foraging behavior may, therefore, affect virus deposition.[15] In a different 2019 study, some wild bumblebees (*Bombus* spp.) near honeybee apiaries had DWV, while bumblebees that were not near apiaries did not, and viruses were found on flowers only near apiaries. This suggests viruses are passing from managed honeybees to wild bumblebees through shared floral resources.[16]

Honeybee (*Apis mellifera*) parasitized by *Varroa* mite (center) and another honeybee with signs of Deformed Wing Virus (bottom left).
Photo ©2020 Piscisgate

Photo ©2020 Whole Foods Market

(email, July 3, 2019). "We need to have many replicates, and the controls need to work. These are, in fact, animal clinical studies. If I had the money, we would be screening 100 species of wood-decomposing fungi. [I expect we] would find many that would have specificity against different sets of viruses and extend longevity in bees."

After September 11, 2001, Stamets became involved with Project BioShield, a collaborative federal effort to discover new ways to protect Americans from chemical, biological, radiological, and nuclear threats. He submitted more than 500 fungal extracts, many of which showed significant effects against small pox, flu viruses, and herpes in vitro. Later, Stamets submitted to the US National Institutes of Health (NIH) 20 isolated compounds produced by agarikon mycelia as they rot wood. Nine of these compounds were found to have significant effects against different viruses (also in vitro).[5] This work later caused him to wonder if fungi could also have antiviral properties in bees.

Several observations led Stamets to notice a connection between fungi and bees. One July morning in the 1980s, he noticed bees from his two beehives all over the wood chips in his garden giant (*Stropharia rugosoannulata*, Strophariaceae), or king stropharia, patch. For about 40 days, from morning to dusk, he observed the bees move from their hives to the garden giant patch and ingest the mycelia. The garden giant is normally red but turned white. Stamets suspects the bees interfered with the garden giant's melanin pathways, which could suggest a possible mode of action by which fungal extracts could help bees. He wrote about this in his book *Growing Gourmet and Medicinal Mushrooms* (Ten Speed Press, 1993), and then it faded from memory until later.

Later, Stamets' observation of bears' scratching trees was also important. When bears scratch trees, it creates wounds in the tree from which bees can collect plant materials to make propolis (or bee glue, a complex, sticky, resinous, wax-like mixture that bees use as cement and disinfectant and to embalm dead hive invaders). The wounds also allow fungi, like the red-belted polypore (*Fomitopsis pinicola*, Fomitopsidaceae), to enter and break down the tree. The fungi thus create a place for bees and other cavity dwellers to move in. "Lots of cavity-dwelling organisms take advantage of myceliated wood once an entry wound is created," Stamets said. "We need cavity dwellers, and some bees are cavity dwellers."

Of the estimated 5.1 million fungal species,[17] Stamets chose to test reishi and amadou for several reasons. During his work with Project BioShield, reishi extracts were found to be highly active against bird flu viruses. And, as Stamets explained, amadou was found in a pouch carried by the Iceman, also called Ötzi, a naturally mummified 5,300-year-old Neolithic hunter who was discovered in 1991 in a glacier in the Ötztal Alps on the border of Italy and Austria. He likely used amadou, which is highly flammable and burns slowly, as a fire starter and medicine.[18,19] Also, according to Stamets, beekeepers have used amadou as a fuel to smoke beehives to calm the bees while they work. Moreover, amadou produces a compound that is highly active against the tobacco mosaic virus, which was the first virus ever discovered and causes mosaic-like mottling on the leaves of tobacco (*Nicotiana tabacum*, Solanaceae) and other species in the Solanaceae family.[20] These factors intrigued Stamets.

Some fungi may also be able to control *Varroa* mites that transmit viruses to bees. *Varroa* mites are tough, Evans said. Like many pests, they have developed resistance to single-chemical treatments over time. Plus, it is difficult to eliminate the mites without also harming the bees. However, preliminary studies by researchers at WSU suggest that

Metarhizium (Clavicipitaceae) fungi may be toxic to *Varroa* but not harmful to bees.[3] The ideal temperature of beehives is typically about 95°F, and *Metarhizium* fungi typically grow at about 90°F, Stamets said. So, through natural selection, WSU researchers are trying to develop heat-tolerant phenotypes of *Metarhizium* so they can be applied in beehives to kill *Varroa*.

Other recent research also supports that fungi can benefit bees. A 2018 study examined the symbiotic relationship between the Brazilian stingless bee *Scaptotrigona depilis* and a fungus in the genus *Zygosaccharomyces* (Saccharomycetaceae). This fungus, found in the bees' brood cells, is eaten by and provides ergosterol to the developing larvae, which allows successful pupation. Insects cannot make sterols on their own and must obtain steroids through their diet to survive.[21] According to Stamets, *Ganoderma* species are known for their steroidal compounds, so this may suggest a possible mode of action by which his extracts could help bees.

Stamets is "perplexed" that science took so long to realize the potential benefits of fungi for bees. "It shows that solutions are hiding in plain sight," he said. Evans, however, is "not as much surprised, but just glad that other options are on the table now." He thinks this discovery reflects recent interest in bee health and how multidisciplinary science has become. It combines mycology (the study of fungi), entomology (the study of insects), virology (the study of viruses), botany, and more.

Down the Road and Bee-yond

Using fungal extracts to improve bee health is both "highly sustainable" and "ecologically rational," according to Stamets. *Ganoderma* species grow in tropical and temperate regions, and amadou commonly grows on birch (*Betula* spp., Betulaceae) trees in northern areas and beech (*Fagus* spp., Fagaceae) trees in southern areas.

Polypore fungi, which form large fruiting bodies with pores or tubes on the underside, such as reishi and amadou, are found in forests around the world. "So it is a vast genomic library that we can tap into," Stamets said. It is possible that fungi found in one area can be used to benefit bees in that area. Often, it is not necessary to wild-harvest entire fungal specimens. By taking small tissue samples, the mycelia can be propagated in vitro anywhere in the world. Stamets' company, Fungi Perfecti (Olympia, Washington), grows tons of mycelia per week using in vitro propagation, he said.

Stamets envisions having a widespread, decentralized network of fungal production facilities. "We should reinvent mushroom cultivation facilities as environmental training and learning centers," he said. "I have been saying that for decades. Mushroom production facilities should be reinvented as keystone components in the fabric of sustainable ecosystems, and this fits in perfectly."

Honeybees *Apis mellifera*
Photo ©2020 Kuttelvaserova Stuchelova

Watercolor of a forest landscape by Jamichael Henterly commissioned by Paul Stamets. Image courtesy of Paul Stamets

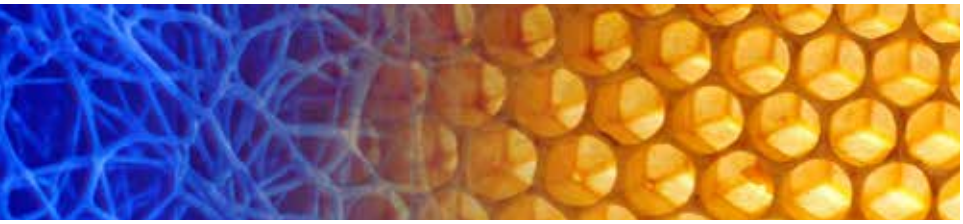
The mycelial extracts are "extremely easy to deploy," Stamets said. Many or most beekeepers already regularly feed sugar water to their bees to maintain health. Because the mycelial extracts are water-soluble, they can be added directly to the sugar water feeding system, at one drop of extract per 100 drops of solution. The extracts could also be sprinkled in the hive, Evans said, but it is probably easiest to add them to the sugar water, as has been done for some other products.

Stamets' company also developed a bee feeder, similar to a hummingbird feeder, so everyone can help bees in their own yards. The feeders can be filled with a solution containing the mycelial extracts. Stamets commits that his company will give away 10,000 of these feeders for free. (More information is available at www.fungi.com/bees.) He also is developing another feeder that will hopefully be able to track bee visits and pollen loads, with the intention that citizen scientists and others around the world will upload the data to a portal to monitor progress. He estimates that millions of these feeders will be needed to reverse loss of bee populations.[2,4]

All of Stamets' experiments so far were conducted on *A. mellifera*, specifically female worker bees, but he believes the extracts "absolutely" can benefit both managed and wild bees. Wild bees, which sometimes are outcompeted by *A. mellifera*, are also important pollinators. Photographic evidence shows two wild bumblebee species, *Bombus mixtus* and *B. melanopygus*, together at a bee feeder containing the reishi extract and sugar water (see image on page 69). "I saw them coexisting," Stamets said. "Both of them were going happily on their way, in and out of the bee feeder and not being aggressive to each other, but just sharing the resource. That was fun to see." From his experience, wild bumblebees usually find the bee feeder first, in about one to two weeks. The honeybees usually find it later and in greater numbers.

Stamets anticipates that the cost of the extracts will be low in general. At first, he and his team grew the mycelia using organic brown rice (*Oryza sativa*, Poaceae) as the substrate, or the material on or from which an organism lives, grows, and/or obtains its nourishment. After switching to birch sawdust, the extract reportedly became more than 10 times more potent, meaning the viral load decreased more than 10 times more. Stamets noted that, unlike brown rice, wood contains lignin, a complex organic polymer that lends rigidity to vascular plants and some algae. He suspects that, when the mycelia are grown on wood instead of brown rice, more bee-beneficial compounds are produced as the mycelia delignify (or remove lignin from) the wood, which increases the potency. According to him, a ten-fold increase in potency results in about a ten-fold decrease in cost. He and his colleagues are still experimenting to make the extracts more potent and thus less expensive.

Using fungal extracts to improve bee health is both "highly sustainable" and "ecologically rational," according to Stamets.

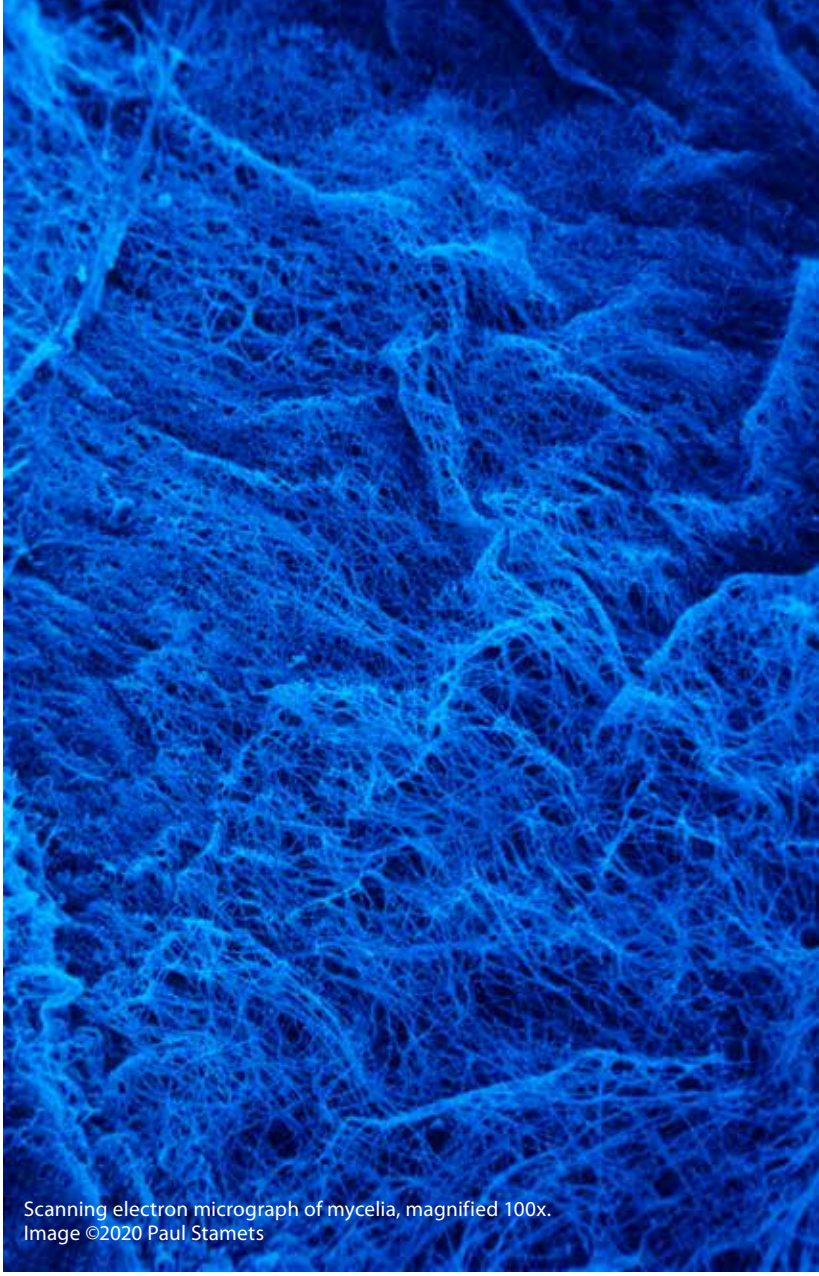


The possibility that the viruses could become resistant to fungal extracts in general is a concern, Stamets said, but it shouldn't prevent the problem from being addressed. He thinks the proliferation of the viruses, in the first place, is partly, or largely, due to widespread deforestation, which has decreased the quantity and variety of woods on which mycelia grow, thus reducing the production of natural compounds that could otherwise help control the viruses. Because the extracts contain many compounds, instead of just one, it is less likely that the viruses will become resistant, Stamets said. Plus, it is thought that the extracts reduce viruses indirectly by improving the overall immunity of the bees, instead of directly affecting the structure of the viruses. If true, that also means resistance may be less likely, Evans noted.

"We need all hands on deck and can't let the perfect be the enemy of the good," Stamets said. "We can twiddle our thumbs and worry about resistance as this massive extinction event unfolds. Or we can begin to take action and deploy these now, because we are in a full-blown crisis."

Stamets noted that, in general, very little is being spent on saving bees, "which have such an important economic impact. It is disproportionate. If we lose bees, then people will better appreciate how important they were when the cost of food skyrockets and a large percentage of our food disappears from the marketplace or becomes so exceptionally rare that only the wealthy can afford it.

"I think I found something fundamental to the foundation of nature: that these polypore fungi and their mycelia are keystones that immunologically protect and connect animal inhabitants of forest lands, from bees to birds, bats, bears, pigs, and people," Stamets said. HG



Scanning electron micrograph of mycelia, magnified 100x.
Image ©2020 Paul Stamets

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